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Testosterone, Estrogen, and Breeding Behavior in an Arctic Bird, the Lapland Longspur

by

Kathleen Ellen Hunt

A dissertation submitted in partial fulfillment
of the requirements for the degree of

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(Chairperson of Supervisory Committee)

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Doctoral Dissertation

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University of Washington

Abstract

Testosterone, Estrogen, and Breeding Behavior in an Arctic Bird, the Lapland Longspur

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Chairperson of the Supervisory Committee
Professor John C. Wingfield
Department of Zoology

Successful breeding in the Arctic requires precisely timed changes in reproductive behavior and physiology, and usually only one reproductive attempt per year is possible. Hormone and behavior patterns of Arctic breeders may therefore be different from those seen in lower-latitude species with longer breeding seasons. During 1991-1996, I investigated relationships of hormones and breeding behavior in an Alaskan population of Lapland longspurs (*Calcarius lapponicus*), socially monogamous passerine birds that breed only in the Arctic. Male Lapland longspurs had a very high, brief peak of circulating testosterone (T) for the first few days on the breeding grounds, during which they performed aerial song displays over a "territory" with indistinct boundaries, and were not aggressive toward simulated territorial intrusions (STIs) . T then fell to intermediate levels as males guarded their mates for approximately one week, singing less and exhibiting high aggression to STIs. Finally, T, song, and aggression declined to low levels during incubation. I investigated these patterns further by implanting free-living birds with hormones (controls received empty implants). During mate-guarding, males with implants of testosterone blockers (ATD and flutamide) had decreased aggression and normal song. However, during incubation, T-implanted males had increased song and normal (low) aggression. Parental care of T-implanted males was initially low, but recovered to near-normal levels as nestlings grew. All experimental groups had normal nestling growth and normal nest success. Finally, estradiol-implanted females did not show changes in sexual behavior, nest-building or parental care, but did tend to beg more from their mates. Overall, Lapland longspurs have some hormone-behavior patterns that seem different from those seen in lower-latitude species, namely: 1) Males may have two functional levels of testosterone: at intermediate levels of T, aggression is T-dependent and song is T-independent, but very high levels of T stimulate increases in song. 2) Neither sex is very responsive to

reproductive hormones after the young hatch. These patterns may be related to the Laplands' very brief courtship, brief reproductive opportunities, and the need to concentrate on parental care of their single brood.

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DEDICATION

I dedicate this dissertation to my parents, Barbara and Shane Hunt, and to my friend and companion Jerry Muhasky. I could not have done this without their encouragement and their love.

CHAPTER 1: Temporal Patterns of Territorial Behavior and Circulating Testosterone in the Lapland Longspur and Other Arctic Passerines

INTRODUCTION

Male passerine birds commonly show certain breeding behaviors that vary in intensity and duration from species to species. For instance, early in the breeding season, males typically spend much time singing, courting females, and defending their territory from rival males. They reduce or cease these activities later in the season. Such behaviors are important for synchronizing the male's and female's physiology and behavior, from final maturation of the gonads to the shift from sexual to parental behavior. These complex behavioral interactions have been shown to have an intimate relationship with steroid hormones, such as testosterone (T), estradiol and their metabolites (e.g. Balthazart, 1983; Harding *et al.*, 1988; Wingfield & Ramenofsky, 1985; Wingfield & Moore, 1987). Furthermore, the temporal patterns of circulating hormone levels (e.g. testosterone) in different species appear to be linked to breeding strategies and mating systems, suggesting a broad interrelationship of behavioral ecology and endocrine mechanisms (Wingfield *et al.*, 1990). However, most investigations on the interrelationships of testosterone and aggression have focused on temperate-zone species in which the breeding season may be protracted (i.e. 2-5 months). None have addressed the temporal progression and integration of reproductive events in the Arctic, where breeding seasons are usually only 4-5 weeks.

Species from mid-latitudes, such as the Puget Sound white-crowned sparrow (*Zonotrichia leucophrys pugetensis*) and song sparrow (*Melospiza melodia*), have multiple-purpose territories that males defend throughout the breeding season. Territorial defense includes singing, patrolling, and agonistic encounters with challenging males. During aggressive encounters, the territorial male approaches the intruder and gives songs, threat postures, short flights (fluttering wings and short flights from perch to perch around the intruder), and direct attacks, remaining within 5 meters until the intruder is expelled from the territory (see Wingfield & Moore, 1987; Wingfield & Hahn, 1994). Males also use song as an advertisement or courtship signal to attract mates. These behaviors have been shown to be activated and prolonged by testosterone (e.g. Balthazart, 1983; Harding *et al.*, 1988; Wingfield & Moore, 1987; Wingfield, 1994). Once plasma levels of testosterone decline at the end of the breeding season, then high levels of spontaneous aggression, and of courtship-related activities, also decline (see Nowicki & Ball, 1989). These behaviors are typical of passerines breeding at northern mid-latitudes. Most species that have been studied breed in wooded or semi-open habitat (shrubs and thickets), and many maintain multiple-purpose territories for several months.

Arctic birds, in contrast, have a very short breeding season. Comparison of hormone-behavior adaptations in this temporally restricted nesting cycle with the more protracted breeding cycle common in lower-latitude species raises a number of questions. Do breeding behaviors (such as territorial and mate-guarding aggression, song, and courtship) have different intensities or durations in birds that must complete breeding in the fleeting arctic summer? Do circulating testosterone levels show a pattern that is different in any way (e.g., in the amplitude, timing, or behavioral correlates of the seasonal T peak) from that seen in lower-latitude species? If so, what aspects of the Arctic might be most important in exerting the selective pressures that result in different breeding strategies? Here we compare the testosterone-behavior interrelationships of a passerine that breeds only the Arctic, the Lapland longspur, with three other passerine species that breed both in the Arctic and at lower latitudes. As we are comparing several different aspects of behavior, we have arranged this chapter by topics. We will first discuss the natural history of the four species, and then describe methods and results for temporal patterns of testosterone, and then methods and results for measurements of aggressive behavior. Finally, we will compare the four species and discuss the ecological context of these hormone-behavior relationships.

NATURAL HISTORY OF THE FOUR STUDY SPECIES

General Methods

We studied a population of Lapland longspurs (*Calcarius lapponicus*, "Laplands"), Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*), American tree sparrows (*Spizella arborea*) and savannah sparrows (*Passerculus sandwichensis*) at Toolik Lake (68° 38'N, 149° 38" W), Alaska, during the summers of 1989 - 1994. Birds were captured in Potter traps baited with seeds or in Japanese mist nets, and each individual (except for savannah sparrows) was banded with a unique combination of color bands for subsequent observation and identification in the field. In each year, we identified mated pairs and observed behavioral patterns and general reproductive biology.

Natural History of the Lapland Longspur

The Lapland longspur is a small passerine that breeds throughout much of the Arctic tundra. It has a circumpolar distribution and is thought to be the most abundant arctic breeding bird (Bent, 1965). Males arrive on the breeding grounds in mid-May (e.g. Irving, 1960) and within a few days they show display flights accompanied by song over an apparent "territory" (or at least they show these aerial displays over the same small patch of ground, about 50-100m in diameter; L.B. Astheimer and K. Hunt, unpublished observations). A displaying male typically flies up to a height of about 50m, holds his wings out, and glides downward in a large spiral while singing several times. Before starting the glide, he often will fly in a large circle that roughly outlines the perimeter of his "territory", uttering a series of short call notes. During this phase, males will sometimes (but not always) chase other males out of their territories. They also actively court females during this phase, and thus the aerial displays may serve a courtship function. Pairing usually occurs rapidly, following which display flights are greatly reduced. As the female begins nest-building and becomes sexually receptive, males follow their mates closely, presumably mate-guarding to prevent extra-pair copulations (Montgomerie, 1988), and often chase other males away. This mate-guarding phase lasts 6-8 days. During this time, females construct a fur-lined or hair-lined cup nest on the ground by a tussock of grass, and lay (typically) 4-6 eggs. Females then incubate the eggs for 11-13 days, during which the male sings only rarely, and no longer follows the female. Until mid-incubation (typically mid-June), the female is capable of renesting if the clutch is lost (see Chapter 5 for details). After mid-incubation, territorial defense by males decreases markedly.

Our field observations indicate that after the eggs hatch, both parents feed the young, which fledge at about 10 days of age. Adult males and females continue to feed fledglings until independence, at about 30 days. During this parental phase, song frequency and territory defense by males declines to near zero, though occasional song bouts are still heard. Males forage in small groups which may travel over many territories ("cruising"), and will tolerate other males close to the nest, sometimes even tolerating another male singing a few meters from the nest. In this late stage, both sexes forage over a wide area, and are frequently found up to 1 km away from their own nests, often in other Laplands' territories (and near their nests) with little aggression from the residents. They will also congregate at unusually rich sites for food, such as at our seed-baited trap sites.

Clearly, male Lapland longspurs do not defend a classical multiple-purpose territory, and instead appear to show a brief series of territorial behaviors, which begin with the aerial displays and songs, and finishing just a week later with mate-guarding behavior. Our general observations of the Lapland longspurs at Toolik lake concur with data published for this species breeding at Barrow, Alaska (Custer & Pitelka, 1977); Bylot Island, Northwest Territories, Canada (Drury, 1961) and Eqalungmiut Nunaat, West Greenland (Fox *et al.*, 1987). It should be pointed out that the aerial displays and lack of a clearly defined multiple purpose territory may be typical of other passerines breeding on grassland/shrub-steppe habitats at lower latitudes -- for example, the bobolink, *Dolichonyx oryzivorus*, (Bent, 1965) and the dickcissel, *Spiza americana* (Zimmermann, 1996)

Natural History of White-crowned, Tree, and Savannah Sparrows

Gambel's white-crowned sparrow, American tree sparrow and savannah sparrow are abundant species throughout the northern North American continent. Gambel's white-crowned and savannah sparrows have breeding ranges extending far south of the Arctic circle (Bent, 1968). Breeding distribution of the American tree sparrow is confined to arctic and subarctic regions, but it does not breed on open tussock tundra or the high Arctic (Bent, 1968) as does the Lapland longspur. One of these species, the white-crowned sparrow, has been studied in detail in relation to the endocrine control mechanisms underlying the activation of territorial aggression by testosterone (e.g. Wingfield & Moore, 1987). Both white-crowned sparrows and American tree sparrows defend multiple purpose territories at Toolik Lake, usually around patches of willow (*Salix* spp.) that may reach a height of up to 2 meters in sheltered river beds and gullies. White-crowned and tree sparrows both construct nests in the branches of these small willows. Savannah sparrows also defend multiple purpose territories, preferring tussock tundra in lower flat areas often associated with patches of willow. Savannah sparrows build cup nests directly on the ground in the sides of grass tussocks, similar to the nests of Lapland longspurs.

These three species of passerines are similar to the Lapland longspurs in the broad outline of their breeding season, with a similar clutch size (4-6) in a single brood, a possibility of renesting until mid-June if the first nest is lost, and similar division of parental duties. Early in the season, males of all three species sing at high frequency and also guard their mates from other males. These behaviors then decline during the parental phase. However, they differ from the Lapland longspur primarily in the following ways: First, the three sparrow species have lower breeding densities than the Laplands, due to the sparrows' preference for willow habitat. Our study site at Toolik Lake usually has 40-50 pairs of Lapland longspurs compared to an average of 10-15 pairs for each of the other three species. Second, all three sparrow species tend not to be as tolerant of conspecifics as the Lapland longspurs; the sparrows have more precise territory boundaries, defend their territories longer, are not as apt to form flocks during the breeding season, and will not tolerate close proximity of a conspecific even when foraging. Third, the sparrows do not have aerial song displays, singing instead from slightly elevated perches within their territories.

TEMPORAL PROFILE OF TESTOSTERONE IN RELATION TO BREEDING PHENOLOGY AND TERRITORIAL AGGRESSION

Changes in Circulating Testosterone Levels

We took blood samples by puncturing of the wing vein and collecting the blood into heparinized microhematocrit tubes. Samples were kept on ice until centrifuged (usually within 4 hours). Plasma was stored at -20°C and later transported frozen to the University of Washington in Seattle. We then measured plasma levels of testosterone by radioimmunoassay after partial purification of steroid extracts on diatomaceous earth/glycol micro columns. This assay procedure, along with inter- and intra-assay variations, has been described in detail by Ball & Wingfield (1986). Data are reported here as mean ± SEM. All statistical tests were two-tailed.

There was considerable dimorphism in plasma levels of testosterone between male and female Lapland longspurs (Fig. 1.1; data for this figure provided by Lee Astheimer and William Buttemer). This is typical of most passerine species studied to date (Wingfield & Farner, 1993). Males showed highest levels for just a few days early in each year (e.g., around May 27 in 1990, Fig. 1.1). Females never attained such high levels and showed very little temporal organization of circulating levels of testosterone with date (Fig. 1.1).

We organized the hormone data from males in 1992 both by calendar date (Fig. 1.2, upper panel) and by stage in the breeding cycle (display, mate-guarding, incubation and feeding of young) for individuals of known breeding status (Fig. 1.2, lower panel). Lapland longspurs appear to be highly synchronous (perhaps due to the rapid temporal progression of the breeding cycle), and the temporal patterns of testosterone in males appeared essentially identical when analyzed either way. Plasma levels of testosterone in 1992 were highest during the three days when males were showing aerial displays and singing at greatest frequency (the "display phase"; mean = 6.95 ± 1.5 ng/ml), with several individuals showing very high T levels of over 10 ng/ml. These levels were higher than in both the pre-settlement phase immediately before the display phase (mean = 1.90 ± .43 ng/ml), and the mate-guarding phase immediately after (mean = 1.64 ± .66 ng/ml). By the time incubation was underway, plasma levels of testosterone were basal (mean = 1.09 ± .33 ng/ml). Analysis with single-factor ANOVA revealed significant differences in T levels among the four phases ($p = 0.0004$). Post-hoc pair-wise analyses with Fisher's Protected Least Significant Difference test showed that the display phase had significantly higher T levels than the pre-settlement, mate-guarding, or incubation phases, while the latter three phases did not differ from each other.

Male white-crowned sparrows and American tree sparrows also showed declines in levels of testosterone as the season progressed (Fig. 1.3; 1989 white-crowned sparrows: $t_9 = 2.392$, $p = 0.0404$; 1989 tree sparrows, $t_9 = 3.212$, $p = 0.0075$; Student's t-test). For ease of comparison, we include the 1992 Lapland data on the same figure ("early" = display, "late" = incubation). The data for the sparrow species are essentially identical to those presented by Wingfield and Farner (1978a,b) for a population of *Z.l. gambelii* breeding in the vicinity of Fairbanks, Alaska (64°N). The decline of testosterone levels as the parental phase of the breeding cycle ensues (late season) is typical of avian species in which males provide significant parental care (e.g. Wingfield *et al.*, 1990). However, testosterone levels in the sparrows were elevated for 2-3 weeks early in the breeding season (when males were establishing territories and courting females, Wingfield & Farner, 1978a; Wingfield & Moore, 1987) - much longer than the 2-3 days of elevated circulating levels of testosterone in male Laplands.

Quantification of Aggressive Behavior

Males of all species were tested with simulated territorial intrusions (STI) to assess their aggressive behavior. This procedure has been described in detail elsewhere (Wingfield, 1985). Briefly, a free-living adult male is challenged by playing back tape-recorded conspecific songs, and the aggressive behavior of the male is recorded as he tries to locate and drive off the apparent intruder. In many studies, a caged, live conspecific decoy is placed alongside the speaker to provide visual as well as auditory stimuli. Typically, males fly up to a perch (e.g. in a willow), sing in reply, and then approach the source with threats and wing quivers (flights), showing all these behaviors to varying degrees throughout the breeding season (e.g. Wingfield, 1985; Wingfield & Moore, 1987; Wingfield & Hahn, 1994). However, this pattern of response may not hold for all species. For example, the Lapland longspur prefers open tundra where vegetation may only be 10 cm high. These birds thus rarely show numerous flights in response to a simulated territorial intrusion, but instead land near the decoy and walk around it with threat postures (Drury, 1961; K. Hunt unpublished observations). Male Laplands often do not sing during STI, and almost always come very close to the decoy (<1 meter), even when not aggressive. Because of these behavioral differences, we will describe in some detail the STI procedure used with the Laplands.

Adult male Lapland longspurs were tested with a STI as follows: A caged adult male Lapland was placed 20 meters from the focal male's nest (or in the center of an apparent territory, designated as the area over which he performed most of his song flights). Conspecific song, recorded at Toolik lake in 1991, was played over a speaker placed alongside the decoy's cage. The playback tape mimicked the natural frequency of Lapland longspur singing. During the STI, an observer stood 20 meters from the decoy (40 m from the nest) and recorded the focal male's response for a ten-minute period. Blinds were not used, as Lapland longspurs at Toolik Field Station are accustomed to humans. Four different behaviors were recorded: 1. Number of Songs throughout the ten-minute period. Because of the dual role of song as a courtship signal as well as a territorial signal, song was not automatically considered to be indicative of aggression. 2. Time spent within 5 m of the decoy (as in Wingfield, 1985). We regarded time within 5 m of the decoy as an indicator of general interest in the decoy, but not necessarily of aggression. 3. Time spent in Threat Posture (defined as body held horizontally, wings drooped slightly and bill pointed straight ahead or tilted up - see Drury, 1961); and 4. Number of Attacks, defined as physical striking of the decoy's cage with the bill or feet. We considered these last two variables to be indicators of aggression.

STIs were performed on Lapland longspurs at three major phases in the breeding cycle: during the song display phase immediately after arrival, during the mate guarding and egg-laying phase, and during the incubation phase. STIs were performed on white-crowned sparrows early in the season (comparable to display and mate-guarding stages in the Lapland longspur) and late in the season (during incubation and feeding of young). STIs were performed on savannah and tree sparrows only late in the season, during incubation and feeding of young.

All Lapland longspur individuals were of precisely known breeding status. For the other three species, individual breeding status was not always known, and was inferred from the general population status. We regard this as generally reliable, since breeding is tightly synchronized in all these species at Toolik.

Results of Simulated Territorial Intrusions

Male Lapland longspurs gave the greatest number of songs during the *display phase* (Fig. 1.4; Kruskal-Wallis test, $H_2 = 10.045$, $p = 0.0066$), but were virtually silent during mate-guarding and incubation. Male Laplands also spent less time within 5 m of the decoy during the display phase compared with mate-guarding and incubation (Fig. 1.4; $H_2 = 6.894$, $p = 0.0318$). Our impression was that during the display phase, males continued their usual aerial song displays with little concern for the STI. In contrast, during the *mate-guarding phase*, males spent much time by the decoy, and time in threat posture and the number of attacks were highest (Fig. 1.4; $H_2 = 19.004$, $p = 0.0001$, and $H_2 = 11.645$, $p = 0.003$ respectively). During the *incubation phase*, aggressive posture, attacks, and song were all low, but males remained close to the decoy, generally appearing "interested" but not aggressive. Males often foraged near the decoy or sat still by the decoy's cage, sometimes apparently going to sleep. Subsequent analysis with Mann-Whitney U tests (Table 1.1) generally concurred with these Kruskal-Wallis test results, although some comparisons did not quite reach significance (e.g. songs and attacks in display vs. mate-guarding).

