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## RESEARCH ARTICLE

# An anti-narcolepsy drug reveals behavioral and fitness costs of extreme activity cycles in arctic-breeding songbirds

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## ABSTRACT

Sleep loss impairs cognitive function, immunological responses and general well-being in humans. However, sleep requirements in mammals and birds vary dramatically. In circumpolar regions with continuous summer light, daily sleep duration is reduced, particularly in breeding birds. The effect of an anti-narcolepsy drug (modafinil) to putatively extend wakefulness was examined in two species of closely related arctic-breeding passerine birds: Lapland longspurs (*Calcarius lapponicus*) and snow buntings (*Plectrophenax nivalis*). Free-living adult males were implanted during the nestling phase on day 4 (D4; 4 days post-hatching) with osmotic pumps containing either vehicle or modafinil to extend the active period for 72 h. Nestlings were weighed on D2 and D7 to measure growth rates. Additionally, focal observations were conducted on D6. Male longspurs receiving modafinil made fewer feeding visits and spent less time at the nest but tended to spend more time near the nest than controls. We observed no change in longspur nestling growth rates, but fledging occurred significantly later when males received modafinil, suggesting a fitness cost. In contrast, modafinil had no measurable impact on male or female snow bunting behavior, nestling growth rates or time to fledging. We suggest male longspurs compromise and maintain vigilance at their nests in lieu of sleeping because of the increased predation risk that is characteristic of their tundra nesting habitat. Snow buntings are cavity nesters, and their nests do not require the same vigilance, allowing males to presumably rest following provisioning. These life-history differences between species highlight the role of predation risk in mediating behavioral modifications to prolonged wakefulness in arctic-breeding songbirds.

KEY WORDS: Arctic, Modafinil, Parental care, Sleep, Songbird, Vigilance

## INTRODUCTION

The purpose of sleep is not fully understood but the benefits of a good night's rest are well established (Rolls et al., 2011). Sleep is typically associated with an overall reduction in responsiveness, motor activity and metabolism (Siegel, 2009; Lesku and Rattenborg, 2014), and a lack of sleep can have serious cognitive and physiological consequences in humans (Goel et al., 2009). In contrast, the amount of sleep per night required by many birds and mammals is quite variable (Campbell and Tobler, 1984; Lima et al., 2005; Siegel,

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2009). While most animals studied to date require sleep or sleep-like behavior, certain mammals may sleep for only a few hours per night (e.g. giraffes, elephants), whereas some bat species sleep for more than 20 h (Siegel, 2009). Many species of birds also differ in their sleep requirements, which may change during breeding and migration (Rattenborg et al., 2004; Lima et al., 2005; Fuchs et al., 2006; Jones et al., 2010).

Trade-offs may occur as individuals modulate wakefulness while on alert to the presence of predators or conspecifics (Lima et al., 2005). Reproductive effort can challenge survivorship (Montgomerie and Weatherhead, 1988; Stearns, 1989; Santos and Nakagawa, 2012) and maintaining vigilance behavior, adequate feeding rates and brooding time may compromise sleep. In pink-footed geese (*Anser brachyrhynchus*), vigilance in parents is maintained through a head-up posture, proximity to other geese, and active defense of the brood at the expense of sleeping and reduced self-feeding time (John and Inglis, 1978). These behaviors are also apparent in tundra swans (*Cygnus columbianus*) (Earnst, 2002). Additionally, brooding female great tits (*Parus major*) infected with an ectoparasite allocate less time to sleeping than uninfected parents but maintain provisioning rates despite setting aside time for anti-parasite behavior (Christe et al., 1996). Clearly, sleep can be compromised while maintaining nestling health. However, in species that regularly undergo seasonal sleep loss, the amount of sleep required to preserve nestling care remains unknown.

At higher latitudes, extreme photoperiodicity surrounding the solstices creates seasons of constant day or constant night (i.e. the sun remains above and below the horizon, respectively). For birds that breed north of the Arctic Circle in summer, continuous illumination can allow for adaptive sleep patterns that many species use to their advantage. For example, pectoral sandpipers (*Calidris melanotos*) contrast long periods of activity with short periods of sleep (Lesku et al., 2012), a behavior that seems to offer a competitive reproductive advantage, with the most active males tending to sire the most offspring (Lesku et al., 2012). In addition, semipalmated sandpipers (*Calidris pusilla*) utilize several diel rhythms during the breeding season, which may also function to increase mating opportunities (Steiger et al., 2013). Furthermore, Lapland longspurs (*Calcarius lapponicus*) are typically quiescent between midnight and 04:00 h Alaska Daylight Time (AKDT) during the breeding season but will maintain territorial aggression throughout the 24 h period if challenged by another male (Ashley et al., 2013, 2014; Steiger et al., 2013). While it is clear that some species in arctic regions experience seasonal changes in sleep patterns, little work has investigated how parental care and reproductive success may be influenced by manipulation of these patterns.

The extent to which sleep requirements in arctic-breeding songbirds affect their ability to provide adequate parental care is poorly understood. Here, we investigated the effects of an anti-narcolepsy drug, modafinil (Sheng et al., 2013), on nesting success of two free-living songbird species: Lapland longspurs and snow

buntings (*Plectrophenax nivalis*). Modafinil has been shown to increase wakefulness in laboratory mammals while minimizing undesirable behaviors sometimes associated with other stimulants (Edgar and Seidel, 1997). Both species of interest breed in the circumpolar region and experience 24 h of continuous light during the summer. In addition, these two species are closely related, although not sister species (Klicka et al., 2003). Snow buntings nest in tussock cracks, rocky hillsides and man-made structures such as nest boxes, eaves or any cavity that offers protection (Montgomerie and Lyon, 2020). Lapland longspurs nest almost exclusively in open tundra and are naturally more prone to nest predation from jaegers (*Stercorarius* spp.), glaucous gulls (*Larus hyperboreus*), arctic foxes (*Vulpes lagopus*) and ermine (*Mustela erminea*) (Hussell and Montgomerie, 2020). As snow buntings nests are better protected, their nest defense strategies differ from those of Lapland longspurs, despite their sympatry (Montgomerie and Lyon, 2020). Males of both species provide parental care by feeding nestlings, although there are some key differences. Male snow buntings actively feed nestlings at rates similar to females (Montgomerie and Lyon, 2020) but provide little to no defense of the nest when nestlings require provisioning, whereas male Lapland longspurs provide nest defense and surveillance but feeding rates vary considerably and are rarely commensurate with those of females (Hussell and Montgomerie, 2020). Differences in life-history traits between the two species may lead to a difference in the amount of sleep required for reproductive success and whether sleep loss will negatively affect reproductive behavior.