Males of the three species that defend multiple-purpose territories also gave songs and approached the simulated intruder (Figs. 1.5 and 1.6). White-crowned sparrows showed no significant changes in number of songs in STIs performed early in the season vs. late in the season (Fig. 1.5, upper panel). However, males approached the simulated intruder more closely early in the season than late ($p < 0.02$, Mann-Whitney U test). For STIs performed during the parental phase (incubation and feeding of young), male white-crowned sparrows, tree sparrows, and savannah sparrows all gave more songs than male Lapland longspurs (Figs. 1.4, 1.5 and 1.6). Male savannah sparrows also showed many flights around the intruder (Fig. 1.7) and spent almost 50 percent of the STI period within 5 meters of the tape recorder, even though all birds tested were feeding young.

DISCUSSION

Patterns of Testosterone, Aggression, and Song

The Lapland longspurs do show some differences when compared to the white-crowned, American tree, and savannah sparrows. Laplands differed particularly in 1) their type of territory defense, 2) the phenology of their testosterone peak, 3) the nature and duration of their aggressive responses to STI, and 4) the correlation of the testosterone profile with behavior.

1. Territory defense. Our behavioral observations show that the Lapland longspur males defend loose "nest areas" with indistinct boundaries, and are tolerant of the close proximity of conspecific males at all times other than during mate-guarding. After mid-incubation, territory boundaries fluctuate to the point of vanishing, and males frequently forage off-territory with small flocks of neighboring males. In contrast, white-crowned and song sparrows breeding at mid-latitudes show a more gradual shift from high song rate to low song rate upon entering the parental phase; greater aggression toward conspecifics at all times in the breeding period; and less of a tendency to flock or to "cruise" through other territories (Wingfield & Hahn, 1994). Our data for white-crowned, American tree, and savannah sparrows at Toolik Lake clearly show a pattern similar to those passerines tested at mid-latitudes, and different from the Lapland longspur.

2. Timing and amplitude of testosterone peak. The Lapland males showed an unusually high and brief peak in testosterone. The Laplands' testosterone peak is up to five-fold higher than, for example, white-crowned sparrows (Figs. 1 and 2; Wingfield & Farner, 1978a; Wingfield & Moore, 1987). (Note that the plasma samples from both species were assayed in the same lab, and in many cases in the same assay.) In fact, Lapland longspurs have the highest levels of circulating testosterone of any wild species studied to date (Wingfield & Farner, 1993). This high T peak lasts only 1-3 days compared to the 2-3 week peak of most other species. The peak is so brief in duration that we may have missed it in many individuals sampled during the first few days after arrival at Toolik Lake.

3. Response to simulated territorial intrusion. Lapland longspurs showed high song rates during STI only at the very first stage of the season, the display stage, when their spontaneous song rate is highest. Lapland song rate during STI declined abruptly after this. In contrast, white-crowned sparrows sang in response to STI throughout the season , and white-crowned, tree, and savannah sparrows all sang more than Laplands during late-season STI (Figs. 1.4, 1.5, 1.6). Laplands showed greatest aggression toward the decoy, as measured by threat postures and attacks, during mate-guarding. Note that this peak in STI-measured aggression lagged about a week behind the peak in spontaneous song rate. Laplands do not tend to respond to STI with flights around the decoy. In contrast, the white-crowned, song, and savannah sparrows responded with numerous flights. There is noticeable variation among the three sparrow species in all the behaviors measured, with

the white-crowns being least like the Laplands and the savannah sparrows most like the Laplands. This may possibly relate to the savannah sparrows' similar tussock-nesting habits.

4. Behavioral correlates of the testosterone peak. The peak of circulating testosterone levels in Laplands (10-20 ng/ml) occurs when the males are singing the most (display phase), *not* when they are most aggressive toward STI (mate-guarding phase). Testosterone declines to an intermediate level by the time the males are most aggressive. In contrast, testosterone in the other three species peaks at about 3-5 ng/ml, and stays at that level for 2-3 weeks while the male is most aggressive and is also mate-guarding (e.g. Wingfield & Moore, 1987; Wingfield & Hahn, 1994).

Our impression is that in Lapland longspurs there is a slight temporal division between maximum song and maximum aggression, with song peaking a week earlier. Maximum song rate correlates with the Laplands' unusually high, brief testosterone peak; maximum aggression correlates with the more usual 2-4 ng/ml level of testosterone seen a week or two later. Testosterone is known to affect both singing and aggression in many species, so much so that song is often considered a type of aggression, and testosterone is sometimes said to promote both behaviors simultaneously. However, song has a dual role -- it is not only an aggressive behavior toward rival males, but also a courtship behavior that serves to attract potential mates. Male Lapland longspurs, in fact, may be using song more for courtship than for aggression, as indicated by their tendency to sing in aerial display, and not to sing during aggressive responses to STIs. It is tempting to see a possible dual-level effect of testosterone in Lapland longspurs, with very high levels of testosterone promoting song and aerial displays, and thus perhaps courtship of females, and lower levels promoting inter-male aggression. The data presented here are just correlations, however, and controlled experiments are needed to examine these questions more closely (see Chapters 2 and 3).

The Arctic As A "Unique" Ecosystem

Overall, it is clear that Lapland longspurs are different in several aspects of their behavior and endocrinology when compared with white-crowned, American tree, and savannah sparrows. Why might Lapland longspurs be different? Laplands are arctic specialists limited to breeding on the tundra, while the other three species have extensive breeding ranges in non-arctic regions as well. For these, the low-arctic tundra is the extreme northern extent of their ranges. Might the differences discussed above be due to special selective pressures seen in the Arctic? The Arctic has several features that might affect breeding biology, including:

1. A very short summer (breeding season limited to mid-May to mid-July).
2. Low vegetation; the tallest trees are riparian willow thickets, 0.5-2m in height.
3. Essentially continuous daylight in June.
4. Large insect bloom, also in June.
5. Erratic snowstorms probable in the early breeding season, and possible anytime.

Most of these factors, individually, are not unique to the Arctic (with the exception of continuous daylight, which occurs only the Arctic and the Antarctic). For instance, birds living at high elevations also experience frequent spring snowstorms, and birds of temperate grasslands also live in low vegetation. If the Arctic is indeed unique, it is in the *combination* of these factors. In particular, the low, open tundra habitat combined with a very short breeding season may lead to certain behavioral and physiological adaptations, as follows:

1. There can be one clutch only per season, with a limited window for renesting if nest is lost. Therefore, mate-guarding may be intense but brief. Individuals may switch rapidly from courtship to mate-guarding behaviors at the beginning of the season.
2. Abundant treeless tundra habitat means that nest sites may not be limited and territory quality will possibly not be very variable. Thus, strong defense of a territory may not be necessary throughout the entire breeding season.
3. The very short time for arrival, setting up territories, and finding a mate (#1), in conjunction with the abundant tundra habitat (#2), may lead to priority being given to courtship and mate-guarding instead of territory defense. Territories may have loose boundaries and may be defended only briefly.
4. The treeless "grassland-like" habitat may promote flight song displays or other aerial displays by males.
5. Ground-nesting may result in high nest predation, with accompanying selection pressure for cryptic nests, "wariness" around the nest, and sentry behavior.

Thus, the abundant tundra nesting habitat combined with the very short window for finding mates may have led Laplands to develop their grassland-like aerial song display combined with their loose "nest area" territories. Their brief, high testosterone peak may serve as a trigger to switch behaviors rapidly from migration to song and then to mate-guarding, thus initiating breeding very rapidly.

The Lapland longspur is just one arctic specialist. On the basis of this one example we cannot answer the broader question of whether the mechanisms and/or temporal patterns of behavior and endocrinology are different in arctic animals from those in temperate-zone animals. We suggest that specific comparisons between Lapland longspurs, other arctic-specialized breeders (such as Smith's longspurs, *Calcarius pictus*; redpolls, *Carduelis flammea*; and snow buntings, *Plectrophenax nivalis*) and lower-latitude grassland breeders (such as McCown's longspurs, *Calcarius mccownii*; chestnut-collared longspurs, *Calcarius ornatus*; bobolinks, *Dolichonyx oryzivorus*; and others) will reveal whether the hormone-behavior interactions of this arctic specialist represent unique adaptations to the Arctic, or are more general features of birds inhabiting grassland and open tundra biomes.

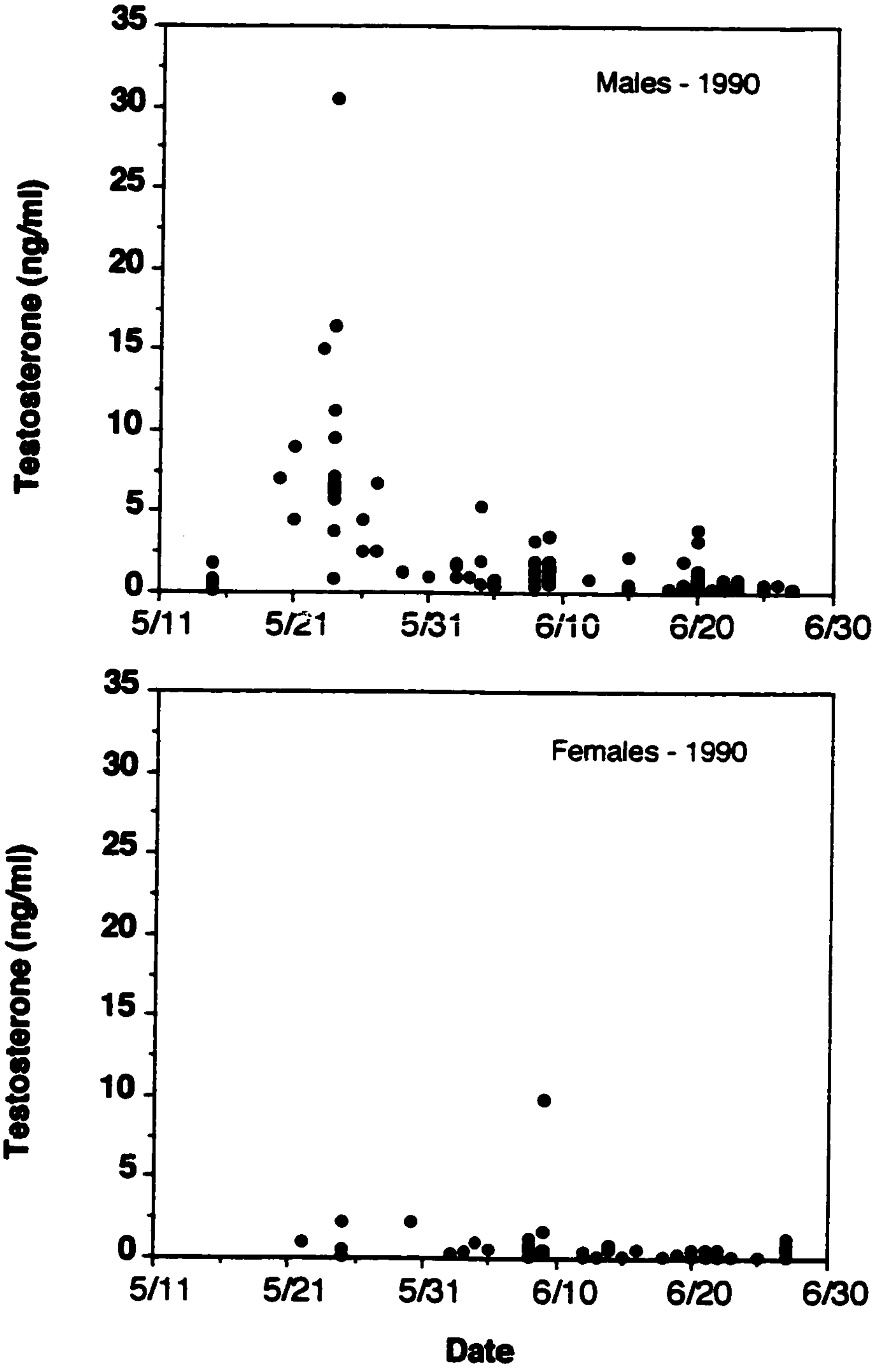

FIGURE 1.1. Plasma levels of testosterone in male and female Lapland longspurs in relation to calendar date at Toolik Lake, 1990. Sample sizes were 122 males and 76 females. (Data for this figure provided by Lee Astheimer and William Buttemer.)

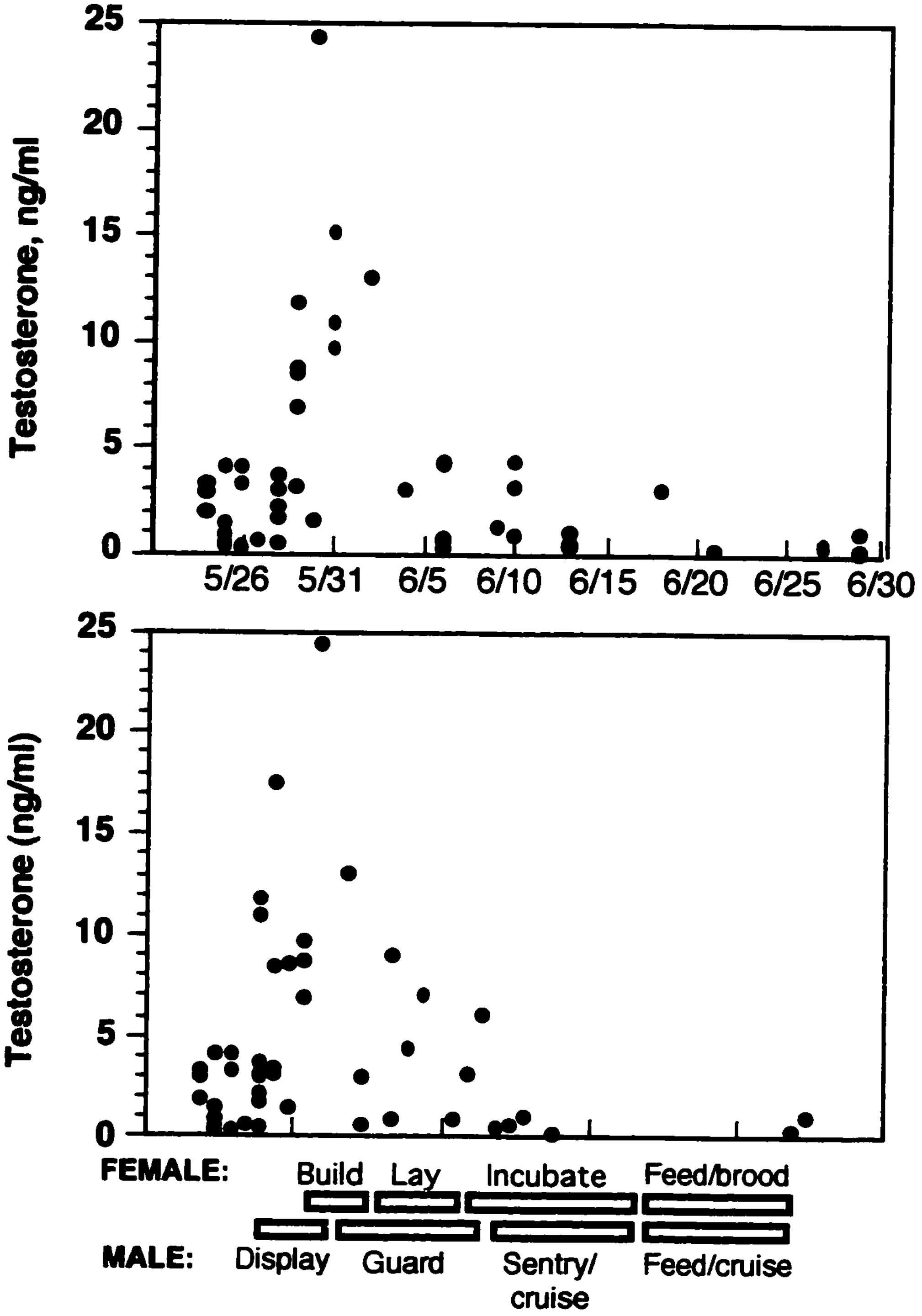

FIGURE 1.2. Comparison of plasma levels of testosterone in relation to calendar date (upper panel) and stage in the reproductive cycle (lower panel) of male Lapland longspurs at Toolik Lake, 1992. Sample size = 44.

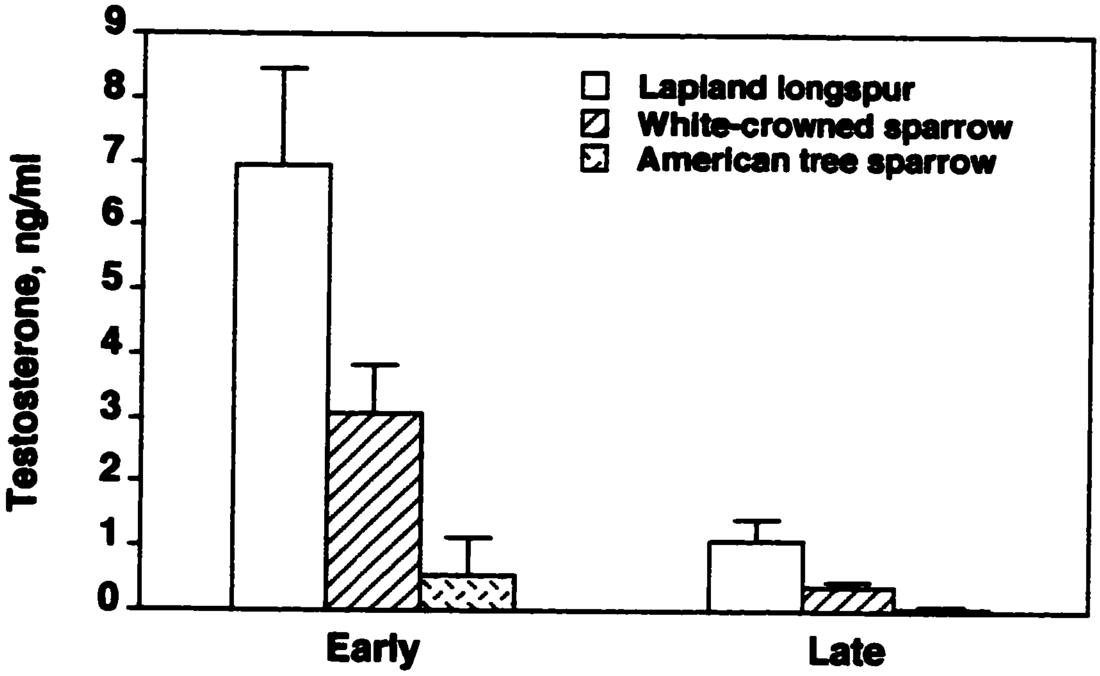

FIGURE 1.3. Plasma levels of testosterone in male Lapland longspurs, male Gambel's white-crowned sparrows, and male American tree sparrows, early (pre-breeding) and late (parental phase) in the breeding season. Data are shown as means ± SEM.

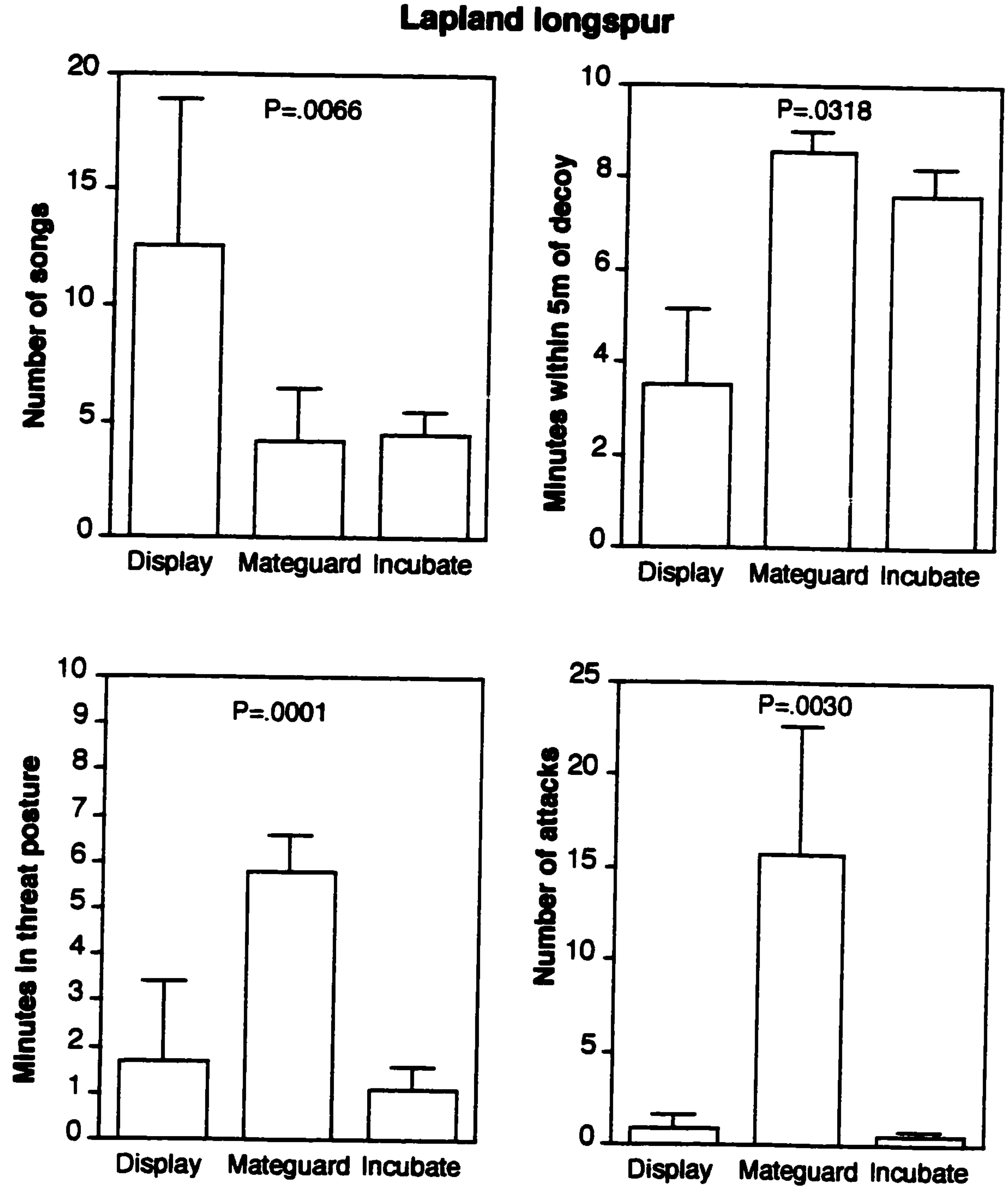

FIGURE 1.4. Behavioral responses of male Lapland longspurs to simulated territorial intrusions during the display phase (n=6), mate-guarding (n=22), or incubation (n=25). Data are shown as means ± SEM. P-values from Kruskal-Wallis tests are shown at top.

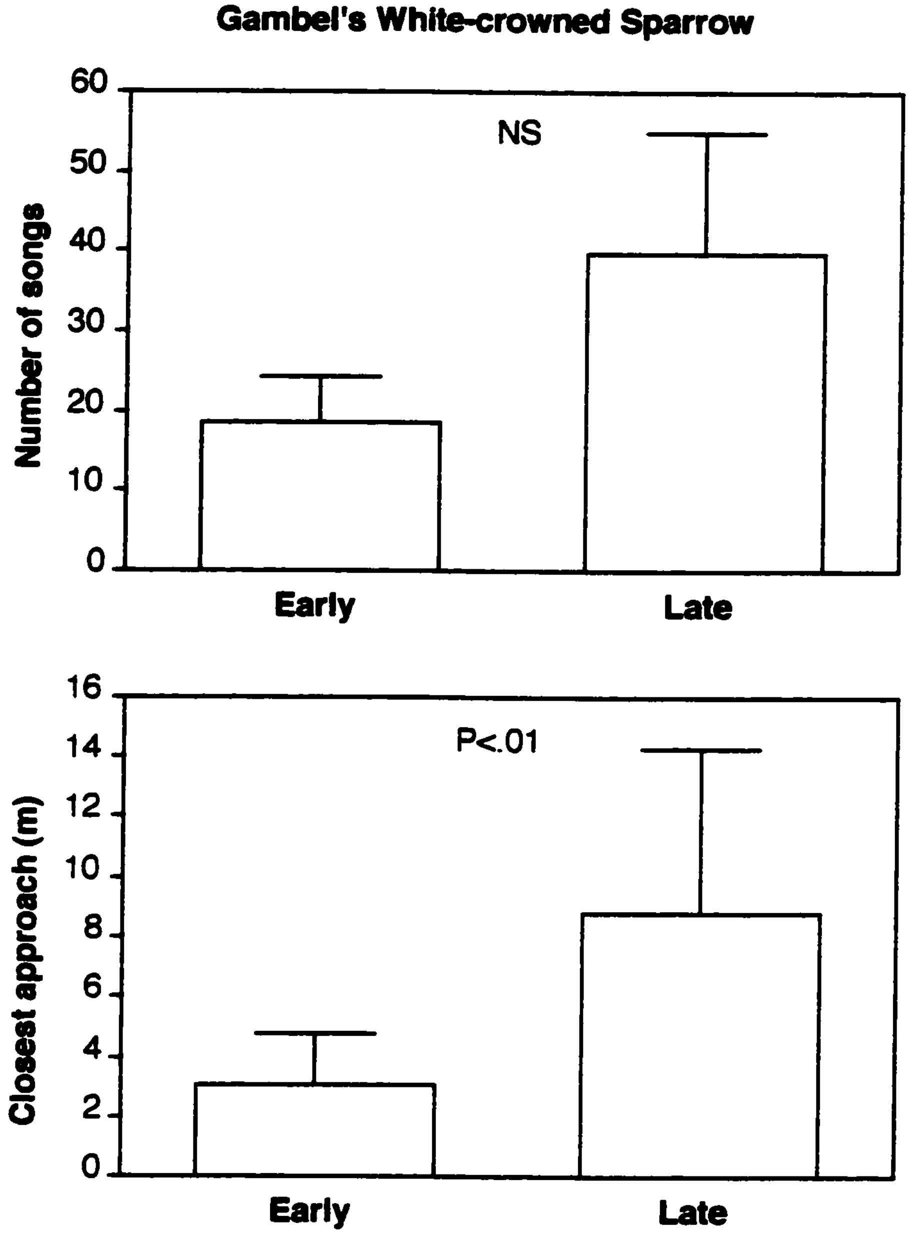

FIGURE 1.5. Behavioral responses of male Gambel's white-crowned sparrows to simulated territorial intrusions, both early (pre-breeding) and late (parental phase) in the breeding season. E = early and L = late in the breeding season. Data are shown as means ± SEM. Significance levels from Mann-Whitney tests are shown at top (NS = not significant = $P > .05$). Data for this figure provided by Lee Astheimer and Bill Buttemer.

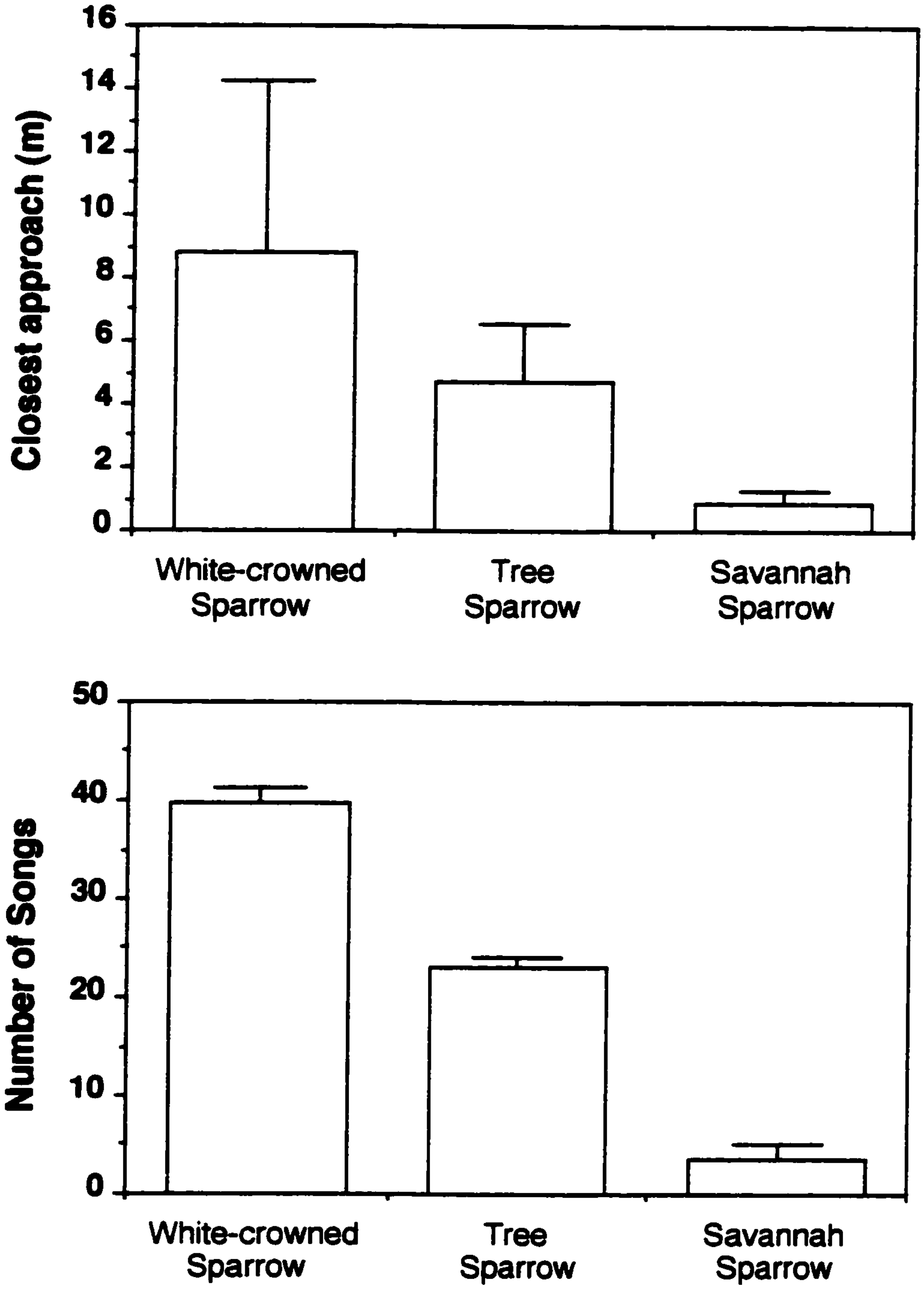

FIGURE 1.6. Behavioral responses of male Gambel's white-crowned sparrows, American tree sparrows, and savannah sparrows to simulated territorial intrusions late in the breeding season. Data are shown as means ± SEM.

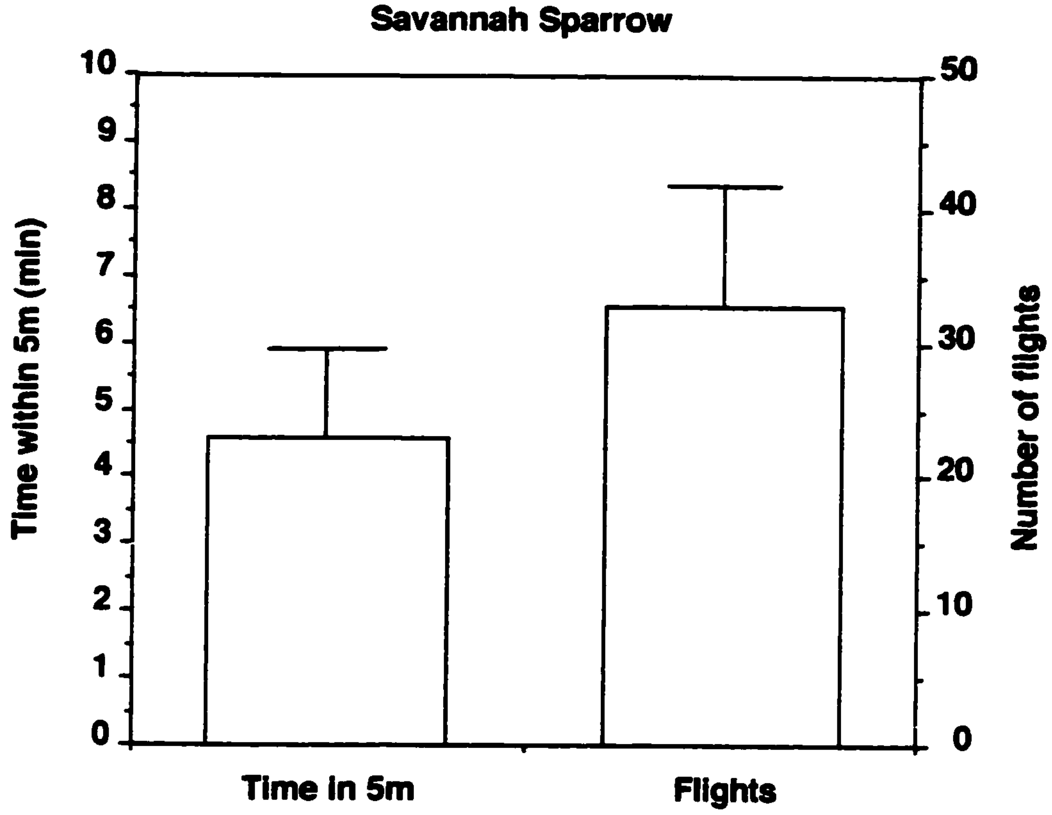

FIGURE 1.7. Additional behavioral responses of savannah sparrows to a simulated territorial intrusions late in the breeding season. Data are shown as means ± SEM.

Table 1.1. Mann-Whitney U test results for the responses of male Lapland longspurs to simulated territorial intrusions during the display phase (D), mate-guarding (M), and incubation (I). The table shows Z-values, corrected for ties, with P-values in parentheses. Sample sizes were n=6 for display, n=22 for mate-guarding, and n=25 for incubation.

	D vs. M	M vs. I	D vs. I
#Songs	1.676 (.0937)	2.024 (.0430)	3.136 (.0017)
Time in 5m	2.572 (.0101)	0.688 (.4913)	2.200 (.0278)
Threat posture	2.194 (.0282)	4.204 (.0001)	0.372 (.7099)
#Attacks	1.766 (.0775)	3.220 (.0013)	0.153 (.8785)

CHAPTER 2. The Effect of Testosterone Implants on Aggression and Song of Male Lapland Longspurs During Incubation

INTRODUCTION

The Arctic breeding season is extremely short. In most of the Arctic, above-freezing temperatures occur only from mid-May to mid-August (Bunnell *et al.*, 1975; Pielou, 1994). Birds nesting at these latitudes must initiate breeding as early as possible, and progress rapidly and efficiently through the stages of nesting before the brief summer ends. For instance, some arctic species may find a mate, court, build a nest and initiate laying within one week of arrival from migration (Carey, 1988). Seasonally breeding birds in temperate latitudes have a much more protracted breeding season, which typically lasts three to four months, with courtship and pair formation sometimes taking an entire month within a population (Immelmann, 1971; Wingfield, 1984a). Given the rapid temporal progression of reproductive behavior in arctic birds, we expected that they might show different relationships of steroid hormones and breeding behavior than are seen in temperate-zone birds.

Sex steroid hormones play a major role in initiating and maintaining breeding behavior in temperate-zone birds and many other vertebrates (e.g. Harding, 1983; Balthazart, 1983). The role of testosterone (T) in male breeding behavior is especially well studied. Castration of males of these temperate-zone species generally reduces aggression and virtually eliminates song, whereas testosterone implants restore both aggression and song, although there are some exceptions (e.g. Harding, 1983; Wingfield, 1994; Wingfield *et al.*, 1994). In free-living males, elevated levels of plasma T usually coincide with increased singing and territorial aggression, and T declines when males begin feeding young (Wingfield *et al.*, 1990; Wingfield & Farner, 1993). Circulating T is generally closely associated with male song, which often plays a dual role in courtship and aggression (e.g. Searcy, 1988; Ketterson & Nolan, 1992). In contrast, T has a subtle association with physical aggression. Different species vary in the temporal patterns of plasma T levels and of aggressive behavior. Some of this variation is correlated with mating system and with periods of social instability (Wingfield *et al.*, 1990; Beletsky *et al.*, 1995), but much additional variation remains unexplained.

Studies of arctic birds may clarify how ecological factors such as length of the breeding season affect the relationship between testosterone, male aggression and song.

The Lapland longspur, *Calcarius lapponicus*, is the most abundant tundra-nesting songbird of the Arctic (Irving, 1961), and is an ideal subject for such studies. These birds have a highly compressed breeding season, which typically lasts only six weeks from arrival of the migrating adults to fledging of the young. Male Lapland longspurs have an unusually brief and high peak of testosterone at the beginning of the breeding season, when they have a stage of intense song display (Hunt *et al.*, 1995). Song display, territory formation, courtship, pair-bonding and egg-laying take just one to two weeks, followed by 12 days of incubation, nine to 10 days of feeding nestlings and two weeks of feeding fledglings (Williamson, 1968a,b; McLaughlin & Montgomerie, 1985). Individuals within a given population are usually very synchronized as they proceed through these stages of breeding (Williamson, 1968b).

Given the brevity of reproductive events within this species, we wanted to know whether this arctic bird shows the same hormone-behavior relationships seen in temperate-zone birds. We tested whether aggression and song are influenced by T in Lapland longspurs, as they usually are in temperate-zone birds. We administered T implants to free-living Lapland longspur males in mid-June, during incubation, when males normally have low T levels and have generally ceased singing and acting aggressive. Our null hypothesis was that Lapland longspurs are similar to temperate-zone birds, and thus we predicted that T-implanted males would show increased aggression and increased song compared to control males.

METHODS

Study Species

Male Lapland longspurs typically arrive from migration at Toolik during two or three days in mid-May. Resident males immediately begin nearly continuous singing, often while doing gliding flights over their territories (the "display stage" of Hunt *et al.*, 1995). Males sometimes show mild aggression during the display stage (K. Hunt & T.P. Hahn, unpublished data). Resident females arrive within a few days and immediately pair up with males, i.e. within 1-2 days (Williamson, 1968a,b). Lapland longspurs at Toolik are socially monogamous. As soon as a male has a mate, he usually sings less frequently and begins following his mate as she builds a nest, lays 4-5 eggs and initiates incubation ("mate-guarding" stage: Hunt *et al.*, 1995; also see Montgomerie, 1988). During the 7-10 days of the mate-guarding stage, males are highly aggressive toward conspecific male intruders and toward experimental playback of conspecific song (Hunt *et al.*, 1995). Females may re-nest if the nest is lost during early incubation (K. Hunt, unpublished data). As incubation proceeds, males cease responding aggressively toward intruders and virtually cease singing (Hunt *et al.*, 1995). Males do not incubate eggs and do not feed the incubating female (Lyon & Montgomerie, 1987). By late incubation, males are commonly found foraging in small flocks, sometimes a kilometer from the territory that they had defended just three weeks before (K.Hunt, unpub. data; Tryon & MacLean, 1980). When the eggs hatch, both sexes feed the young (McLaughlin & Montgomerie, 1985).