The major aim of this study was to pharmacologically extend the active period of free-living male Lapland longspurs and snow buntings using short-term (72 h) modafinil exposure through osmotic pump infusion. We hypothesized that males receiving modafinil would extend activity cycles and putatively nestling provisioning, as measured indirectly by higher nestling growth rates compared with controls during this period. Alternatively, extended wakefulness from modafinil treatment could exact life-history costs upon behavior and nestling success, and instead reduce nesting success if males are prone to the detrimental effects of sleep loss.

## MATERIALS AND METHODS
### Captive pilot experiments
All experiments described below were approved by the Institutional Animal Care and Use Committee (IACUC) at Western Kentucky University and appropriate state, federal and tribal entities for field studies in northern Alaska (see below).

We performed captive experiments with zebra finches, *Taeniopygia guttata* (Vieillot 1817) (Fig. S1), and Lapland longspurs, *Calcarius lapponicus* (Linnaeus 1758) (Figs S2 and S3), as proof-of-concept studies to assess the validity and dose of modafinil as a stimulant before administering this drug in free-living birds.

To assess the stimulatory effect of modafinil, we measured each subject's distance traveled (in cm) over time using video tracking software (Limelight, Actimetrics). Birds were held in individual cages modified with a Plexiglas top to allow a clear camera view from above. Birds were acclimated to 12 h light:12 h dark (with lights off at 20:00 h, Central Standard Time, CST) before monitoring, and then two birds were given 75 mg kg⁻¹ body mass modafinil in 50% water/50% dimethyl sulfoxide (DMSO) solution via subcutaneous injection, and two were given vehicle (50% water/50% DMSO). Injections occurred at 20:00 h CST, and birds were then immediately placed in cages. Video tracking started 10 min later.

We also performed similar studies in captive Lapland longspurs captured in Utqiaġvik (formerly known as Barrow), Alaska (71°N, 156°W) in the summer of 2018, except that continual infusion of modafinil was tested instead of a single injection. Lapland longspurs were brought to Kentucky for behavioral studies and housed on a 12 h light:12 h dark photoperiod. To allow for infusion, we used osmotic pumps (Alzet 1003D, 100 µl) to administer the drug over a 3 day period. One bird received 75 mg kg⁻¹ body mass modafinil dissolved in 50% DMSO and 50% polyethylene glycol, and one received vehicle. The pumps were implanted at 17:30 h CST according to the procedure in 'Osmotic pump implantation', below, and birds were placed in video tracking cages thereafter on a 24 h light:0 h dark photoperiod and allowed to recover for 6.5 h before video tracking recordings began (00:00 h). Osmotic pumps remained in birds for 72 h, after which they were removed. As above, we used Limelight tracking software.

### Field site and study species
Free-living Lapland longspurs and snow buntings, *Plectrophenax nivalis* (Linnaeus 1758), were studied in the general vicinity of Utqiaġvik, Alaska, during the summer of 2019 (2 June to 14 July). As Lapland longspurs nest in tundra, most of our field sites for this species were located in coastal tundra within walkable distance of the Barrow Arctic Research Station or Gaswell (Cakeeater) Road.

The majority of snow buntings were studied at the Naval Arctic Research Laboratory and Ilisaġvik campus, where artificial nest boxes have been erected to monitor this particular population for several decades. In addition, these birds also nest in other man-made structures on the campus, such as vehicles, machinery and other abandoned structures. However, in 2019, there were several snow buntings that nested in the coastal tundra either in polygon tundra frost heaves or underneath artificial debris (e.g. discarded building supplies such as plywood).

### Nest monitoring
During the month of June, we actively searched for nests of both species and monitored them until hatching. Thereafter, we monitored these nests daily to ascertain hatching date (defined as one or more chicks hatched from a clutch). Over the course of the field season, we found and monitored 59 Lapland longspur nests and 46 snow bunting nests. At 2 days post-hatching (D2), nestlings were weighed, and their toenails were marked with colored nail polish for individual identification. Handling time was limited to keep the chicks as warm as possible and nestlings were kept inside a heated cooler to protect them from environmental exposure. On day 4 post-hatching (D4) we identified, captured and implanted the resident male with an osmotic pump filled with modafinil (*n*=13 for each species) or vehicle (*n*=14 for each species; see 'Osmotic pump implantation', below). Sample sizes were determined based upon previous behavioral studies that have utilized osmotic pumps in free-living birds (e.g. Soma et al., 2000). Resident males were identified by passively observing their territorial behavior as well as mate-guarding behavior of females and assessing their territorial response to a live decoy with song playback. If a male was aggressive towards the decoy, then we attempted to capture and implant this particular male (see 'Osmotic pump implantation', below). On day 6 post-hatching (D6), we assessed parental behavior of focal male and female birds (see 'Parental behavior observations', below). On day 7 post-hatching (D7), we weighed, banded and obtained blood samples from each nestling (used for paternity analysis in another study). Afterwards, the nest was monitored for fledging or signs of depredation on a daily basis.