General Methods

We studied Lapland longspurs at Toolik Lake, Alaska (68° 38'N, 149° 38' W), during the summers of 1991, 1992, 1994 and 1996. Birds were captured in Potter traps baited with seeds, and traps were checked at least every 20 min. We took a blood sample from each bird, checked the bird's hormone implants (if any), and measured the length of the cloacal protuberance, an androgen-dependent sexual organ. We checked general condition by measuring body mass to the nearest gram, scoring fat stores in the furcular and abdominal areas (by visual assessment and ranking on a scale from 0 = no fat, to 5 = bulging fat deposits; Wingfield & Farner, 1978b), and noting molt status. Each bird was banded with an aluminum U.S. Fish & Wildlife Service band and a unique combination of color bands for subsequent visual identification and observation.

We measured cloacal protuberances (CPs) along the antero-ventral surface from base to tip with a small ruler, rounding all measurements to the nearest mm. For comparison of CP lengths of T-implanted and control birds, we used only those measurements that were taken when the birds' mates were incubating eggs (roughly June 3-15), since this was when we did our behavioral observations. We also measured CP length during incubation from 20 unimplanted males known to be breeding residents. All CPs were measured by K.H.

Blood Sampling & Radioimmunoassay

We obtained blood samples by puncturing the alar vein and collecting approximately 250-300 ul of blood into heparinized microhematocrit tubes. This amount was approximately 1% of the body mass of these 25-30g birds. The tubes were stored temporarily in small field coolers filled with ice packs, and were centrifuged within six hours. We then stored the plasma at -20°C and later shipped it on dry ice to the University of Washington. We measured plasma levels of T (and other steroid hormones not presented here) by radioimmunoassay, after partial purification of steroid extracts on diatomaceous earth/ glycol micro columns (Ball & Wingfield, 1987). Twenty ul of tritiated steroid was added to every sample to determine the percentage of steroids recovered. Inter-assay variation was 15%, intra-assay variation was 11%, and mean recovery percentages were 70%.

We tried to capture and bleed each implanted bird at least twice, once before and once during incubation. Many individuals were captured more often. No birds were bled more than once a week. Testosterone levels presented here do not include those samples taken directly after a simulated territorial intrusion, since T levels in some species are known to rise transiently (for a few hours) in response to aggressive encounters (Wingfield & Wada, 1989). We were unable to measure baseline levels of corticosterone due to the time delay involved in checking the traps.

Testosterone Implants

We wanted to expose males to high, but physiologically normal, testosterone levels during incubation, when males normally sing little and are not aggressive. Thus, we implanted males during late May and early June, when females were building nests and laying eggs, a mean of 12.03 ± 1.45 days before observing behavior. A few males were implanted when their females had just begun incubation. Each bird was implanted only once throughout 1991-1994. The implant procedure itself does not seem to affect birds' behavior for more than one day after implanting, as we frequently observed birds on the first or second day after implanting that were behaving normally.

Subcutaneous T implants were made with crystalline T packed into 12- or 20-mm lengths of silastic tubing sealed with silastic medical adhesive (i.d. 1.47mm, o.d. 1.96mm; Dow Corning, Midland, Michigan). Testosterone implants of this type begin to release measurable levels of T within a few hours, and then continue to release T at a slowly declining rate for approximately 60 days (e.g. Wingfield, 1984b; Ketterson *et al.*, 1991). Because we had varying results with different implant procedures, we describe the procedures in detail. We anesthetized birds in the field with a light dose of an inhalant anesthetic, methoxy-fluorane (Metofane; Pitman-Moore, Mundelein, Illinois). We then inserted the implants through a small incision in the skin, either on the side between the wing and knee or on the back between the wings, and sealed the incision with a veterinary skin bond (Nexaband; Tripoint Medical, Raleigh, North Carolina). This procedure took about 2 min, and the birds were alert and ready for release within 10 min. We implanted birds in pairs, one bird receiving T implants and the other receiving empty (control) implants of the same number and placement. We checked the implants each time a bird was captured and removed them at the end of the season whenever possible. Measurement of circulating levels of T allowed us to assess the effectiveness of the T implants.

In 1991, we implanted 7 males with one 12-mm testosterone implant ("low-T" group; effective implant length = 10 mm) on the left side, and an empty 12-mm implant on the right side. Six control birds similarly received two empty 12-mm implants. Implants were on each side so that we could compare 1991 birds to subsequent experiments involving implants on each side. Some males were left unimplanted. We expected that a single 12-mm T implant would raise plasma T levels to about 3 ng/ml, which is comparable to maximum natural levels for most other passerines (e.g. Hegner & Wingfield, 1986b, Wingfield & Farner, 1993), but is below the Lapland longspurs' natural peak of 5-20 ng/ml (Hunt *et al.*, 1995).

In 1992 and 1994, we implanted males with multiple T implants ("high-T" group) designed to increase T to the maximum levels seen in the field. In 1992, we gave birds two 20-mm implants, one on each side (effective total length = 36 mm). Control birds received two 20-mm empty implants each, and other birds were left unimplanted. Several birds lost these long implants; high-T birds that lost both implants were excluded from analysis, while those birds that retained at least one 20-mm implant were included in our analysis. Final 1992 sample sizes were 9 high-T birds and 5 control birds.

In 1994 we modified the T implant size and placement, giving each bird either three or four 12-mm implants (effective total length = 30mm or 40mm, respectively), positioned either as two on each side, or all on the back between the wings. Implants between the wings tended to stay in place without causing health problems. Birds with two 12-mm T implants on each side, however, sometimes developed inflamed knees, perhaps due to the Lapland longspurs' habit of walking with long strides rather than hopping. We recaptured such birds, removed their implants and excluded them from the study. A few birds did lose implants, and only those birds that retained at least two 12-mm implants were kept in our analysis. Final 1994 sample sizes were 7 high-T birds and 6 control birds.

In 1996 we implanted 10 males with three T implants between the wings for the purpose of studying song rate. These birds were not included in the simulated territorial intrusion experiment. Control birds in 1996 were not implanted.

Response to Simulated Territorial Intrusions

We chose to study territorial aggression during incubation, when males are typically not aggressive, rather than earlier in the season when they are more aggressive, for two reasons. First, we wished to see if elevated testosterone alone is sufficient to cause changes in behavior, in the absence of cues that normally accompany the natural T peak (e.g. sexually receptive females), and in the presence of cues that may be suppressive (e.g. incubating females). Second, since we expected that testosterone would increase both aggression and song, we wanted to do our studies at a time when control birds normally have low aggression and song so that any experimental effect would be visible. We expected that any response to T implants during incubation would give us clues to the function of the natural T peak earlier in the season.

We studied aggression and song in free-living males with simulated territorial intrusions (STIs). This technique involves "challenging" a resident bird with presentation of a decoy and playback of conspecific song. This combination of visual and auditory cues is a highly effective stimulus for territorial aggression in most bird species (e.g. Wingfield & Hahn, 1994), and Lapland longspurs respond vigorously to STIs during mate-guarding (Hunt *et al.*, 1995). Briefly, an observer (K.H.) placed a live caged male decoy and a tape player, with a recording of local Lapland longspur song, 20 m from the focal bird's nest. The observer then walked 20 m further away from the nest and started the tape player. The STI was considered to have begun when the focal bird first came within 20 m of the decoy. We defined the start of the STI in this way because Lapland longspur males often spend much time off-territory, especially during incubation, and song attenuates rapidly in the windy conditions of the tundra. Therefore, a male may easily be out of hearing and visual range when the tape is started, and so we began behavioral observations only when the male came close enough to the decoy that he was probably able to hear and see it.

For 10 min after the start of the STI, four different behaviors were recorded:

1. Time Spent Within 5 m of the decoy (e.g. Wingfield, 1985). We regarded this variable as an indicator of general interest in the decoy, but not necessarily of aggression.

2. Number of Songs given during the 10 min of the STI. Birds occasionally began singing soon after the STI had ended. Therefore, we also noted whether birds sang at all (i.e. at least once) during the 30 min after the start of the STI, though we could not obtain these data for all birds.

3. Time Spent in Threat Posture, defined as body held horizontally, wings drooped slightly and bill pointed straight ahead or tilted up (Drury, 1961).

4. Number of Attacks, defined as physical striking of the decoy's cage with the bill or feet. We considered these last two variables to be indicators of aggression, so we combined them into a third variable, Occurrence of Aggression. A bird was scored as "aggressive" if he showed any threat posture and/or any attacks, and as "not aggressive" if he never showed threat posture and never attacked. We feel confident that these two variables can be combined because Lapland longspurs almost always show threat posture before and while attacking. A separate study of Lapland longspurs during mate-guarding indicates that threat posture and number of attacks are indeed strongly and significantly correlated (see Chapter 3).

Seven male decoys were used throughout the study. Because Lapland longspur males each have only a single song type, we used one song type on the STI tape. The tape consisted of repeated cycles of 10 repetitions of the same song (recorded in 1991) followed by a 1-min pause. We used the same song type throughout the study.

STIs were presented in 1991 to 7 low-T birds, 5 empty-implanted birds and 6 unimplanted birds, in 1992 to 9 high-T birds, 5 empty-implanted birds and 2 unimplanted birds, and in 1994 to 7 high-T birds, 2 empty-implanted birds, and 3 unimplanted birds. In 1996 we tested 6 high-T birds with a different song type, for comparison with responses of high-T birds from earlier years. No bird was tested more than once throughout the multi-year study, so all data represent the first STI experienced by each bird on his territory.

Spontaneous Song of Individuals

We counted songs of free-living male Lapland longspurs during 10-30 min focal observations during the display, mate-guarding and incubation stages. An observer walked to the center of the male's "territory" (the area over which the male flew during the display stage), found and identified the male by color bands, and watched him for 30 min or until he went out of sight. Data were recorded as number of songs per 30 min, and were adjusted if necessary for birds that went out of sight before 30 min. If the bird could not be found at all on territory (a common occurrence for Lapland longspurs during incubation), the observer left the territory after 30 min and tried again later. Some birds could not be found despite repeated visits to their territories, and were not included in our analysis. We could not determine whether these birds were off their territories, or whether they were on territory but were not detected.

We alternated focal observations on control males with focal observations on T-implanted males, and observed birds between 0900 and 1830 hours. In 1991, we gathered song rate data for control birds and low-T birds during display, nest-building, laying (the latter two are successive substages of the "mate-guarding" stage), and incubation. We intended to gather similar song rate data for high-T birds in 1994. Unfortunately, a unusual series of snowstorms in late May and mid-June prevented us from measuring mate-guarding and incubation song rates for almost all of our high-T birds, because some birds disappeared, and most of the surviving birds lost their nests.

Spontaneous Song Within Small Populations

The focal observations were hampered by a small sample size of T-implanted birds, and also may have been biased toward birds that sing. Therefore, in 1996 we conducted surveys of overall song occurrence in two locations near Toolik Lake, one with many T-implanted males and one with no T-implanted males. Environmental conditions and Lapland longspur population density were virtually identical at the two locations; thus any difference in song occurrence should reflect differences in the tendency of males to sing. On 7-9 June (early incubation), 10 birds caught on the same hill (the "high-T area") were implanted with three 12-mm T implants in the back. The high-T area was approximately 1 km east-west and 500 m north-south and contained the territories of at least 11 males (8 high-T males and 3 unimplanted males), and possibly 4 other occasionally seen males. We also studied another population on a different hill (the "control area"), which was 1.5 km north of the high-T area. The control area was slightly larger, approximately 1 km east-west and 700 m north-south, and contained the territories of at least 14 unimplanted males and possibly 4 other occasionally seen males. Both survey areas consisted of gently sloping south-facing slopes of *Eriophorum vaginatum* tussocks mixed with dwarf heath shrubs (e.g. *Betula* spp., *Salix* spp., *Vaccinium* spp.; Bliss *et al.*, 1973; Bunnell *et al.*, 1975), and both have supported similar high densities of Lapland longspurs throughout our study. There is an intervening hill between the high-T and control areas, and banded longspurs from one area were never seen in the other area.

Song surveys took place on 11-14 June, which was the middle of incubation for all known nests. A song survey consisted of an observer visiting 10 pre-determined survey sites in each area and counting songs. The survey sites were spaced 150-200 m apart. At each survey site, the observer waited until any alarm calls had ceased, and then for the next 10 min recorded the number of songs heard, the number of males that sang, and the mean number of songs per singing male. Songs heard from the same direction were assumed to be given by the same male, and some males were identifiable by their unique songs. There was occasionally uncertainty about exactly how many males were singing, e.g. 3 versus 4 males. In such cases, we chose the lower number if the survey was in the high-T area and the higher number if the survey was in the control area. All 20 sites were surveyed three times (once between 0500 and 0800 hours, once between approximately 1100 and 1400 hours, and once between approximately 1400 and 2200 hours) and the results were averaged for each site. Song surveys in the high-T and control areas were precisely matched for time of day, and weather conditions were nearly identical in both areas.

Statistical Analysis

We combined the empty-implanted birds and the unimplanted birds into a single "control" group, after testing all variables for effects of year and implant status (we found one year effect on one song variable: 1991 control birds tended to sing less than controls from other years.). Similarly, we combined high-T birds from 1992 and 1994 into a single "high-T" group, after testing for year effects (no differences were found). Thus, our subsequent data analyses included three groups: control, low-T, and high-T. All statistical tests were two-tailed. Data are reported as means ± SEM unless otherwise indicated.

We often sampled different individuals on different schedules. For instance, some birds were captured many times through the study, while others were captured only once or twice. This mixed sampling schedule was unavoidable because some birds were difficult to capture while other birds were unexpectedly recaptured many times. Since birds may theoretically be affected by their previous capture history, a mixed sampling schedule may introduce variation into the data. However, previous capture history of male Lapland longspurs does not affect testosterone, luteinizing hormone, mass, fat, cloacal protuberance length or song rate (K. Hunt, unpublished data), and therefore we think that the use of a mixed sampling schedule is not a major concern for this study.

We performed a log-transformation of the plasma testosterone data (log[X+1]) to remove effects of heteroscedasticity, and then compared plasma T levels among treatment groups with Student's T-test (for birds before implantation), or one-way ANOVA (for birds with implants). Post hoc pair-wise comparisons were done with Fisher's Protected Least Significant Difference test (PLSD). We also analyzed T data for increases or decreases after receiving implants, using Wilcoxon signed rank tests for each of our three treatment groups. We compared cloacal protuberance lengths, mass, abdominal fat and furcular fat among the three treatment groups with one-way ANOVA.

We analyzed continuous behavioral data (time within 5 m, time in threat posture, number of attacks, number of songs during the STI, number of spontaneous songs during focal observations) among the three experimental groups with Kruskal-Wallis tests, corrected for ties, followed by post hoc pair-wise comparisons with Q tests for data with ties (Zar, 1984, p.201). We tested each of the two categorical behavioral variables (song occurrence and aggression occurrence) for effect of experimental group, using X^2 tests on 3x2 contingency tables (Siegel & Castellan, 1988, p.111; Agresti, 1990). We also tested song occurrence and aggression occurrence for correlation with each other using the phi coefficient of correlation for 2x2 tables (Siegel & Castellan, 1988, p. 232). Finally, we analyzed log-transformed T levels in singing versus non-singing birds, and in aggressive versus non-aggressive birds, with Student's T-tests.

Focal observations of song rate were analyzed with Kruskal-Wallis and Mann-Whitney U tests for effect of reproductive stage and implant. Due to the need for independent samples for these analyses, we analyzed only one focal observation from each bird. This reduced the sample sizes for the analysis on effect of reproductive stage (control birds: display, n = 20; mate-guarding, n = 13; incubation, n = 7; low-T birds: display, n = 4; mate-guarding, n = 4; high-T birds: display, n = 4; incubation, n = 4). For the analysis of effect of implant during the display stage, we chose 8 control birds that matched the T-implanted birds for date. For song surveys of the high-T and control areas, we averaged the data from the three surveys of each of the twenty sites, and then analyzed the resulting twenty means with Mann-Whitney U tests for effect of population (high-T versus control).

RESULTS

Typical Patterns of Song, Aggression and Testosterone

The breeding season of male Lapland longspurs is summarized in Figure 2.1 (modified and expanded from Hunt *et al.*, 1995; dates are for a typical year with no severe snowstorms). For the control population used in this study, the mean plasma T level during the display stage, when song is frequent, was 8.40 ± 0.92 ng/ml (median = 8.89 ng/ml, range = 0.19 - 19.97 ng/ml, n = 34), with circulating T in many displaying Lapland longspur individuals reaching 10-20 ng/ml. During mate-guarding, when aggression peaks, testosterone levels were intermediate (mean = 2.57 ± 0.34 ng/ml; median = 1.38, range = 0.14 - 10.99, n = 50; Fig. 2.1). During incubation, testosterone levels declined to a mean of 0.98 ± 0.24 ng/ml (median = 0.46, range = 0.12 - 8.11, n = 37). After hatching, testosterone levels were nearly at baseline (mean = 0.52 ± 0.11 ng/ml; median = 0.30 ng/ml, range = 0.07 - 3.58 ng/ml, n = 38). Males were leaner during the display phase than later in the season (mean mass during display phase = 26.8 ± 0.3 g, n = 45; mean mass during incubation = 27.8 ± 0.2 g, n = 37; t = -0.3000, p = 0.0036).

Plasma Testosterone

T implants raised plasma T levels (Fig. 2.2). Before receiving their implants, T birds and control birds had essentially identical T levels, as expected ($T_{55} = 0.164$, $p = 0.8704$; Fig. 2.2, upper panel). After receiving implants, however, high-T birds, low-T birds and control birds had significantly different plasma T levels from each other ($F_{2,46} = 270.1$, $p < 0.0001$, Fig. 2.2, lower panel). Post hoc comparisons with Fisher's PLSD revealed that high-T birds had higher plasma T than both other groups, and low-T birds had higher T than control birds ($p < 0.0001$ for all three pair-wise comparisons). We also analyzed T levels of just those birds for which we had both a before-implant sample and a with-implant sample. The "before" samples were taken during mate-guarding. Control birds showed a significant decrease in circulating T levels after receiving an implant (Wilcoxon signed ranks test, $Z = 4.015$, $p < 0.0001$, $n = 21$), which was expected due to the normal seasonal decline of T (Hunt *et al.*, 1995). However, high-T birds showed a significant increase in T ($Z = 2.903$, $p = 0.0037$, $n = 12$). Low-T birds showed no change in T, although we had only two low-T birds for this analysis ($Z = 1.342$, $p = 0.1797$, $n = 2$). In general, our high-T birds had plasma T levels during incubation that were at or slightly above a typical T peak of the display phase (Figs. 2.1 and 2.2; Hunt *et al.*, 1995).

Within the control group, empty-implanted and unimplanted birds had similar mean T levels during mate-guarding (empty-implanted: 7.715 ± 1.112 ng/ml; control-implanted: 6.215 ± 1.703 ng/ml) and during incubation (empty-implanted: 0.53 ± 0.117 ng/ml; unimplanted: 0.331 ± 0.095 ng/ml).

Cloacal Protuberance (CP) Length and Body Condition

High-T birds, low-T birds, and control birds had significantly different cloacal protuberance lengths, but did not show differences in body mass, furcular fat, or abdominal fat (Table 1.1). Post hoc pair-wise comparisons with Fisher's PLSD test revealed that high-T birds had significantly larger CPs than did low-T birds ($p = 0.0333$) or control birds ($p = 0.0021$). CP lengths of low-T and control birds were not significantly different from each other, however ($p = 0.7809$).

Behavioural Responses to Simulated Territorial Intrusions

High-T birds, low-T birds and control birds differed significantly in the number of songs given during the 10-min STI, but showed no significant differences for time within 5 m, time in threat posture, or number of attacks (Fig. 2.3; number of songs, $H_2 = 6.592$, $p = 0.0370$; time in 5 m, $H_2 = 4.234$, $p = 0.1204$; time in threat posture, $H_2 = 1.468$, $p = 0.4801$; number of attacks, $H_2 = 2.533$, $p = 0.2818$). The three groups also did not differ in overall aggression occurrence (Table 2.2; $X^2 = 0.978$, df = 2, $p = 0.6133$). Post hoc comparisons revealed that high-T birds sang significantly more often than control birds during the 10-min STI ($Q = 2.555$, $p = 0.022$), but the other two pairwise comparisons were not significant. Analysis of song occurrence showed that high-T birds were also significantly more likely to sing at least once during the 30 min of observations than were either low-T birds or control birds (13 of 15 high-T birds sang, 2 of 7 low-T birds sang and 4 of 22 control birds sang; $X^2 = 17.774$, df = 2, $p = 0.0001$). High-T birds showed a non-significant trend to spend less time within 5m of the decoy than low-T or control birds (Fig. 2.3), possibly because singing birds often left the decoy's vicinity to perform a song flight. Plasma testosterone levels were significantly higher in birds that sang versus birds that did not sing, regardless of experimental treatment (Fig. 2.4; mean T of singers = 18.37 ± 3.79 ng/ml, mean T of non-singers = 3.16 ± 1.45 ng/ml; $t_{29} = 6.397$, $P<.0001$), but plasma testosterone levels were not different in aggressive birds versus non-aggressive birds (Fig. 2.4; mean T of aggressive males = 7.74 ± 2.04 ng/ml, mean T of non-aggressive males = 11.57 ± 3.93 ng/ml; $t_{28} = 0.016$, $p = 0.9874$). Finally, song occurrence and aggression occurrence were not correlated, either for all birds together or for each experimental group separately (Table 2.2).

Spontaneous Song

Control birds and low-T birds showed a significant decline in spontaneous song rate during the breeding season (Table 2.3; control birds: $H_3 = 17.472$, $p = 0.0002$; low-T birds: $U = 16.0$, $p = 0.0209$). High-T birds showed a nonsignificant trend toward a decline (Table 2.3; high-T birds: $U = 11.5$, $p = 0.3065$). The three groups did not differ from each other during display or incubation (display: $H_2 = 3.960$, $p = 0.1381$; incubation: $H_2 = 2.564$, $p = 0.2775$), and low-T and control birds did not differ from each other during nest-building or laying (nest-building: U = 46, p = 0.1908; laying: U = 28.5, p = 0.6651). No high-T birds could be observed during nest-building or laying.

In the song surveys of small populations, the high-T area had significantly more songs and singing males per song site than did the control area (Fig. 2.5; mean number of songs: U = 78.5, p = 0.0311; mean number of singing males: U = 88.0, p = 0.0041). The mean number of songs per singing male was similar in the two populations (Fig. 2.5, upper panel; U = 58.0, p = 0.5453), and alarm calls were equally likely to be heard in both populations (U = 57.0, p = 0.5645).

DISCUSSION

The results suggest that song in Lapland longspurs is strongly T-influenced throughout the breeding season, but that territorial aggression is not. Male Lapland longspurs with high-T implants were more likely than control birds to sing. Such increased song is a classic response of a socially monogamous male passerine bird to exogenous testosterone (e.g. Nowicki & Ball, 1989; McDonald, 1991). High-T birds did not, however, show increased aggression in response to simulated territorial intrusions during incubation. The increased plasma testosterone, CP length, and increased song occurrence in the high-T birds demonstrated that their implants delivered T at a level that was physiologically effective for at least some measures. High-T birds had normal body mass and fat stores, indicating that they probably were not stressed by the high levels of testosterone.

The lack of an effect of T on aggression is striking, because in other species of birds, T implants given during the breeding season generally result in at least some increase in aggression (e.g. Moore, 1984; Hegner & Wingfield, 1987; Beletsky *et al.*, 1995). Exogenous T is especially likely to result in increased aggression if a male is exposed to novel social stimuli, such as a simulated territorial intrusion (Wingfield *et al.*, 1990). The Lapland longspur is notable in that T implants given during the breeding season caused no discernible change in territorial aggression even when males were challenged with an novel intruder. Male Lapland longspurs are capable of aggression, and in fact they are typically very aggressive toward simulated territorial intrusions done a few weeks earlier during mate-guarding (Hunt *et al.*, 1995, Chapter 3). Apparently high T alone is not sufficient to maintain high aggression in this species. Nevertheless, high T may play a subtle role in promoting Lapland longspur aggression; i.e., to display territorial aggression, perhaps a male must experience both elevated testosterone and certain environmental and social cues, such as the presence of a fertile female. It is also possible that neuroendocrinological changes may underlie the Lapland longspurs' lack of aggressive response to testosterone during incubation. For instance, if the neurological circuits that affect aggression reduce their sensitivity to testosterone (or its metabolites), a T level that is sufficient to cause aggression early in the breeding season may become insufficient later in the season. This would still leave the question of why Lapland longspurs have evolved to have T-influenced song, but not T-influenced aggression, late in the breeding season.

Our results suggest that the major role of the Lapland longspurs' very high plasma T peak may be to promote very frequent singing during the display phase, rather than promoting physical aggression *per se*. The Lapland longspurs' natural T peak coincides with the peak in singing, rather than with the peak in aggression, which occurs one to two weeks later (Hunt *et al.*, 1995). Furthermore, our 1996 song surveys showed that a population of high-T birds showed an increase in the number of songs and number of singing males compared to a control population. Song rate differences were not apparent in earlier years when birds were observed individually, possibly because our focal observations may be biased toward birds that sing. The song surveys further suggest that though T may increase a male's tendency to initiate a bout of singing, T may not affect his song rate after he has begun to sing.

If the major role of the T peak in Lapland longspurs is to promote initiation of singing, what, then, is the function of their song? The likely possibilities are attraction of females, advertisement of territory ownership to rival males, or both (Searcy, 1988; McDonald, 1991). The Lapland longspurs' peak in song frequency occurs at the same time as pair formation, suggesting that song may be a courtship signal, but we have been unable to test this hypothesis because of their very brief courtship (24-48 h). Lapland longspur song appears not to be simply part of a suite of "aggressive behaviors", since song occurrence is independent of occurrence of threat posture and attacks (this study), and also because song frequency declines during mate-guarding, when territorial aggression is strongest (Hunt *et al.*, 1995). None the less, Lapland longspur song may be related indirectly to territoriality, perhaps to territory advertisement rather than to the physically aggressive aspects of territorial behavior. This distinction between T-influenced "advertisement" (song or patrolling) and T-independent "physical aggression" has been seen in some other species (Andrew & Rogers, 1972; Wingfield, 1994). Understanding the function of Lapland longspur song in detail will probably require experiments with captive birds or perhaps devocalization experiments in the field.

Adaptations to the Arctic?

Lapland longspurs seem to be different from most temperate-zone passerines in several ways. First, Lapland longspurs have an unusually brief, high T peak. Mean circulating T levels during the display stage is over 8.0 ng/ml, which is well above the typical mean T peak of 2-5 ng/ml seen in most other male passerines studied during the early breeding season (Beletsky *et al.*, 1995). A few other passerines are known to have high average T peaks of about 8.0 ng/ml, such as the Pacific northwest race of the song sparrow, *Melospiza melodia morphna* (Wingfield & Hahn, 1994) and an Appalachian mountain population of dark-eyed juncos, *Junco hyemalis carolinensis* (Ketterson *et al.*, 1991). The significance of unusually high peak T levels in these species is unknown. Second, Lapland longspurs seem to have a high threshold of response to T (at least during incubation). Low-T implants caused no obvious behavioral or physiological effects in male Lapland longspurs during incubation, despite causing an increase in plasma T to about 2.5 ng/ml, a level that would be near-maximal in many non-arctic birds (e.g. Hegner & Wingfield, 1986a; Logan & Wingfield, 1995). Third, even when given high T, Lapland longspurs responded only with song and not with increased aggression. A similar relationship has recently been seen in snow buntings, *Plectrophenax nivalis*, an arctic bird which shows increased song and decreased aggression when given T implants (M. Romero, J.C. Wingfield, and R. Suydam, unpublished data). In contrast, most non-arctic passerines show at least some increase in aggression when given T, although the link with aggression is often subtle and complex (Wingfield *et al.*, 1990).

The similar pattern emerging in snow buntings suggests that the Lapland longspurs' system of a high and brief T peak, with a strong effect of T on song but not on aggression, may somehow be related to breeding in the Arctic. We will speculate here on some possible evolutionary advantages of this system. First, breeding in the Arctic demands unusually rapid and well-timed changes in behavior (Carey, 1988). The Lapland longspurs' high T peak may somehow facilitate their abrupt transition from migration to pair-bonding and territoriality, which takes place in just a few days (West *et al.*, 1968; Hunt *et al.*, 1995). In contrast, many temperate-zone species have a much more gradual transition into pair-bonding and territoriality, with a pre-nesting period lasting several weeks (e.g. prairie warbler, *Dendroica discolor*, Nolan, 1978; savannah sparrow, *Passerculus sandwichensis*, Wheelwright & Rising, 1993; also see Immelmann, 1971). Second, once breeding is under way, the brevity of the Lapland longspurs' T peak and their high threshold of sensitivity to T may enable males to limit intensive song display to just a few days. Frequent aerial song displays are undoubtedly energetically expensive, and displaying males are lean (this study). Third, the uncoupling of aggression from high T, such that aggression is seen only during mate-guarding, may indicate that Lapland longspurs gain few benefits from aggression at other times. The arctic tundra in summer offers unlimited nest sites for these ground-nesting birds, food is abundant from early June on, and females do not re-nest after mid-June (Seastedt & MacLean, 1979; Tryon & MacLean, 1980; Custer *et al.*, 1986). The reduced benefits of late-season aggression may explain the almost total lack of aggression shown by male Lapland longspurs after incubation has started, even when exposed to high levels of testosterone.

In summary, high-testosterone implants cause male Lapland longspurs to sing, but not to become aggressive, when their mates are incubating. Low-testosterone implants also increase plasma T levels, but to a lower level that does not affect CP length, song, or aggression. Lapland longspurs offer an interesting system for studying the differing roles of testosterone on aggression and on song, and for understanding the ecological factors that may affect the relationship between hormones and behavior. We speculate that the hormone-behavior patterns demonstrated in this study may be adaptations for breeding in the Arctic. If so, other monogamous arctic birds should show similar patterns, and perhaps so would monogamous temperate-zone birds that have "arctic-like" hormone profiles or breeding behavior (e.g. birds with unusually high T peaks, or birds with very short courtship). Phylogenetic factors may be investigated with studies of other *Calcarius* species, such as the Chestnut-collared Longspur, *C. ornatus*, and McCown's Longspur, *C. mccownii*, neither of which breed in the Arctic. These comparative studies will clarify whether the Lapland longspurs' hormone-behavior relationships are indeed adaptations for breeding on the arctic tundra.

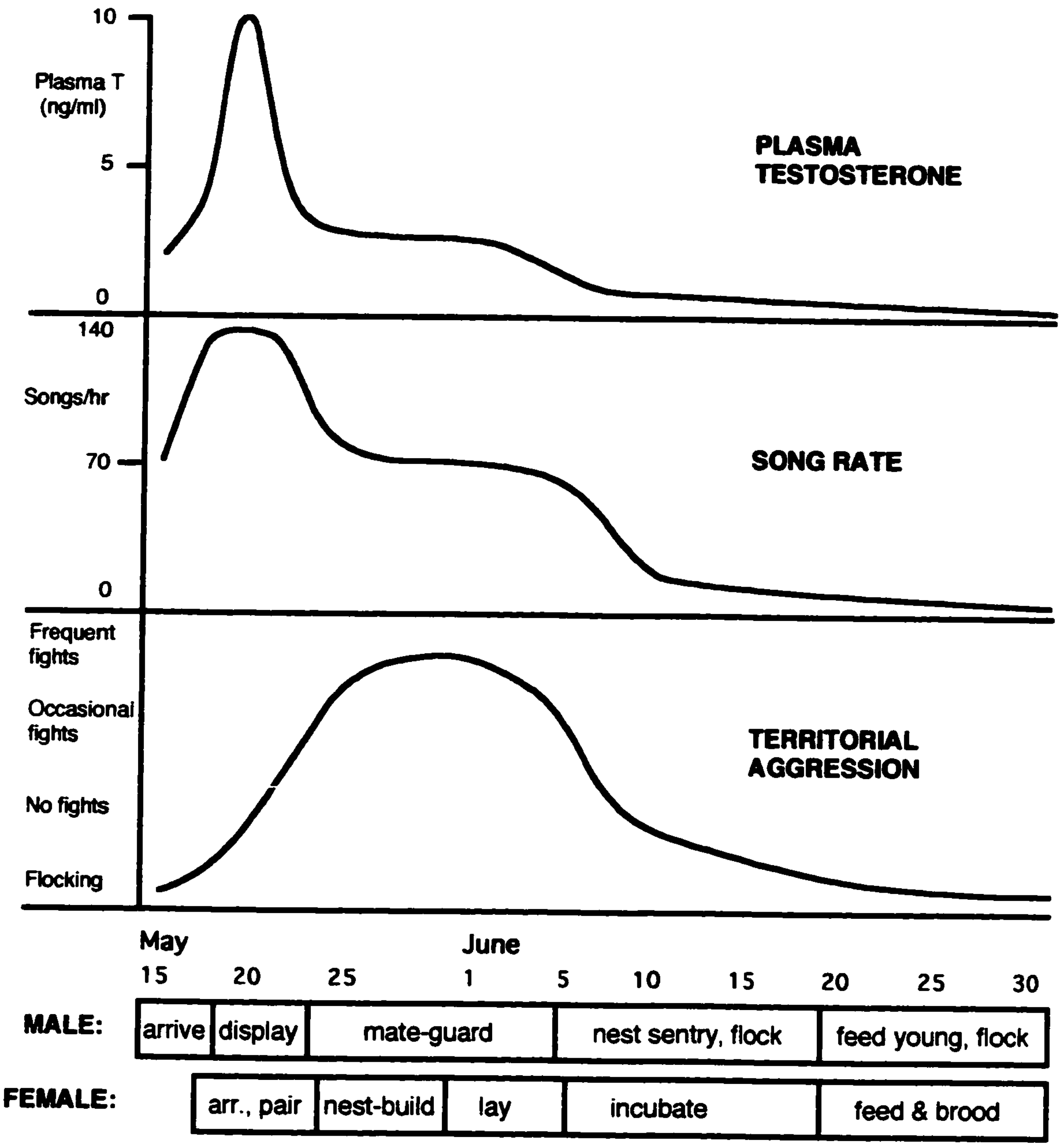

FIGURE 2.1. A typical Lapland longspur breeding season. Plasma testosterone, song rate, and territorial aggression of male Lapland longspurs are shown schematically, with typical activities of male and female Lapland longspurs shown at bottom. Testosterone and song rate data are from observations of spontaneous song in control males presented in this paper, and aggression data are from Hunt et al. (1995). Dates are for a typical year with no severe snowstorms.

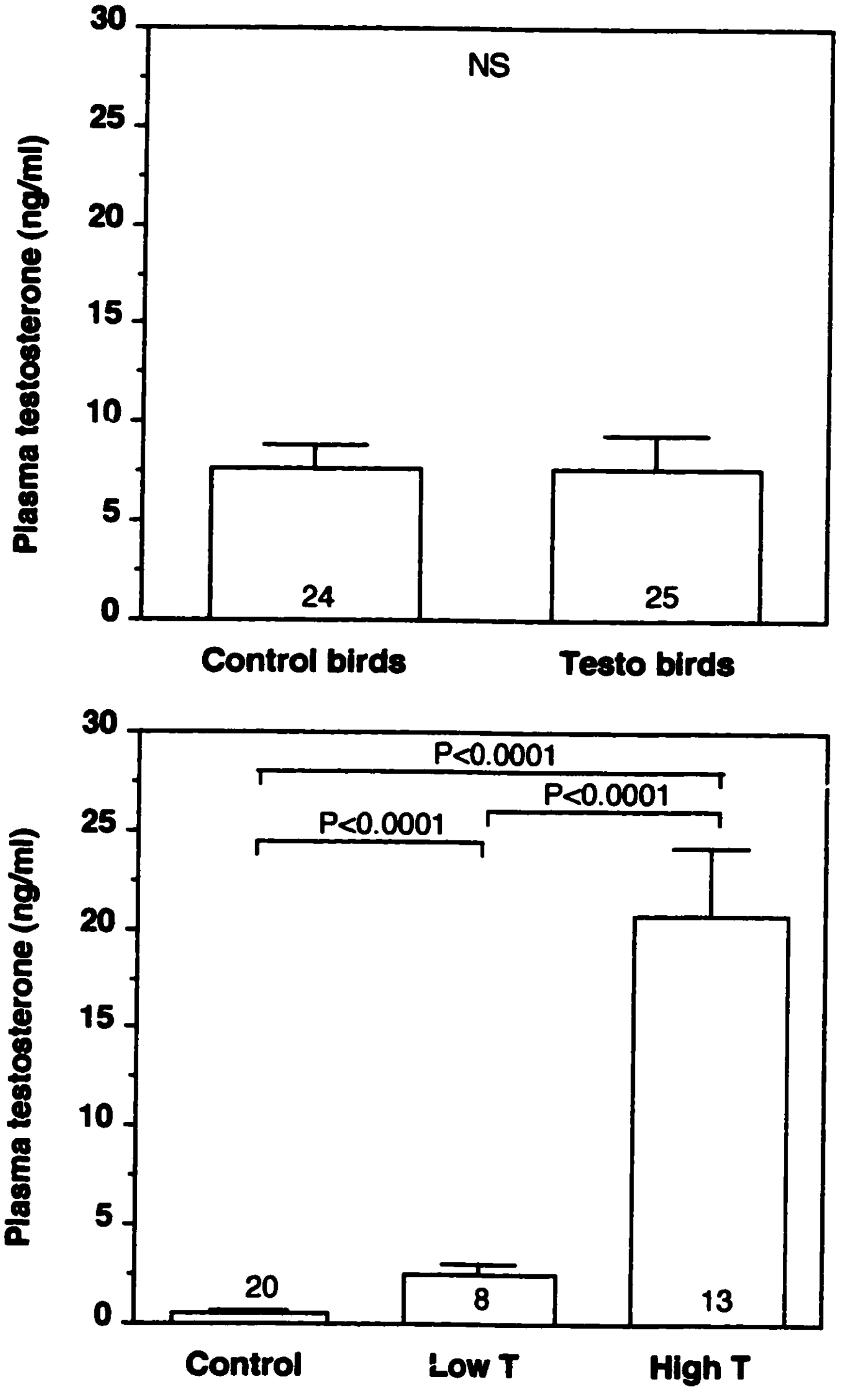

FIGURE 2.2. Top: Mean testosterone levels (± SEM) during mate-guarding in male Lapland longspurs before receiving implants. Bottom: Mean testosterone levels (± SEM) during incubation in male Lapland longspurs implanted with control, low-testosterone, or high-testosterone implants. Sample sizes are indicated at bases of bars. NS = not statistically significant.