## Osmotic pump implantation

On D4, we attempted to capture resident adult males at each nest and implant them with an Alzet 1003D osmotic pump. The pump released 100 μl h$^{-1}$ of modafinil, at a dose of 75 mg kg$^{-1}$ body mass day$^{-1}$ for 3 days or vehicle (1:1 ratio of polyethylene glycol and DMSO). The dose was based upon captive study results (see Results, 'Captive studies'). Treatments were coded A versus B for Lapland longspurs and C versus D for snow buntings and assigned using a balanced design. Decoding of treatments occurred after the field season when data were analyzed. Males were captured using Potter traps or clap traps baited with seed and were accompanied by a live decoy and acoustic playback. Upon capture, males were measured for tarsus length, wing length and body mass (to the nearest 0.25 g). Fat score was ascertained by estimating the extent of furcular and abdominal fat deposition using a semiquantitative scale (0–5 with 5 having the highest fat stores) as developed by Wingfield and Farner (1978). These two scores were then averaged for each bird. Osmotic pumps were implanted underneath the skin between the shoulder blades of birds. Pumps contained 100 μl of solution with a release rate of 1 μl h$^{-1}$. For implantation, the surgical site was first prepped using a cotton ball soaked with 70% ethanol to expose the skin directly underneath the neck and the scapular feathers. Then, a small incision (approximately 1 cm) in the skin between the bird's shoulder blades was made using fine-scale micro-dissecting scissors that was just wide enough to slide the osmotic pump underneath the skin. The incision was sealed with veterinary surgical glue. Subjects were also banded with a unique combination of plastic colored leg bands for individual identification, as well as an aluminium leg band (US Geological Survey). Birds were then released on an adjacent territory after being warmed in a bird bag for 5–10 min, in an attempt to prevent birds from associating the stress of capture with their territory. Following fledging, we attempted to recapture males, explant osmotic pumps, and assess body condition. Because of the number of days between implant and explant, we were unable to assess the immediate effects of modafinil. Instead, we used fat score and mass to assess body condition after the effects of the drug had worn off.

## Parental behavior observations

Initially, we aimed to assess behavior of males during their inactive period (00:00 h to 04:00 h AKDT on day 5 post-hatching, D5) to examine whether modafinil treatment was effective at promoting wakefulness during this period. However, we quickly realized that it was difficult to evaluate activity when our presence tended to awaken birds. Thus, it was impossible to ascertain whether activity during this time was due to the stimulant or human disturbance (or both). In addition, parental feeding of nestlings typically wanes during this quiescent period (Hussell and Montgomerie, 2020). Instead, we decided to examine parental behavior ca. 2 days after drug treatment during the middle of their active period, which would provide a measure of how parental care is affected by pharmacologically induced sleep loss from modafinil treatment. On D6, at each nest where a male was implanted, a 60 min behavioral observation was conducted by a single observer. Behavior of the resident male and female was assessed between 09:00 h and 15:00 h AKDT. This time period was chosen to occur when pairs were actively feeding nestlings (Hussell and Montgomerie, 2020). For both species, time-of-day of observations did not differ between treatment groups (mean±s.e.m. time-of-day; Lapland longspurs: control, 11:34 h ± 52 min; modafinil, 10:48 h ±44 min; snow buntings: control, 11:29 h ± 35 min; modafinil, 11:48 h ±43 min; unpaired two-tailed t-test, all P>0.65). The observer remained >20 m away from the nest and was

naive to the treatment of the focal male. The following behavioral parameters were measured: (1) duration spent within 5 m of the nest by the focal male, (2) number of nest visits by the resident male and female, and (3) time spent directly next to or on the nest by the male and female. Females of both species (but not males) will brood younger nestlings (Hussell and Montgomerie, 2020; Montgomerie and Lyon, 2020), but we were not able to differentiate between feeding and brooding in regards to time spent on the nest for females. In some cases, males left the nest area and were considered 'out of view' and thus by default >5 m from the nest. Behavioral observations were recorded on a digital voice recorder.

## Nestling growth

We measured nestling growth rates by examining several measures: (1) percentage change in total nestling mass (calculated from D2 to D7), (2) average growth rate by nest in grams per day (calculated from mass at D2 and D7), (3) largest and smallest nestling mass at D7 for each nest, and (4) highest growth rate (calculated from chicks across nests with the highest value in g day$^{-1}$ for each nest). These comparisons included nestling number as a covariate in all analyses to control for the effects of the number of nestlings on growth rate.

## Statistical analyses

Statistical analyses were performed in RStudio v.1.2.1335 (RStudio running R 3.6.0, Boston, MA, USA) using the function wilcox.test to conduct non-parametric Mann–Whitney U-tests on groups of data, as most of the data were non-normal even after transformation. Unpaired, two-tailed t-tests (t.test function) were conducted for parametric data, which meet the assumptions of normality and homogeneity of variance. ANCOVA were conducted to evaluate the effects of nestling number on D2 (covariable) on estimates of growth rates in Lapland longspur and snow bunting nestlings. As there was no significant effect of the covariate (all P>0.17), except for one measure in snow buntings (see Results, 'Parental behavior'), unpaired, two-tailed t-tests were used. A Fischer's exact test was used to compare the effect of modafinil treatment on captive Lapland longspur activity and the proportion of nests that failed in the field study. Significance was designated at P<0.05.

## RESULTS
### Captive studies

Captive zebra finches receiving modafinil were observed to be more active (Fig. S1) throughout the majority of subjective night compared with controls. More specifically, if cumulative distance traveled is summed into 4 h bins, activity was greater in modafinil birds from 20:00 h to 00:00 h CST (N=2; distance traveled: 4286 and 1893 cm) than in controls (vehicle only, N=2; distance traveled: 450 and 648 cm). During the middle of subjective night (from 00:00 h to 04:00 h CST), modafinil birds were more active (distance traveled: 4025 and 10,121 cm) than controls (distance traveled: 275 and 1008 cm). In the later part of subjective night (04:00–08:00 h CST), activity was greater in control birds (distance traveled: 5714 and 11,348 cm) than in modafinil birds (distance traveled: 3837 and 2692 cm). This reversal in activity patterns is likely because modafinil birds were either exhibiting sleep rebound after the stimulant had been metabolized or the activity rhythm of modafinil birds was phase advanced relative to that of controls (Fig. S1).