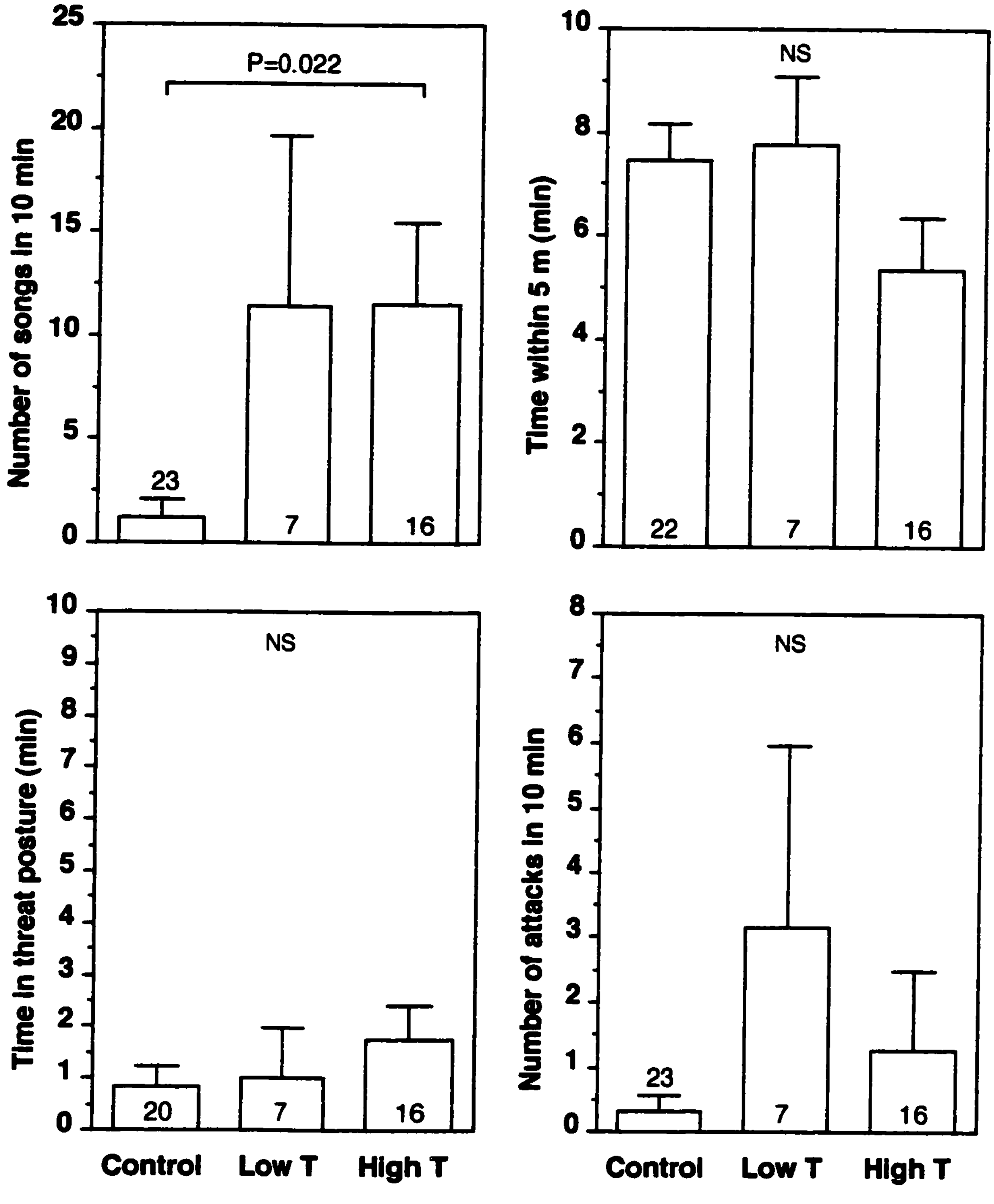

FIGURE 2.3. Behavioral responses of resident male Lapland longspurs implanted with control, low-testosterone, or high-testosterone implants, tested during incubation with simulated territorial intrusions. Males were presented with a live decoy and playback of conspecific song, and responses were recorded for 10 min. Bars are means ± SEM, with sample sizes indicated at bases of bars. NS = not statistically significant.

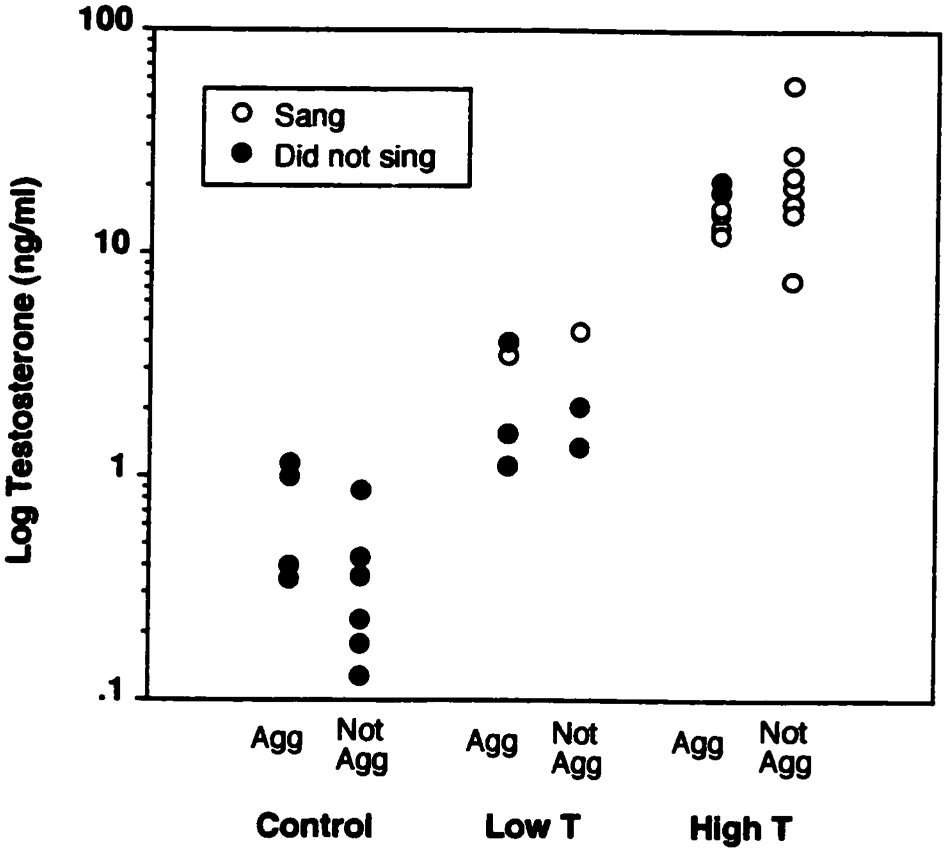

FIGURE 2.4. Plasma testosterone levels (logarithmic scale) of male Lapland longspurs with control, low-T, or high-T implants, in relation to aggression occurrence and song occurrence during simulated territorial intrusions. "Agg" = showed threat posture or attacked. "Not Agg" = never showed threat posture and never attacked.

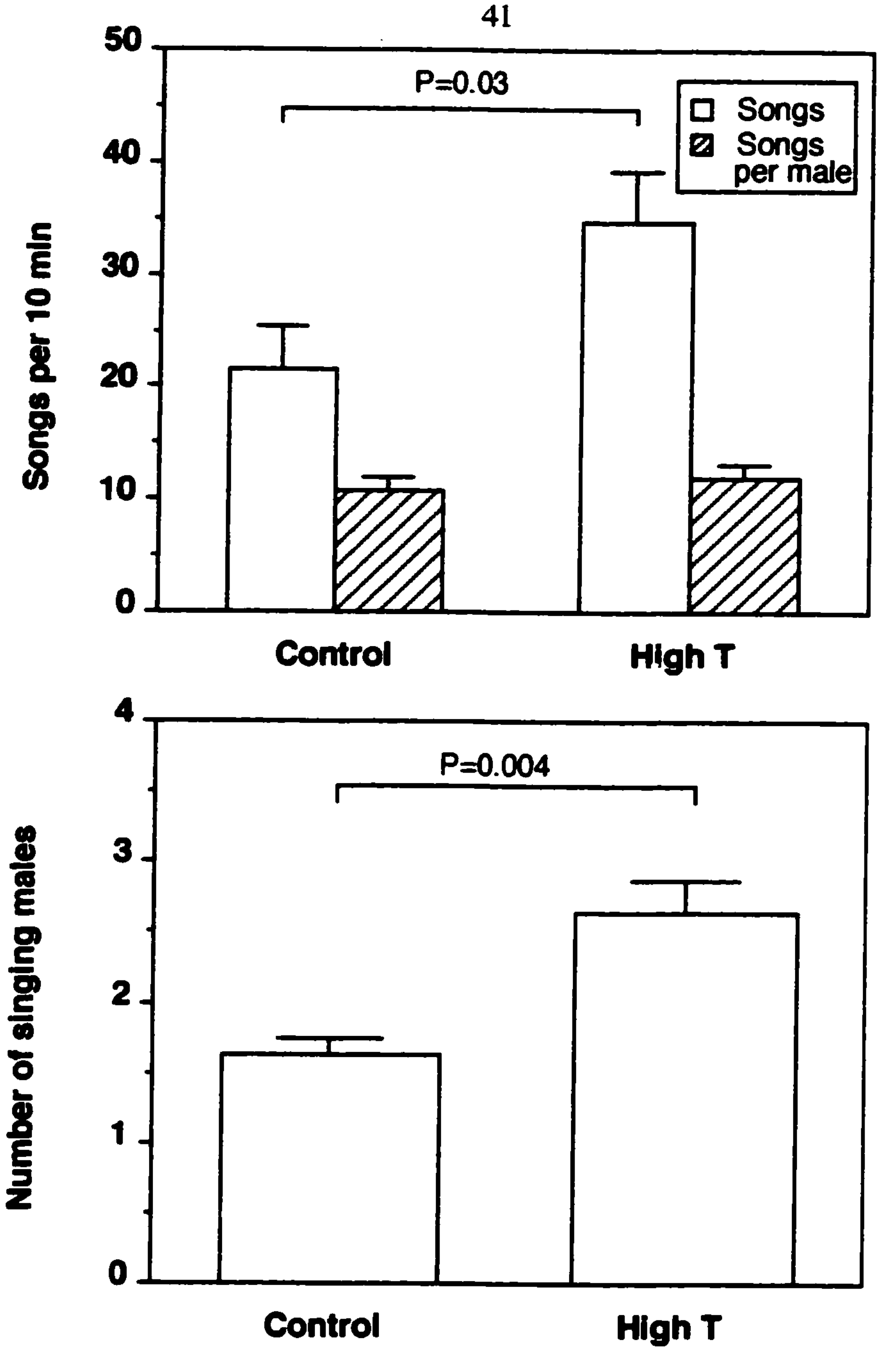

FIGURE 2.5. Results of 10-min song surveys of two populations of Lapland longspurs. Upper panel: mean number of songs heard, and mean songs heard per singing male (± SEM). Lower panel: mean number of singing males heard per site (± SEM). The "High T" population consisted mostly of high-testosterone-implanted male Lapland longspurs, while the "Control" population consisted of unimplanted males. Sample size = ten sites per population. Each site was surveyed three times and the results were averaged. P-values from Mann-Whitney tests are shown at top.

Table 2.1. Cloacal protuberance (CP) length, body mass and fat scores of male Lapland longspurs with control implants, low-testosterone implants, or high-testosterone implants. Control birds for this analysis were selected to match T-implanted birds for date and time of day. Mass and fat data were not available for all birds. Fat was scored on a scale of 0 = no fat to 5 = obese (Wingfield and Farner 1978). Data are shown as means ± SEM, with sample sizes below in parentheses. Bottom two rows show F-values (degrees of freedom in parentheses) and P-values from one-way ANOVA for effect of implant.

	CP length (mm)	Body mass (gm)	Furcular fat	Abdominal fat
Control	6.14 ± 0.20 (22)	27.43 ± 0.38 (13)	1.20 ± 0.25 (10)	1.40 ± 0.27 (10)
Low-T	6.25 ± 0.37 (8)	27.22 ± 0.32 (9)	1.44 ± 0.38 (9)	1.11 ± 0.25 (9) (9)
High-T	7.18 ± 0.25 (17)	28.10 ± 0.38 (13)	1.52 ± 0.21 (11)	1.46 ± 0.35 (11)
F	5.570 (2,44[c])	1.510 (2,32)	0.427 (2,27)	0.399 (2,27)
P	0.0060	0.2362	0.6566	0.6750

Table 2.2. Aggression and song occurrence in male Lapland longspurs observed during simulated territorial intrusions. One control bird and one high-T bird were excluded from this table due to missing song or aggression data. R_ϕ = phi coefficient of correlation for 2x2 tables. P-values are from Fisher exact tests.

	Aggressive		Not aggressive				
	Sang	Did not sing	Sang	Did not sing	R_ϕ	X^2	P
Control males	1	7	3	10	0.131	0.359	>0.9999
Low-T males	1	3	1	2	0.091	0.058	>0.9999
High-T males	5	2	8	0	0.419	2.637	0.2000
All T males	6	5	9	2	0.293	1.886	0.2125
All males	7	12	12	12	0.132	0.307	0.5380

Table 2.3. Number of songs per 30 min of focal observation of individual male Lapland longspurs with control (or no) implants, low-testosterone implants, or high-testosterone implants, at different stages of the breeding cycle. Low-T and high-T birds received their implants after the display stage observations and before any subsequent observations. High-T birds were not observed during nest-building and laying due to inclement weather that caused nest failure. Each bird was observed only once during a single reproductive stage; some birds were observed during several stages.

Group	n	Mean ± SEM	Median	Range
Control males				
Display	31	61.1 ± 7.2	63.0	0-150
Nestbuild	12	26.2 ± 8.4	8.0	0-83
Lay	10	17.3 ± 5.4	15.0	0-50
Incubate	11	8.1 ± 4.4	0.0	0-40
Low-T males				
Display	3	102.0± 16.1	107.0	72-127
Nestbuild	6	54.8 ± 14.3	55.5	4-100
Lay	5	16.0 ± 8.5	11.0	0-49
Incubate	2	4.0 ± 4.0	4.0	0-8
High-T males				
Display	3	30.7 ± 21.9	19.0	0-73
Incubate	7	21.4 ± 14.1	3.0	0-104

CHAPTER 3. The Effect of Testosterone Blockers on Aggression, Song and Nest Success of Male Lapland Longspurs

INTRODUCTION

Testosterone (T) has long been linked with territorial aggression during the breeding season in male vertebrates, but the connection between circulating T and breeding-season aggression is controversial (reviewed in Harding, 1983; Wingfield & Farner, 1993). In some species, male territorial aggression during the breeding season is strongly T-dependent, while in others, factors such as age, experience, and the current social situation are important in addition to T level (e.g. Hegner & Wingfield, 1986; Ramenofsky, 1984; Schwabl & Kriner, 1991; Collis & Borgia, 1992). In fact, in many situations, aggression can be independent of plasma T level (Hegner & Wingfield, 1986; Schwabl & Kriner, 1991). A further subtlety is that aggressive behavior itself often causes an increase in plasma T (Wingfield et al., 1990). This complex relationship of T and aggression offers an ideal system for studying the costs and benefits of having a given behavior under hormonal control. We can ask why aggression is strongly T-dependent in some species, or in some situations, and T-independent in others. If we can understand how these patterns relate to each species' natural environment, we may better understand the adaptive significance of common patterns of T secretion, the different possible relationships of T and aggression, and the methods by which vertebrates can modulate the relationship of hormones with behavior.

One factor that may provide some illumination into these issues is the duration of territorial aggression, which is often reflected in the length of the breeding season. For example, in temperate latitudes, seasonally breeding birds have breeding seasons that are several months long with prolonged territorial defense. In these species, territorial aggression is often activated by T, and T implants may result in an increase in various measures of aggression and territorial defense (e.g. Hegner & Wingfield, 1987; Wingfield & Farner, 1993). In contrast, subtropical and tropical vertebrates often have an extremely long breeding season with nearly year-round territorial behavior. In these tropical species, T's link with aggression is often weak or nonexistent, presumably because there is little advantage to putting a behavior under tight regulatory control by hormones if that behavior is expressed year-round (Levin & Wingfield, 1992). Hormonal control appears to be most useful for behaviors that vary over the year, since a change in the level of the hormone can serve as a cue to change the expression of the behavior. For behaviors that do not vary over the year, such a cue would be unnecessary (Levin & Wingfield, 1992).

One further category has not been investigated, however: species with breeding seasons that are extremely short, with a very brief expression of aggression and territorial behavior. The Lapland longspur (*Calcarius lapponicus*), a tundra-nesting passerine bird, is an example of such a species, with a breeding season only six weeks long. Males show rapid changes in courtship and territorial behavior, particularly during the first two weeks on the breeding grounds (for a full description see Hunt et al., 1995). Males also have a correspondingly abbreviated T profile, with a remarkably high, brief T peak during the first few days on the breeding grounds (mean T = 8.40 ± 0.92 ng/ml). During this T peak, males engage in numerous song flights but only rarely fight other males. After these first few days, T levels drop to intermediate levels (mean = 2.57 ± 0.34 ng/ml), but aggression increases - most males begin to guard their fertile females, and are highly aggressive toward rival males. When females begin incubating eggs, T levels drop further to near-baseline levels (0.98 ± 0.24 ng/ml), and males stop guarding their territories entirely and virtually cease aggressive behavior toward other males. These unusually rapid behavior changes, and the unusually pronounced swings in the T levels, raise the question of whether T promotes aggressive and territorial behavior in this species, as in most temperate-zone birds, or whether aggression is mostly T-independent, as in tropical birds.

Testosterone implant experiments in Lapland longspurs indicate that high levels of exogenous testosterone do not increase territorial aggression during incubation, though they do increase singing (Chapter 2; Hunt et al., 1995). These results demonstrate that high T alone is not sufficient to increase territorial aggression, at least not during incubation. In this respect, these arctic birds are reminiscent of tropical birds. However, it would be premature to conclude that T is not involved in territorial aggression at all in Lapland longspurs. For example, T might be necessary, but not sufficient, for territorial aggression. Another possibility is that T might be involved in aggression during mate-guarding, but not later during incubation. Or, a low level of T may be sufficient for normal levels of aggression, while the higher levels of T provided by the implants may have had no additional effect on aggression.

To investigate the possibility that T may be involved in territorial aggression in male Lapland longspurs in one of these subtle ways, we must experimentally remove T or block the effects of T, and then test for any deficits in behavior. Generally, such experiments use one of two techniques: gonadectomies or chemical blockers. Gonadectomy is quite simple in concept, consisting of surgical removal of the testis, which is the primary source of testosterone. However, it is technically difficult to perform gonadectomies in the field (though it can be done, e.g. Wingfield, 1994). Furthermore, gonadectomies are irreversible (when done correctly), which is a particular concern when working with wild animals. For these reasons, field studies generally rely on the second technique, use of chemicals that block the effects of testosterone. Since testosterone can affect behavior in at least two ways (directly via androgen receptors, and indirectly via aromatization to an estrogen, which then interacts with various estrogen receptors), both of these pathways must be blocked to effectively inhibit T's behavioral effects (e.g. Alexandre & Balthazart, 1986, Harding et al., 1988, Beletsky et al., 1990, Schlinger & Callard, 1990).

In this chapter, we describe a blocker-implant experiment on free-living male Lapland longspurs, designed to test the possibility that T may be necessary for normal territorial aggression during mate-guarding. We gave implants of an androgen receptor blocker and an aromatase blocker to male Lapland longspurs a few days after arrival at the breeding grounds. The next week, during mate-guarding, we measured their response to simulated territorial intrusions (STI). If T is indeed not necessary for aggression during mate-guarding in Lapland longspurs (e.g. if Laplands are like tropical birds), then blocker birds should exhibit normal levels of aggression. However, if some level of T is necessary for normal aggression (e.g. if Laplands are like temperate-zone birds), then blocker birds should exhibit reduced levels of aggression. In addition, we investigated whether control birds experience a rise in T in response to an STI, as occurs in many other species.

METHODS

Study Area & General Methods

We studied male Lapland longspurs near Toolik Field Station (North Slope Borough, Alaska, 68° 38'N, 149° 38' W) in 1992 and 1994. Birds were captured in Potter traps baited with seed. Each bird was assigned a unique combination of colored leg bands and a numbered Fish & Wildlife Service aluminum band. We measured body mass to the nearest 0.1g, and scored furcular and abdominal fat stores on a scale from 0 to 5 (as in Wingfield & Farner, 1978b, with 0 = no fat and 5 = bulging fat deposits). We measured the length of the cloacal protuberance (CP) to the nearest mm (as in Hunt et al., 1997).

Body mass, fat score, and cloacal protuberance length were measured both before and after the birds received implants. The "before implant" measurements are from the display stage, while the "with implant" measurements were taken a mean of 7.247 ± 0.542 days later, during the mate-guarding stage when simulated territorial intrusions were performed. For blocker birds, the "with implant" measurements were taken a mean of 5.722 ± 0.699 days after the birds received their blocker implants.

Implants

I gave ATD (1,4,6-androstatriene-3,17-dione, Steraloids Inc.) and flutamide (α-α-α-trifluoro-2-methyl-4'-nitro-m-propionotoluidide, CGS #16949A) implants to eleven males in 1992 and to ten males in 1994. We refer to these birds as "blocker birds". Each implant consisted of crystalline ATD or flutamide packed into a 12-mm length of Silastic tubing and sealed at the ends with Silastic medical adhesive. Each blocker bird received one ATD implant on the left side, and one flutamide implant on the right side. Empty Silastic implants were given as controls to eight birds in 1992 and eight birds in 1994. In both years, a few males were left unimplanted.

I chose ATD and flutamide because 1) in combination they effectively block both of the two major known pathways by which T affects behavior, 2) they can easily be administered in Silastic implants, and 3) previous experiments indicate that ATD and flutamide are effective androgen blockers in birds (e.g. Archawaranon & Wiley, 1988; Beletsky et al., 1990; Schlinger & Callard, 1990; Schwabl & Kriner, 1991;Vleck & Dobrott, 1993). ATD is an aromatase blocker that also acts to some degree as an androgen receptor blocker (Kaplan & McGinnis, 1989). Flutamide is an androgen receptor blocker (Peets et al., 1975). Note that though both chemicals are thought to block intracellular androgen receptors, it is not known whether either chemical interacts with any membrane androgen receptors which might exist. Both these chemicals will diffuse slowly across the walls of a Silastic implant, resulting in slow release from the implant for at least two weeks (e.g. Beletsky et al., 1990). This is in contrast to most other commonly used androgen blockers, which tend to diffuse out of an implant extremely rapidly, e.g. within one day. One example is fadrozole, a highly specific and effective aromatase blocker often used in lab experiments. At the time of this experiment, fadrozole could only be administered effectively by daily injections, making it a poor choice for field experiments. Fadrozole may now be used in the field with short-acting mini-osmotic pumps, but the pump's effects are probably limited to about one week (K. Soma, pers. comm.).

Blood Sampling & Radioimmunoassay

Blood samples were taken from birds on initial capture, and on any subsequent captures that occurred 7 or more days after any previous blood sample was taken. Because corticosterone level rises sharply within minutes after capture, we monitored traps closely. Whenever birds were captured, we took blood samples as soon as possible, and noted the elapsed time between capture and blood sampling. We used heparinized microhematocrit tubes to draw up 250-300 ul of blood from a small puncture in the alar vein, and then stored the tubes temporarily with ice packs in small field coolers. Blood samples were centrifuged within six hours, and plasma was drawn off and frozen at -20C.

We assayed plasma samples for testosterone and corticosterone using the radioimmunoassay protocol of the Wingfield laboratory, University of Washington. A small amount of radiolabelled testosterone and corticosterone was added to every sample to determine percentage recovery after extraction and separation. Steroids were extracted from plasma with dichloromethane, and were separated on diatomaceous earth/ glycol micro columns. The fractions containing progesterone, dihydrotestosterone, and estradiol were discarded. The testosterone and corticosterone fractions were collected and assayed as described in Ball & Wingfield, 1987 and Wingfield et al., 1991. Intra- and inter-assay variations for these assays have been reported previously (Hunt et al., 1995).

Extra plasma, if any, was saved and assayed for luteinizing hormone (LH) with a post-precipitation, double-antibody radioimmunoassay (Follett et al., 1972; Follett et al., 1975; for details see Wingfield et al., 1991). This assay has been used successfully in a wide variety of bird species, and plasma from photostimulated female Lapland longspurs generates an inhibition curve that parallels the standard curve (data not shown). All samples were run in the same LH assay to eliminate inter-assay variation. Intra-assay variation was less than 10%.

Comparison of Plasma Testosterone

Blocker implants may block the negative feedback of T at the pituitary or hypothalamus, thereby causing an increase in plasma T levels. Though blocker implants can be effective without causing this increase (e.g. Searcy & Wingfield, 1980, Schwabl & Kriner, 1991), if an increase in T does occur, it is a good sign that the implants are working effectively. For comparison of plasma T levels in blocker birds vs. control birds, we analyzed plasma samples that were taken from blocker birds within 20 days of implanting. (Plasma samples that were taken directly after a simulated territorial intrusion were not included in this analysis.) If multiple samples were available from the same bird, we selected the sample that was closest to ten days after implanting. Since T level varies dramatically with date in this species (Hunt et al., 1995), samples from control birds were chosen to match the blocker samples as closely as possibly for date and, secondarily, for time of day. One blocker bird could not be captured for blood sampling. The final sample sizes for this comparison were nine blocker birds and nine control birds (seven from empty-implanted birds and two from unimplanted birds). The blood samples from the nine blocker birds were taken a mean of 12.33 ± 1.55 days after implanting (range of 6 - 19 days), and the samples from the seven empty-implanted birds were taken a mean of 11.29 ± 0.87 days after implanting (range 8 - 14 days). All nine of these blocker birds had known territories, and six had known nests. Eight of the nine control birds had known territories, and at least five (probably seven) had nests.

Blocker implants may also cause upregulation of luteinizing hormone (LH), due to release from negative feedback. Some samples could not be assayed for LH due to small plasma volumes, however, and therefore sample sizes for this LH comparison were small (n = 5 for each group).

Territorial Aggression

Territorial aggression was measured with simulated territorial intrusions (STI), a technique in which the resident male is challenged with presentation of a live caged decoy and playback of conspecific song, and the resident's behavior is monitored for ten minutes. (See Hunt et al., 1997 for details of the STI procedure.) We scored the following five behaviors: 1. Time spent within 5m, a measure of interest in the decoy, but not necessarily of aggression. (Previous studies indicate that time within 5m is not correlated with measures of aggression in this species; see Hunt et al., 1997). 2. Time spent in threat posture, where threat posture is defined as the body held horizontally, bill tilted up, and wings slightly drooped, as in Drury, 1961. 3. Number of attacks, where an attack consists of physical striking of the decoy's cage with the bill or feet. We consider these last two measures, threat posture and attacks, to be measures of aggression. In addition to analyzing each of these measures separately, We also combined these measures into a single measure of aggression, "aggressive score", calculated as follows. Each bird was ranked with all other birds for time spent in threat posture, and was also ranked with all other birds for number of attacks. A bird's aggressive score is the sum of the two ranks. Note that the aggressive score is a combination of two relative measures, and thus only indicates a bird's aggressiveness relative to the other birds in the study. 4. Number of songs. We consider this to be a measure of singing behavior only, and not necessarily of aggression. All birds were also scored for whether or not they sang at all. 5. Number of low flights. This category includes only those flights that are more or less horizontal, thus excluding the high "song flights" used by displaying males. In this species, low flights during an STI do not appear to represent aggression (Hunt et al., 1995), but instead may be thought of as a measure of the bird's general activity level.

Final STI sample sizes were ten blocker birds and 15 control birds. STIs were presented to 11 blocker birds (seven in 1992 and four in 1994), but one blocker bird's data were unusable due to problems with data collection. Eight of the blocker birds for which we have STI data had known nests. An additional seven blocker birds (four in 1992 and three in 1994) could not be found on territory when scheduled for an STI, and were classified as "not found" (Table 3.1). They were not included in the analysis of STI data. At least three of these "not found" birds had nests and/or were seen on their territories later in the season. STIs were also presented to 15 control birds, ten in 1992 (eight empty-implanted and two unimplanted) in 1992, and five in 1994 (two empty-implanted and three unimplanted) in 1994. Eight of the 15 control birds had known nests. All control birds were found on territory when they were scheduled for STI.

Effect of a Territorial Challenge on T Level

To investigate whether a territorial challenge can cause a short-term elevation in T level in Lapland longspurs, We compared T levels of control males who were captured immediately after a simulated territorial intrusion ("STI" group) to control males that had not experienced an STI ("no-STI" group). "STI" males were captured in unbaited traps placed by the live decoy after a 10-minute STI; sample sizes were 16 males during mate-guarding, and 9 males during incubation. "No-STI" males were captured in traps baited with seed, and were selected to match the STI birds as closely as possible for year, date, time of day, and bleeding time. Sample sizes for "no-STI" males were 14 birds captured during mate-guarding and 18 birds captured during incubation. Birds tested during mate-guarding were different individuals than those tested during incubation. Note that this analysis did not include any blocker birds. All STIs for this analysis took place in May or June of 1991-1994.

Nest Success & Nestling Growth

To assess any differences in nest success, blocker and control groups were compared for clutch size, hatching success, fledging success, and cause of nest failure. All nests were checked daily and number of eggs or nestlings noted. Nestlings were weighed daily with a portable digital scale accurate to 0.01g. We averaged masses of nestlings from the same nest, resulting in a mean nestling mass for each nest for each day.

Focal Observations

We did focal observations to examine the possiblity that blocker implants might decrease behaviors other than song and aggression. In general, if blocker birds show reduced aggression, the low aggression might be due to a non-specific reaction to the compounds in the implant, rather than to a specific anti-androgen effect. For example, if ATD or flutamide makes a blocker bird "feel sick" -- independent of any anti-androgen actions -- the blocker bird might respond by reducing his general activity level. This can result in a decrease in many behavior measures, including aggression, but such a decrease would *not* be due to a specific anti-androgen effect. In this situation, many other behaviors, such as preening and flying, might also be decreased, and this may be revealed by focal observations. However, if the blockers' effects are primarily due to specific anti-androgen actions, then behavioral deficits should be limited to those behaviors which are *a priori* suspected to be T-dependent (e.g. song, aggression), and other behaviors (e.g., preening, foraging) should not be decreased.

During a focal observation, we watched the bird from a distance of at least 30m, and recorded any songs, flights, preening, foraging, and any interactions with females or other males. Most focal observations were 30 minutes long, but some focals done early in 1992 were 20 minutes long. Behavioral results from the 20-minute focals were later standardized to rates per 30 minutes. Blocker birds and control birds did not differ in the the mean duration of focal observations. Data from a separate study indicate that once Lapland longspur males have been observed for about 10-15 minutes, further increases in observation time do not reveal noticably different patterns of behavior (K. Hunt, unpublished data).

Singing behavior was recorded as number of songs per 30 minutes, number of song flights per 30 minutes (flights in which at least one song occurred), and the percentage of time during which the bird sang. Birds were also scored for percentage of time spent near a female, percentage of time spent near any other males, and percentage of time spent foraging. We also noted the number of "spontaneous flights", which are flights during which the male did not sing, and which were not obviously related to presence of another bird (e.g., this excluded flights to follow the female or to chase another male). Finally, birds were also scored as to whether or not they were following the female closely (within 10m of the female for 75% or more of the time), whether or not they exhibited any aggression toward another male, and whether or not they preened.

Behavior was lumped into 1-minute bins for time spent foraging, time spent singing, time spent with a female, and time spent with other males. Grouping behavior in time bins in this way amplifies the apparent time spent doing brief activities (e.g. if a bird preened once in a given minute, it was scored as spending the entire minute preening), but still allows comparisons of experimental animals with control animals. In the results section, these data are reported as the "percentage of time" spent doing the behavior. Strictly speaking, this is actually the percentage of one-minute bins during which the behavior occurred. (The percentage of real time during spent doing the behavior is typically lower.) Since more than one behavior may occur in a given minute, these percentages may add up to more than 100%.

In 1992, we did focal observations on ten blocker birds and eight control birds. Birds were scheduled for focal observations once during mate-guarding and again during incubation. If the bird could not be found after thirty minutes of searching, the bird was scored as "not found" and was not included in the analysis of focal observation data. A bird that was "not found" may have been present on territory, but silent and inactive. Alternatively, he may have been temporarily off-territory. In 1994, few birds could be observed due to unusual inclement weather; most nests failed, many birds abandoned their territories, and poor visibility hampered efforts to locate birds. Only one blocker bird and one control bird were observed in 1994, both during a spell of good weather near the end of incubation.

Statistical Analysis

We combined the empty-implanted birds and the unimplanted birds in a single "control" group, after testing to confirm that they did not differ noticably in any measure of physiology or behavior. (See Hunt et al., 1995 and Hunt et al., 1997 for further evidence that empty-implanted and unimplanted Lapland longspurs typically differ very little, if at all, in physiology or behavior.) Thereafter, statistical tests on the effect of blocker implants were done on two groups, "blocker" and "control".

Behavioral responses to STI, focal observations, and hormone levels were compared with Mann-Whitney U-tests. Mass, fat, CP length, and nestling weights were compared with Student's T-tests. Differences in proportions (such as aggressive vs. not aggressive) were scored with Fisher exact tests. Nestling growth data were analyzed with repeated-measures ANOVA on those nests in which nestlings survived until at least day 7 after hatch. There were no differences in year, date, or time of day for any blocker vs. control comparisons reported in this study (p's >0.50; data not shown). Means are reported ± SEM. All tests were two-tailed.

RESULTS

Plasma Testosterone, Corticosterone, & Luteinizing Hormone

Testosterone was significantly higher in blocker birds than control birds (Fig. 3.1; $t_{16} = 2.188$, $p = 0.0439$). This indicates that the blocker implants were working effectively. Blocker implants probably increased T levels by either blocking the negative feedback of T on the pituitary and/or causing a "build-up" of circulating T due to the blocking of aromatase.

No differences were seen in corticosterone or in luteinizing hormone between blocker vs. control birds (Table 3.2; corticosterone, $U = 41.0$, $Z = 0.044$, $p = 0.9648$; luteinizing hormone, $U = 18$, $Z = 1.149$, $p = 0.2506$). Time until bleeding was similar in both groups, with median times under ten minutes (Table 3.2; $U = 48.5$, $Z = 0.712$, $p = 0.4767$).

Body Mass and Fat Score

Before receiving implants, blocker birds were slightly but significantly lighter than control birds (Table 3.3; $t_{46} = 2.253$, $p = 0.0291$). The two groups had similar body mass after implanting, when behavioral observations were done (Table 3.3). No differences were seen in mean fat score of blocker vs. control birds, either before or after implanting (Table 3.3). When the analysis of pre-implant body mass was limited to just those birds for whom we have behavioral data, body mass of blocker vs. control birds was not significantly different (blocker birds = 25.921 ± .351g, control birds = 26.744 ± .430 g, $t_{21} = 1.477$, $p = 0.1545$).

The cause of the unexpected difference in before-implant body mass is presumably chance sampling error, i.e., the birds given blocker implants happened to be slightly lighter than controls. Further investigation revealed that blocker birds show a slight, nonsignificant trend to have smaller winglengths than control birds, so it is possible that the blocker birds were slightly smaller in overall size than were control birds (winglength of blocker birds = 92.3 ± 2.591, n = 17; control birds = 93.125 ± 4.925, n = 19; t_{34} = 1.370, p = 0.1797). There were no apparent differences in age, e.g. both groups contained similar numbers of birds banded in previous years (data not shown).

The blocker birds gained mass as the season progressed, while mass of control birds did not change significantly over time (paired T-tests: blocker birds, t_{17} = 2.512, p = 0.0224; control birds, t_{18} = 1.320, p = 0.2035). These changes in body mass were not reflected in fat score; neither group showed a change in mean fat score as the season progressed (paired T-tests: blocker birds, t_{17} = 1.283, p = 0.2168; control birds, t_{18} = 0.506, p = 0.6189). Further analysis of the body mass data revealed that regardless of implant, birds that are light in body mass during May tend to gain weight during June, while birds that are heavier during May tend to maintain their weight through June (data not shown). Therefore, the gain in body mass of blocker birds is probably an artifact of the blocker birds being, by chance, unusually light before receiving their implants, and is probably not an effect of the blocker implants themselves.

Cloacal Protuberance Length

There were no differences in cloacal protuberance (CP) length between groups, either before or after implanting (Table 3.3). However, there was an effect of time on CP length, as is typical for Laplands during this stage of breeding. Birds of both groups showed a significant increase in CP length during the experiment, from display (before implant) to mate-guarding (with implant) (paired T-tests; blocker birds, t_{17} = 3.449, p = 0.0031; control birds, t_{18} = 2.446, p = 0.0249). The lack of difference in CP length may indicate either that the blocker implants were not affecting peripheral androgen receptors, or that CP length had not yet responded to the change in effective androgen level. Changes in CP length can lag behind changes in testosterone level (Salt, 1954).

Territorial Aggression

Blocker birds exhibited less direct physical aggression in response to STI than did control birds, but blocker and control birds sang at similar rates (Fig. 3.2). Blocker birds spent less time in threat posture (U = 118, Z = 2.385, p = 0.0165), and performed fewer attacks, though the difference in attacks was not significant (U = 103, Z = 1.676, p =

0.0938). Aggressive score was significantly reduced in blocker birds (U = 120.5, Z = 2.524, p = 0.0112). Six of the 10 blocker birds (60%) showed threat posture, and three (30%) attacked the decoy. In contrast, 14 of the 15 control birds (93%) showed threat posture and nine (60%) attacked the decoy. The most aggressive bird was a control bird that attacked the decoy 151 times in ten minutes; this bird's T levels during mate-guarding are not known, but later during incubation he had a normal T level of 0.17 ng/ml. The control birds' aggressive scores were not correlated with their log-transformed T levels (p = 0.7857, n = 11; also see Hunt et al., 1997).