For the osmotic pump captive study, the Lapland longspur receiving modafinil appeared to show an extended active period during the second subjective night when compared with the control (Fig. S2). To further examine this specific period (20:00–08:00 h, but lights on), we compared periods of activity between the modafinil- and

control-implanted birds (Fig. S3). To assess activity during this time, we designated bins (10 min) that registered no movement (reading of zero) as 'inactive'. The modafinil bird was 94.5% active, whereas the control bird was 69.5% active. If we assume that the absence of movement represents sleep, then these data suggest that modafinil is effective in maintaining wakefulness during subjective night of longspurs.

## Nest monitoring

By D6, 38 of the 59 (64%) identified Lapland longspur nests had failed, whereas 7 of the 46 (15%) snow bunting nests had failed. Modafinil treatment did not affect Lapland longspur or snow bunting nest failure rate (Fischer's exact test, all $P>0.69$). On D4, 13 and 14 males of each species were implanted with modafinil or vehicle pumps, respectively. Two male Lapland longspurs that were implanted with modafinil were not from the correct territory and were thus removed from the analysis. Following implantation, an additional 6 nests failed before D6 (3 modafinil, 3 vehicle). Nineteen ($n=8$ modafinil, $n=11$ vehicle) Lapland longspur nests survived until D6 and could be used for behavioral observations, while 16 of those 19 ($n=8$ modafinil, $n=8$ vehicle) survived until D7 and were used to measure nestling growth rates. Two snow buntings that were implanted with modafinil had failed nests before D6. One snow bunting implanted with modafinil was not observed again, and was no longer included in the analysis. Twenty-four snow bunting ($n=10$ modafinil, $n=14$ vehicle) nests were monitored for parental behavior and nestling growth rate. Clutch size averaged 5.2±0.36 and 5.8±0.25 nestlings for Lapland longspurs and snow buntings, respectively. Mean time to fledging was 11±0.32 and 15±0.33 days for Lapland longspurs and snow buntings, respectively.

## Parental behavior

Lapland longspur males receiving modafinil made significantly fewer visits to the nest compared with controls [Fig. 1A; $U=19$, (tied) $P=0.03$]. Males receiving modafinil spent significantly less time on the nest than controls [Fig. 1C; $U=21$, (tied) $P=0.049$] but tended to spend more time within 5 m of the nest than controls [Fig. 1E; $U=22$, (tied) $P=0.07$]. However, modafinil treatment in Lapland longspur males did not modify female behavior. We found no difference between groups for the number of female visits to the nest [Fig. 1B; $U=38$, (tied) $P=0.62$] or time spent by females on or near the nest [Fig. 1D; $U=4$, (tied) $P=0.87$]. We recaptured and explanted three males following implantation but were unable to compare means between groups because of the low sample size.

Male snow buntings receiving modafinil did not exhibit any significant differences in the number of nest visits (Fig. 2A; d.f.=21, $t=-0.25$, $P=0.81$) or time spent at or directly next to the nest (Fig. 2C; d.f.=22, $t=-0.24$, $P=0.81$) compared with males receiving vehicle. Additionally, modafinil treatment in males did not significantly influence parental behavior in females, as measured by the number of nest visits (Fig. 2B; d.f.=8.9, $t=0.75$, $P=0.47$) or time spent at or directly next to the nest (Fig. 2D; d.f.=22, $t=-1.5$, $P=0.14$). We recaptured and explanted nine males following fledging (control $n=5$, modafinil $n=4$). One male had no implant and we assumed it had explanted itself in the 13 days since implantation. The removal of this individual in all analyses did not alter statistical significance of the results. There were no differences in cumulative fat score (d.f.=7, $t=1.38$, $P=0.47$) or body mass (d.f.=7, $t=0.55$, $P=0.60$) between groups following explanting.

## Nestling growth and fledging

The average growth rate for snow buntings and Lapland longspurs was 3.65 and 2.93 g day$^{-1}$, respectively. There were no significant differences in any measure of growth rate for either species (Table 1, Fig. 3A,B). Using ANCOVA, there was a significant effect of nestling number at D2 (covariate) on the mass of the smallest nestling at D7 ($F_{1,20}=6.15$, $P=0.02$) in snow buntings: the greater the nestling number, the lower the body mass of the smallest nestling at D7. There was a significant effect of modafinil treatment on time to fledging in Lapland longspurs (Fig. 4A; d.f.=12, $t=2.35$, $P=0.04$) but not snow buntings (Fig. 4B; d.f.=20, $t=0.38$, $P=0.71$); nests of Lapland longspurs where males received modafinil fledged about 1 day later than controls (control 10.6±1.1 days, modafinil 11.9±0.9 days).

## DISCUSSION

The captive pilot data provide proof-of-concept validation that modafinil treatment increased activity via either single injection or continual infusion in zebra finch and Lapland longspurs, respectively, although the duration and timing of effects were different depending upon the mode of delivery. Osmotic pump infusion of modafinil led to increased activity starting during the second subjective night of exposure, whereas single injection produced more immediate effects. It should be noted that given the small sample sizes of these trials and the fact that sleep was not directly measured, caution is warranted in attributing the effects of modafinil to direct inhibition of sleep. A future study that measures electroencephalographic and electromyographic biopotentials is necessary to identify the specific effects that this anti-narcolepsy drug has upon sleep architecture in birds. Lastly, further investigation is required to understand specific effects of this drug on avian physiology and behavior.

In the field, behavioral observations suggest that modafinil treatment for 2 days modified the parental behavior of male Lapland longspurs, but not snow buntings. Lapland longspur males receiving modafinil made fewer visits to the nest, spent less time on or directly next to the nest, and tended to spend more time within 5 m of the nest compared with controls. We saw no evidence that snow bunting males receiving modafinil modified their behavior when compared with those receiving vehicle. Additionally, female behavior did not change as a result of male implantation in either species. Finally, no change in growth rate was observed between groups in either species despite an obvious lack of parental care in male Lapland longspurs. However, significantly, nestlings of Lapland longspurs receiving modafinil fledged a day later than controls, which implies a potential fitness cost.

In Lapland longspurs, modification of behavior was apparent, suggesting that putative sleep loss from modafinil treatment affects parental care in this species. We speculate that modafinil extended the activity profile of free-living male Lapland longspurs on D4 and D5 to the extent that parental care was compromised on D6 because of sleep loss. In a previous study at Sarcpa Lake (Northwest Territories, 68°N), Lapland longspur nestlings were fed throughout the polar day by parents, but not often between 22:30 h and 02:30 h Mountain Standard Time (MST; Hussell and Montgomerie, 2020). Because we were not able to observe males during their inactive period (ca. 00:00–04:00 h AKDT; Ashley et al., 2013) on D4 or D5 because our presence artificially awakened birds, it is unknown whether males remained active during this time and, if so, whether feeding of nestlings occurred. In the future, this shortcoming should be rectified through use of accelerometers or spatial tracking devices (e.g. GPS) to examine whether males receiving the stimulant were more active and subsequently fed nestlings during this time.

On D6 (ca. 48 h after implantation), male Lapland longspurs receiving modafinil tended to spend more time around the nest, but less time visiting the nest, apparently displaying vigilance behavior
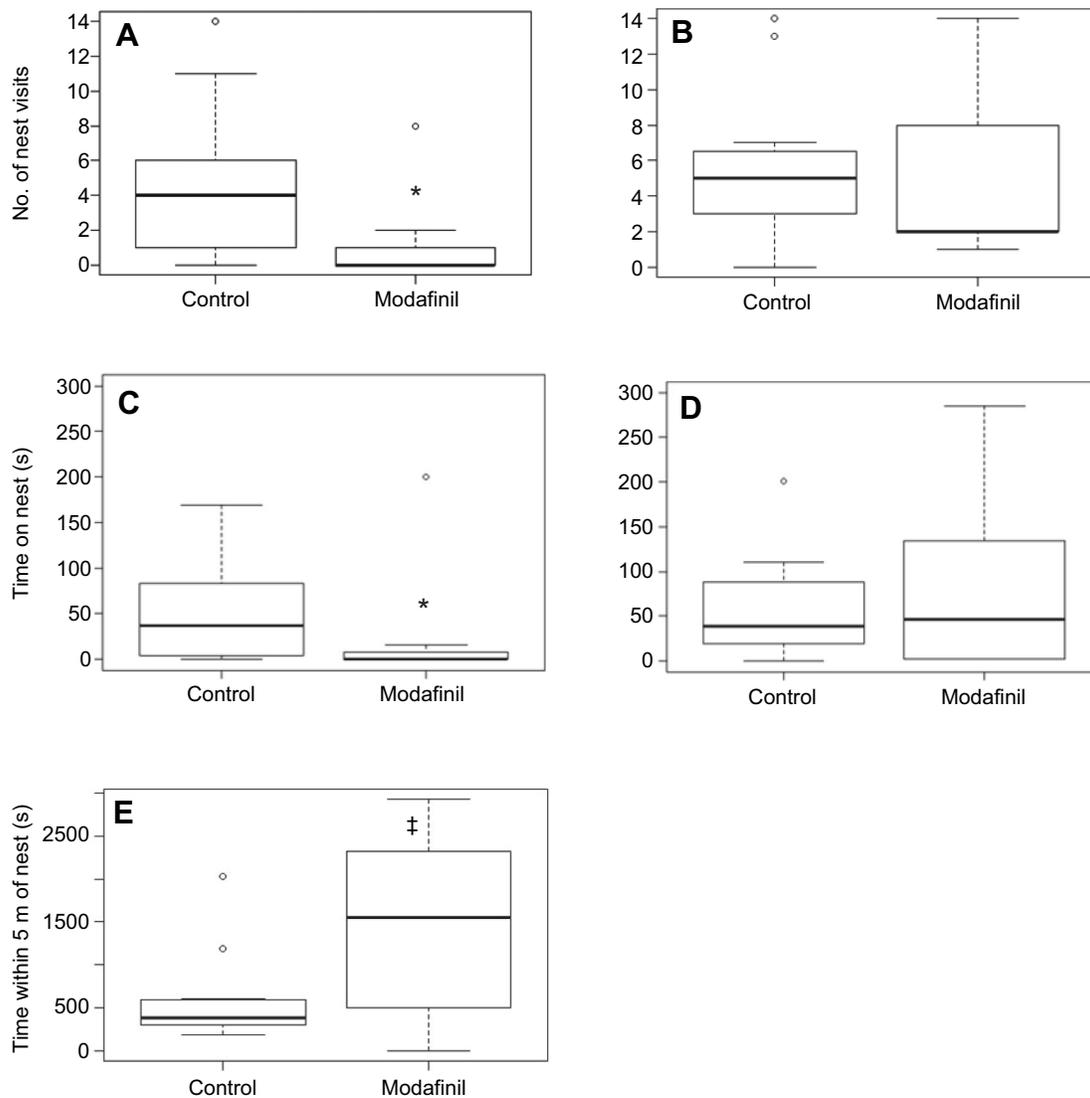
**Fig. 1. Effect of modafinil treatment of male Lapland longspurs on parental behavior at 6 days post-hatching (D6, ca. 48 h after treatment).** Observations (60 min) were conducted to assess the number of feeding visits to the nest for males (A) and females (B), the time spent on the nest for males (C) and females (D), and the time spent within 5 m of the nest (E, males only). Control $n=11$, modafinil $n=8$. Box plots show the 5th, 25th, 50th, 75th and 95th percentiles and outliers (open circles). *$P<0.05$, ‡$P=0.069$; Mann–Whitney $U$-tests.

rather than foraging. Vigilance may be less costly than feeding, during which males typically stay within a few meters of the nest. Feeding typically consists of longer flights away from the nest, foraging and a return to feed the nestlings. High provisioning rates may be too physically demanding for sleep-deprived individuals to accomplish or, alternatively, reduced sleep may decrease motivational behaviors associated with parental care, although more research is warranted.

While clear behavioral changes were observed in Lapland longspurs, snow buntings did not exhibit the same effect. We suggest that ecological differences in nesting site preferences may be partially responsible as these two species are closely related (Klicka et al., 2003). Lapland longspurs construct cryptic nests on exposed tundra, whereas snow buntings lay in nest boxes, holes and man-made objects. Regardless of the difficulty a predator may have finding nests of either species, predation of a Lapland longspur nest is more likely. This is supported by a 64% nest failure rate in Lapland longspurs compared with only 15% in snow buntings during our study season in 2019. A similar disparity between

species in nest failure rate had been documented previously at our study site: for Lapland longspurs, 92% and 88% in 2017 and 2018, respectively; for snow buntings, 5% and 23% in 2017 and 2018, respectively (note that these 2 years experienced abnormally late snow melt, reducing the available breeding habitat for Lapland longspurs, resulting in abnormally high levels of nest failure; N.T.A., unpublished data). Snow buntings therefore do not require the same level of vigilance when protecting their nests and may have more time for resting in between feeding bouts, although the latter supposition requires further evidence. Part of the reason we did not include a metric for snow bunting time within 5 m of the nest is because snow bunting males do not maintain active, consistent vigilance around their nest during this period of the parental phase (W.I.P., personal observation), and our behavioral observations indicate that they spent time near the nest only to provision nestlings. In Lapland longspurs, we frequently observed the male spending time around the nest before or after feeding and exhibiting vigilance behavior (W.I.P., personal observation). The alternative hypothesis is that snow buntings are completely insensitive to
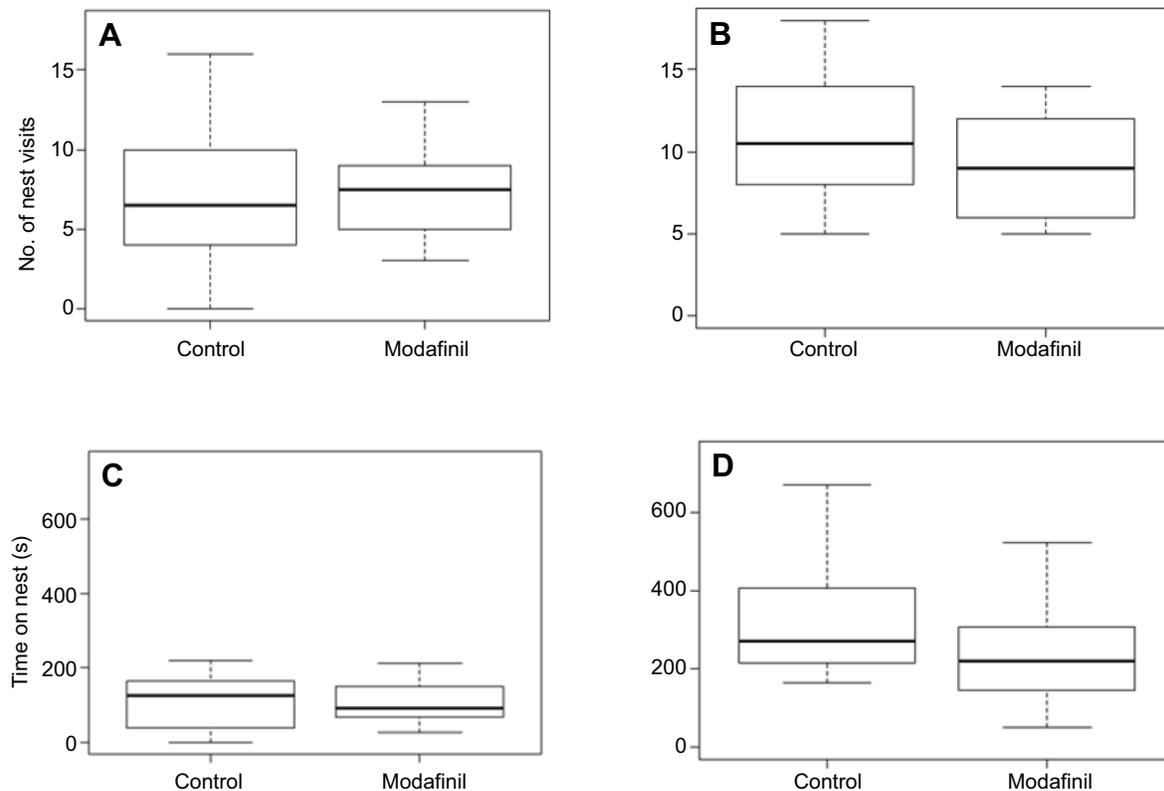
**Fig. 2. Effect of modafinil treatment of male snow buntings on parental behavior at D6 (ca. 48 h after treatment).** Observations (60 min) were conducted to measure the number of feeding visits to the nest for males (A) and females (B) and the time spent on the nest for males (C) and females (D). Control $n$=14; modafinil $n$=10. Box plots show the 5th, 25th, 50th, 75th and 95th percentiles. Unpaired, two-tailed $t$-tests were used.

modafinil, which we find unlikely given that both zebra finches and Lapland longspurs, a close congener, are sensitive to this drug in captive studies.

As seen in other studies (John and Inglis, 1978; Christe et al., 1996; Earnst, 2002), increased parental care at the expense of sleep may be an overall fitness advantage, despite putative detrimental effects on parents. However, it is unclear why Lapland longspur nestling growth rate was maintained through D7 despite an obvious change in male parental care on D6. We initially hypothesized that males receiving modafinil would extend activity cycles (and putatively increase feeding rate), which would lead to increased growth of nestlings. If this had been the case on D4 or D5, then we would expect an increase

in growth rate by D7 compared with controls. However, if males were unable to provide adequate care because of putative extended wakefulness from modafinil, then we would expect females to possibly compensate by increased provisioning relative to controls. Neither was the case. Furthermore, it is unclear why parental care in males decreased but nestling growth and female parental care remained unchanged through D6, as nestling growth rates in some species would be expected to fall following a lack of male support (Tinne et al., 2005) or reduction in food intake (although structural growth rates were maintained) (Moe et al., 2004). It may also be important to note that Sasvari (1986) reported increased nestling mortality in blue tits (*Parus caeruleus*) and in great tits (*Parus major*)

**Table 1. Growth rate calculations for Lapland longspur ($n$=16) and snow bunting ($n$=24) nests where resident males were treated with control or modafinil osmotic pumps**

| Parameter | Treatment | Lapland longspurs | | | | Snow buntings | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean±s.d. | d.f. | $t$ | $P$ | Mean±s.d. | d.f. | $t$ | $P$ |
| Percentage change in total nestling mass | Control | 28.5±6.9 | | | | 28.6±5.5 | | | |
| | Modafinil | 25.2±5.3 | 13.1 | 1.07 | 0.30 | 24.7±4.7 | 21.3 | 1.86 | 0.08 |
| Average growth rate by nest (g day$^{-1}$) | Control | 2.9±0.3 | | | | 3.7±0.3 | | | |
| | Modafinil | 3.0±0.1 | 9.1 | −0.61 | 0.56 | 3.6±0.2 | 21.2 | 1.24 | 0.23 |
| Largest nestling mass (g) | Control | 22.0±0.8 | | | | 27.9±1.6 | | | |
| | Modafinil | 22.1±1.0 | 13.5 | −0.21 | 0.84 | 26.6±1.9 | 17.5 | 1.71 | 0.10 |
| Smallest nestling mass (g) | Control | 17.2±2.4 | | | | 21.5±3.9 | | | |
| | Modafinil | 16.8±2.1 | 13.8 | 0.30 | 0.77 | 19.5±3.7 | 20.1 | 1.24 | 0.23 |
| Highest growth rate by nest (g day$^{-1}$) | Control | 3.2±0.3 | | | | 4.1±0.4 | | | |
| | Modafinil | 3.3±0.1 | 9.6 | −0.92 | 0.38 | 3.9±0.2 | 21.5 | 1.42 | 0.17 |

Percentage change in total nestling mass was calculated from day 2 to day 7 post-hatching (D2 to D7). Average growth rate by nest was calculated by averaging each nestling's growth rate to find the average value per nest. Largest and smallest nestling mass were calculated by assessing the chick with the largest and smallest mass on D7 for each nest. Highest growth rate by nest was calculated by comparing chicks across nests with the highest rate (g day$^{-1}$ value) for each nest. Results of unpaired, two-tailed $t$-tests are shown.
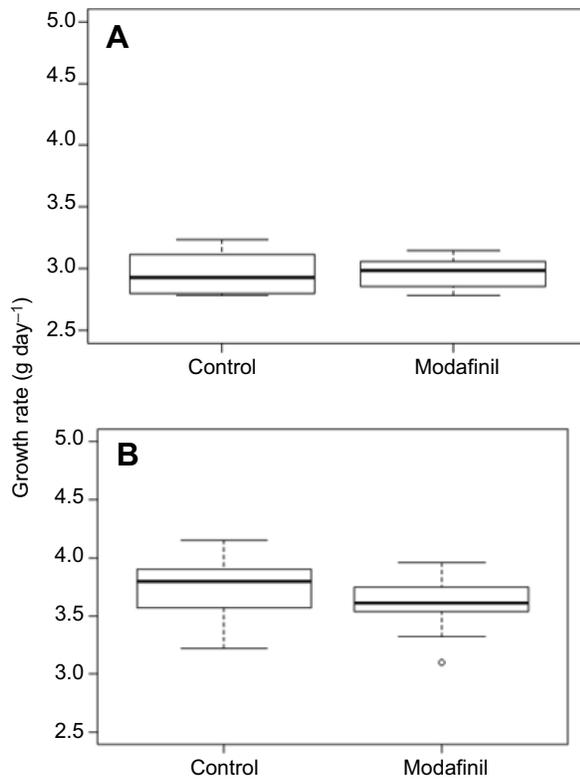
**Fig. 3. Effect of modafinil treatment of resident males on nestling growth rate from D2 to D7.** (A) Lapland longspur and (B) snow bunting nestlings. Growth rate was calculated by averaging the growth rate (in g day$^{-1}$) of each nestling to find the average value for each nest. Lapland longspurs: control $n$=8, modafinil $n$=8; snow buntings: control $n$=14, modafinil $n$=10. Box plots show the 5th, 25th, 50th, 75th and 95th percentiles and outliers (open circles). Unpaired, two-tailed *t*-tests were used.



**Fig. 4. Effect of modafinil treatment of resident males on time to fledging.** (A) Lapland longspur and (B) snow bunting nestlings. Lapland longspurs: control $n$=7, modafinil $n$=7; snow buntings: control $n$=13, modafinil $n$=9. Box plots show the 5th, 25th, 50th, 75th and 95th percentiles and outliers (open circles). *$P$<0.05; unpaired, two-tailed *t*-tests.

following removal of males or females, but not after D6 or D8, respectively, the time when nestlings reach about half their adult body mass. In another study that examined the effect of testosterone implantation upon male parental care in breeding Lapland longspurs, males given testosterone when nestlings were 2–3 days old visited nests and fed nestlings fewer times than control males, and mates of the testosterone-treated males compensated through elevation of feeding rates (Hunt et al., 1999). However, when nestlings were 4–5 days old, testosterone-treated males still fed at lower rates, but females no longer compensated for this (Hunt et al., 1999), suggesting a limit to the ability of females to adjust feeding rates to increased provisioning demands of older chicks. Alternatively, it is possible that males and/or females compensated by increasing the amount of food per nest visit. Time spent on the nest during the nestling phase can be considered a reliable estimate of the amount of food that is delivered in male Lapland longspurs, but less so in females because a portion of the visits to the nest by the female are associated with brooding of chicks rather than feeding (Hunt and Wingfield, 2004). Male Lapland longspurs provided with modafinil exhibited a significant reduction in time spent on or directly next to the nest compared with control males, so it is unlikely that more food was delivered per visit to compensate for lower visitation rates. In addition, female Lapland longspur that were paired with males given modafinil did not spend more time on the nest than controls.

While no change in nestling growth rate was observed between groups, significantly, Lapland longspur nests of males receiving modafinil fledged 1 day later than controls. We speculate that a
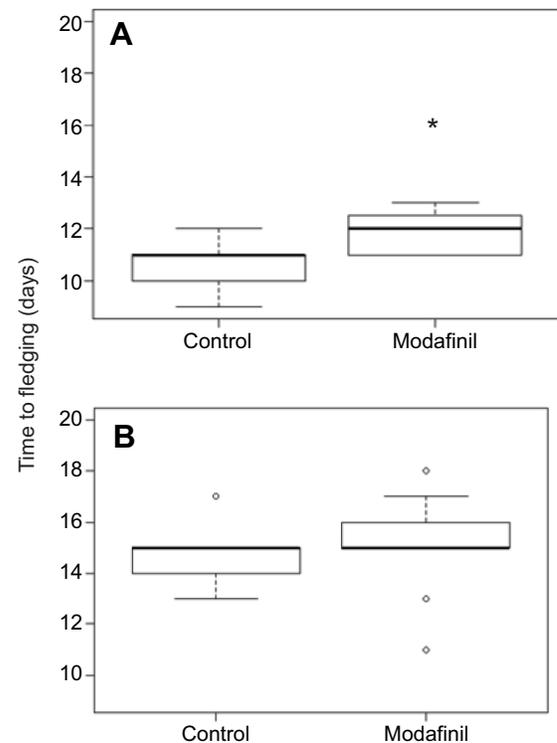
reduction in nestling growth occurred after D7, which we were unable to measure because of an increased risk of premature force-fledging that can occur from human disturbance. Future work should conduct behavioral observations during this post-drug delivery period to ascertain whether modafinil elicited sleep rebound in treated birds. Even though feeding rates in males receiving modafinil were lower than for controls on D6, the effects of this behavior may not have been apparent until after D7, and were manifested in delayed fledging dates. As nest predation rates can be high, fast growth rates and earlier fledging may increase recruitment (Verboven and Visser, 1998; Maness and Anderson, 2013), especially in arctic-breeding songbirds where the window to breed is extremely truncated. The later dates to fledge may have been indicative of lower growth rates, which were probably a result of decreased male feeding rates (likely during a later period we did not measure). This may be considered a slight but important fitness cost in nests of males receiving modafinil. If the female is unable to compensate for the sleep-deprived male, then nestlings may not reach proper fledging mass as early and may be more vulnerable to predation the longer they remain in the nest.

Furthermore, these findings suggest that Lapland longspurs, a ground-nesting species, may be more susceptible, from a fitness perspective, to modafinil than snow buntings, a cavity nester. In captive studies, male Lapland longspurs were physiologically sensitive to 12 h of experimental sleep fragmentation as measured by an elevation in plasma corticosterone; however, birds experienced no decrement in executive function from sleep loss, suggesting behavioral resilience (Hodinka and Ashley, 2020). Previous data from our laboratory (Ashley et al., 2013) and others (Steiger et al., 2013) have shown that male Lapland longspurs have a consistent diel rhythm in activity profiles with a quiescence period of ca. 00:00–

04:00 h AKDT (Ashley et al., 2013). In addition, preliminary data from our lab suggest that the majority of sleep bouts occur during this inactive period (N.T.A., unpublished data). Although a similar activity profile has been observed in snow buntings (N.T.A., personal observation), we propose that there is greater flexibility of male snow buntings in their sleep requirements partially as a result of their protected nest sites that require little to no vigilance.

In summary, we initially hypothesized that males receiving modafinil would temporarily increase parental care and thus induce an increase in nestling growth rates. We observed partially the opposite, although part of this hypothesis could not be tested directly because we were not able to measure parental behavior during their inactive period as a result of logistical constraints (described above). Nonetheless, by measuring parental effort 2 days after treatment to assess responses to pharmacologically induced sleep loss, we report that male Lapland longspurs receiving modafinil altered behavior from a time-consuming, putatively expensive feeding regime to a less active but still potentially beneficial vigilance regime. Additionally, nests where males received modafinil fledged 1 day later. Interestingly, snow buntings did not exhibit any changes from modafinil treatment, which may be in part related to their well-protected nests. As we conducted only pilot studies on effects of modafinil in captive birds, additional research needs to be carried out to pinpoint the specific effects that modafinil has on avian physiology and behavior. While this project sheds light on what is tolerable under conditions of modafinil exposure in arctic-breeding songbirds, more work should be done to examine the interspecific differences in sleep demand and behavioral responses to sleep loss in species living in extreme environments.

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