In contrast to the decreased aggression, blocker birds sang as often as control birds during the ten-minute STI (Fig. 3.2; U = 92.0, Z = 0.943, p = 0.3163). Seven of ten (70%) blocker birds sang, and seven of fourteen (50%) control birds sang (Fisher exact test, p = 0.41). Blocker and control birds also spent a similar amount of time within 5m of the decoy (Fig. 3.2; U = 95, Z = 1.136, p = 0.2559), and blocker birds performed as many low flights as controls (blocker birds: mean = 9.600 ± 4.819, n = 10; control birds: mean = 6.200 ± 1.422, n = 15; U = 87, Z = 0.669, p = 0.5034).

Effect of a Territorial Challenge on T Level

The control Lapland longspurs showed no change in circulating T level in response to an STI (Fig. 3.3). T levels of STI and no-STI groups were similar, both during mate-guarding and during incubation (mate-guarding: U = 117, Z = 0.208, p = 0.8353; incubation: U = 110, Z = 1.493, p = 0.1356).

Nest Success, Nestling Growth, & Paternal Care

Blocker birds had similar nest success compared to control birds (Table 3.4). Eight blocker nests were monitored, six in 1992 and two in 1994. The six 1992 nests had unusually good hatching rates - all eggs in blocker nests hatched, and all six 1992 nests produced fledglings. Three blocker nests suffered unexplained disappearances of small nestlings, which is a fairly common occurrence at this study site (Table 3.4). In 1994, a year of unusually severe weather and generally poor nest success, only two blocker nests were monitored. One was abandoned during a snowstorm (several control nests were abandoned during the same storm). The other blocker nest was initiated late; eggs in this nest hatched and the nestlings survived until at least day 5, when the study ended. (Lapland longspur chicks normally fledge at days 8-10). Overall, 6 of 7 (86%) blocker nests produced at least one fledgling. (This data does not include the late 1994 nest.) This is not significantly different from the 15 of 26 (58%) control nests that produced at least one fledgling (Table 3.4; again, this does not include some late nests that could not be followed until fledging).

Nestling growth was also similar in blocker nests and control nests (Fig. 3.4). There were no significant differences in a repeated-measures ANOVA on those nests that could be followed to day 7 (n = 10 control nests and n = 6 blocker nests; $F_{1,14} = 0.524$, $p = 0.4809$). Similar results were obtained when analysis was limited to days 1-4 (n = 11 control nests and n = 7 blocker nests; data not shown). Interestingly, when a sample of nests of testosterone-implanted males was included in the ANOVA on days 1-7, results approached significance ($F_{2,21} = 3.143$, $p = 0.0640$), primarily due to blocker nests having slightly greater nestling weights than T nests as young neared fledging age.

Two blocker males showed unusual parental behavior. Blocker male #39165 was found sitting on a nest with eggs during a routine nest check, and was later videotaped in the nest brooding the nestlings. Incubation of eggs and brooding of young are both unusual behaviors for a male Lapland longspur. Blocker male #39162 was videotaped feeding young regularly at a neighbor's nest. The neighbor male (a control-implanted bird in another study) and his female were also feeding the young regularly. The males occasionally encountered one another at the nest's edge, but did not respond noticably to each other's presence, and all three adults attempted to defend the nest whenever observers approached. #39162 had lost his own nest in a storm and his mate had disappeared; paternity of the young at the neighbor's nest is not known. This is the only case we are aware of in which two Lapland longspur males were observed to feed nestlings at the same nest.

Focal Observations

During focal observations, blocker birds did not differ from control birds in any measure of singing, flights, foraging, preening, or interactions with other females or males (Table 3.5). This indicates that the blocker implants were likely not interfering with the blocker birds' general activity levels or general health.

However, control birds were more likely than blocker birds to be found on territory when they were scheduled for a focal observation (Table 3.1). (This analysis was restricted to data from 1992, when good weather permitted regular scheduling of observations and prolonged searching for birds.) Seven of the eleven blocker birds could not be found for a scheduled focal observation during mate-guarding or incubation, while just one of twelve control birds could not be found (Fisher exact test, p = .0272). When a blocker bird was "not found" for a focal observation, he presumably was either off his territory temporarily, or was on territory but was silent and inactive. Most of the blocker birds that were "not found" had known mates and nests, and were seen repeatedly on their territories later in the season.

DISCUSSION

Male Lapland longspurs with testosterone blockers sang at normal rates in response to STI, but had lowered levels of physical aggression and sometimes could not be found on their territories. These results suggest that there is a minimum level of T that is necessary for normal levels of aggression and territoriality during the week of mate-guarding, which is the only time of the year in this species when territorial aggression is very common and when the response to STI is typically strong. Additionally, plasma T levels appear not to rise in response to an STI, possibly indicating that T levels in this species are not very responsive to social cues.

It is worth noting that the effect of the blocker implants seemed to be limited only to behaviors related to territorial aggression. This implies that the blocker implants were indeed having a specific anti-androgenic effect, and were not simply making the blocker birds "feel sick." For example, during the simulated territorial intrusions, blocker males showed normal levels of non-aggressive behaviors such as song, low flights, and time within 5m. Focal observations (of males that were found on territory) also showed no differences between blocker males and control males in various behaviors such as time spent foraging, number of songs, time spent mate-guarding, etc. Additionally, blocker males successfully courted mates, appeared to feed young normally, and had normal nest success. And finally, blocker birds exhibited normal physiological measures, such as mass after implanting, fat stores, and corticosterone levels.

Some blocker birds had low body mass before implanting as compared to controls. It is possible that these blocker birds may have spent more time foraging off-territory than control males, as a result of differences in body mass. However, the subset of blocker birds that we observed for behavioral data had normal body mass (including all birds scheduled for observations, whether or not they were found). Therefore, we do not think that our behavioral results can be explained solely by a tendency of blocker birds to forage off-territory as a result of low body mass early in the season.

The decreased aggression and normal song of blocker birds are especially interesting when compared to the results of a T-implant experiment done on the same population (see Hunt et al., 1997). In that experiment examined, the effect of T implants was studied during incubation, when territorial aggression is rare and response to STI is typically weak. Results indicated that high T, by itself, is not sufficient to cause elevated levels of physical aggression during incubation, but is sufficient to promote increased singing during incubation. Comparison of these two studies raises two questions. First, why is T necessary for aggression during mate-guarding, but not sufficient to increase aggression during incubation? Second, why is T apparently not necessary for song during mate-guarding, but is sufficient to increase song during incubation?

In considering these questions, we must keep in mind two important differences between these two experiments. First, the two experiments focused on different levels of T. The T-implant experiment studied effects of very high T levels of around 10-20 ng/ml (levels that are typical of the display phase). In contrast, the blocker experiment occurred when T levels of controls are typically at intermediate levels of 3-5 ng/ml (Hunt et al., 1995). Second, the experiments involved STIs performed at different stages: STIs were done during incubation for T-implanted birds, while STIs were done during mate-guarding for blocker-implanted birds.

Testosterone & Aggression

There are at least two possible explanations for the differing effects of T on aggression at different times of the breeding season. First, an intermediate level of T may be necessary but not sufficient for normal territorial aggression, and higher levels of T may have no additional effect. Territorial aggression may require both some minimum level of T *and* certain environmental cues, such as a nearby fertile female. In this scenario, the decreased aggression of blocker birds would be due to the effective level of T falling below that minimum level, while the lack of aggression in T-implanted birds would be due to a lack of appropriate environmental and social cues. During the mate-guarding stage, a male has many interactions with his own fertile female, other fertile females, and neighboring males. Song is frequent and male-male aggression is common. In contrast, during incubation, females are not fertile, and most males cease singing and entirely stop acting aggressive -- in fact, they begin to form small flocks. At this stage, the lack of appropriate social cues may prevent territorial aggression from occurring, regardless of T level.

A second possibility is that at different times of the breeding season, Lapland longspurs may respond differently to a given level of T (all other factors being identical). A study of the effect of T on parental behavior (Chapter 4) indicates that as the season progresses, male Laplands can alter their behavioral response to a given level of T. Additionally, preliminary results from a study of aromatase density in brains of unimplanted male Laplands shows that aromatase activity in several brain regions is high during display and mate-guarding, and then declines significantly as the birds enter incubation (R. Bindra, K. Soma, & B. Schlinger, unpub. data). This might have the effect of reducing expression of any behaviors that are mediated through aromatization of T. If aggression of Lapland longspurs is mediated through aromatization, as it is in some other bird species (e.g. Beletsky et al., 1990; Schlinger & Callard, 1990), this reduction in aromatase could explain the lack of aggression seen in T-implanted Laplands during incubation. Alternatively, it is possible that the decreases in aromatase activity are an effect of decreased T levels in incubation, and therefore might not be the direct cause of behavioral changes. Finally, it is not yet known whether androgen receptor density or estrogen receptor density might undergo similar changes during the breeding season in Lapland longspurs. Further studies of aromatase density and receptor density in brains of control and T-implanted Lapland longspurs may resolve these questions.

Testosterone & Song

An additional puzzle is why T implants increased song in incubation (Hunt et al., 1997), but T blockers did not inhibit song (this study). In this study, blocker birds sang at normal rates during STIs during mate-guarding, and also sang normally during focal observations in both mate-guarding and incubation. (Sample sizes for focal observations were small, and it is possible that larger sample sizes might reveal a slight decrease in singing rates of blocker birds. However, the present data do not indicate any trends in that direction.) Interestingly, T implants increase singing only if the implants raise T levels to very high levels (above about 8 ng/ml); smaller T implants have no effect on song (Hunt et al., 1997). It appears that different levels of T have different effects on song behavior: very high T (above 8 ng/ml) is sufficient to increase song rate, but lower levels of T (below about 4 ng/ml) do not affect song rate. This means that during mate-guarding or incubation, when T levels are generally below 4 ng/ml, normal song rate may occur virtually independently of T level. However, if a bird experiences very high T levels - either naturally during the display phase, or artificially with a high-T implant - the very high T may trigger exceptionally frequent singing. Lapland longspur males do seem to have two different levels of song frequency. During the brief display phase (when T is very high), they sing virtually constantly, while after the display phase (when T is at intermediate or low levels) they only perform occasional song bouts interspersed with other activities (see Hunt et al., 1997 for details). Perhaps the high-T implants that were given during incubation raised T to such a high level that the birds, in essence, responded with display-phase song rates.

Testosterone & Parental Care

Finally, though this study did not focus on parental care, there are some hints that testosterone blockers may enhance paternal care. This is interesting in light of a concurrent study indicating that T has a slight inhibitory effect on paternal care when chicks are young (Chapter 4). Nestlings of blocker males had slightly increased mass compared to the nestlings of the T-implanted males discussed in Chapter 4, and this trend approached significance. Anecdotal observations indicated that two blocker males exhibited unusual "extra" paternal care: one fed at a neighbor's nest, and the other took on some of the female's duties (incubation and brooding), behaviors that have not been reported before in male Lapland longspurs. It is possible that these behaviors may occur occasionally in normal male Lapland longspurs at low rates or under special circumstances. For instance, the bird who fed at a neighbor's nest might have been responding to the loss of his own nest, and it is possible that he may have been the father of some young in his neighbor's nest (a small percentage of Lapland longspurs are bigamous, and extra-pair copulations are seen occasionally). Yet, none of the many control males who lost nests were found to be feeding at neighbor's nests. Taken together, the nestling mass data and the anecdotal observations of unusual parental care hint that T may indeed have a subtle inhibitory effect on paternal care in this species.

In summary, the hormone-behavior interactions of Lapland longspurs are similar to temperate-latitude birds in some respects (e.g., high T increases song, blockers inhibit aggression), but not in others (e.g., aggressive experiences do not affect T level, high T does not affect aggression, and blockers do not affect song). The blocker experiment revealed several unexpected interactions of T with territorial aggression and song in Lapland longspurs. Some minimal level of T appears to be necessary for aggression during mate-guarding, but during incubation, high levels of T do not increase aggression above normal levels. In contrast, singing can occur almost independently of T for most of the breeding season, but at the very beginning of the breeding season, very high T may trigger extremely frequent singing for a few days during the display stage. The Lapland longspurs' very brief song display stage, their need for territorial defense and mate-guarding behavior only during the second week on the breeding grounds, and their need for precisely timed transitions in behavior, may have led them to partially uncouple song and aggression from testosterone. After the week of mate-guarding, aggression may be independent of T level. In this respect, Lapland longspurs in June (after mate-guarding) are similar to temperate-zone birds in fall and winter, when aggression is often independent of T (e.g. Logan & Wingfield, 1990; Schwabl, 1992; Wingfield, 1994). We might consider Lapland longspurs as having only one week of typical "breeding-season behavior" by temperate-zone standards -- the week of mate-guarding. A similar relationship of T with song and aggression has recently been seen in snow buntings, in which T implants increased song and actually decreased aggression during incubation (M. Romero, unpublished data). Further investigations of other arctic birds, and of other other socially monogamous birds with long or short seasons, may clarify whether these hormone-behavior relationships are common adaptations when breeding must be completed as rapidly as possible.

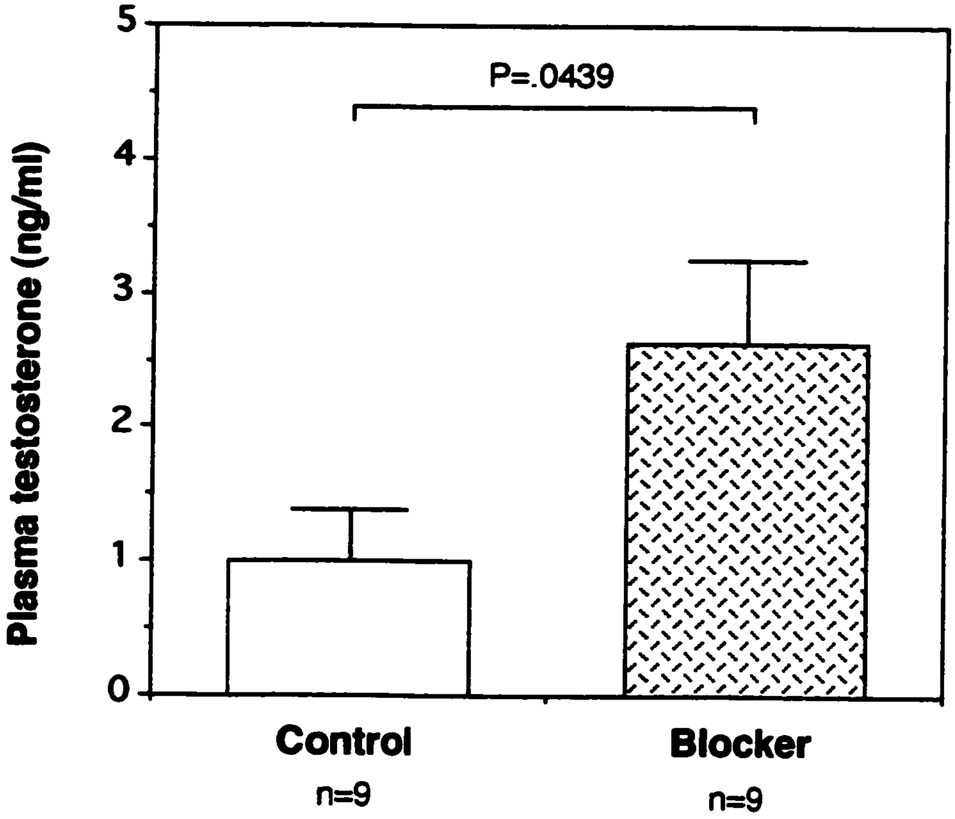

FIGURE 3.1. Testosterone levels of blocker-implanted and control male Lapland longspurs. Data are shown as mean ± SEM. P-value from t-test is indicated.

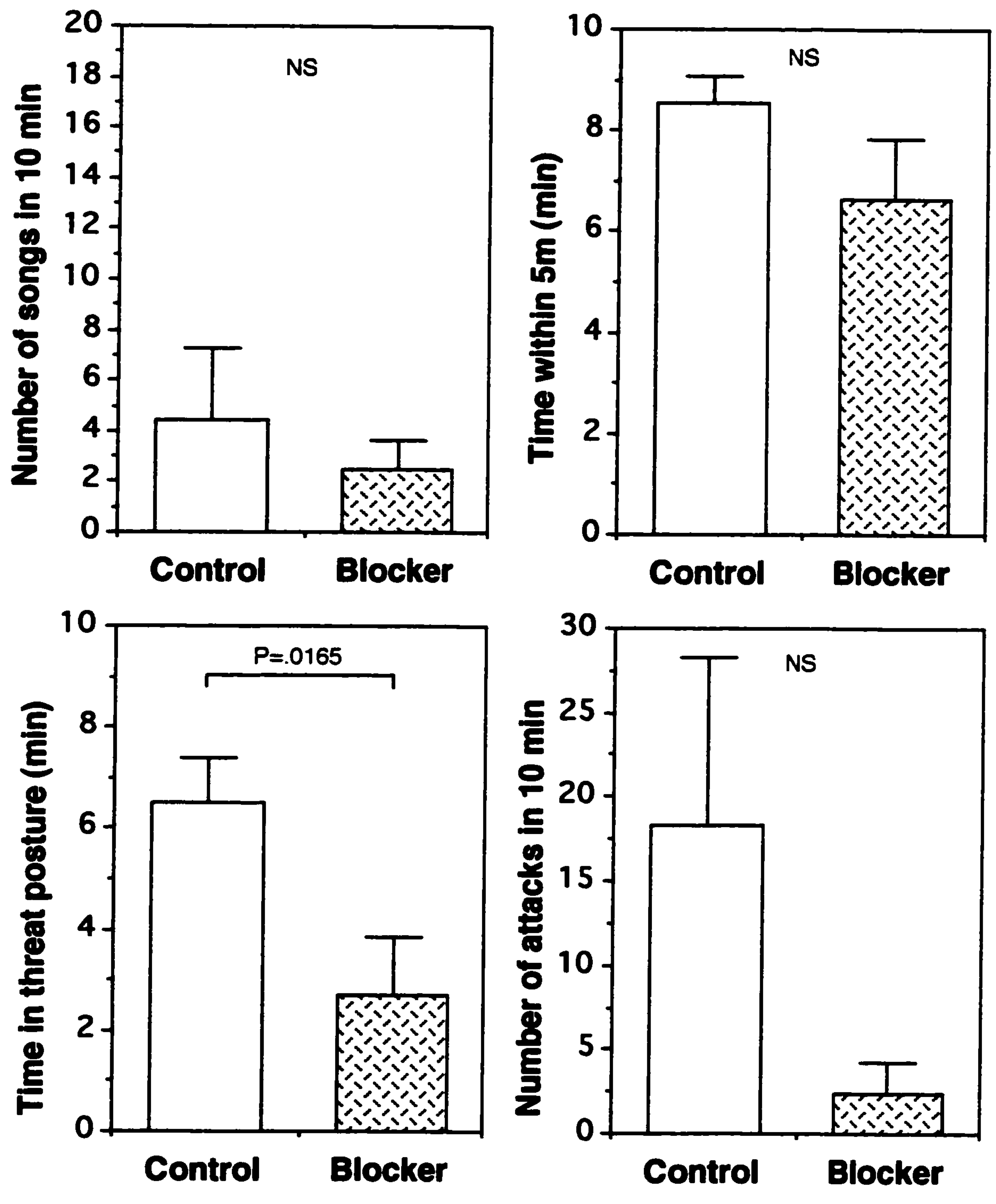

FIGURE 3.2. Behavioral responses to simulated territorial intrusions presented to blocker-implanted and control male Lapland longspurs during mate-guarding. Data are shown as mean ± SEM. Sample sizes were n=10 for blocker birds and n=15 for control birds. Where results are significantly different, the p-value from a Mann-Whitney test is indicated. NS = not significant.

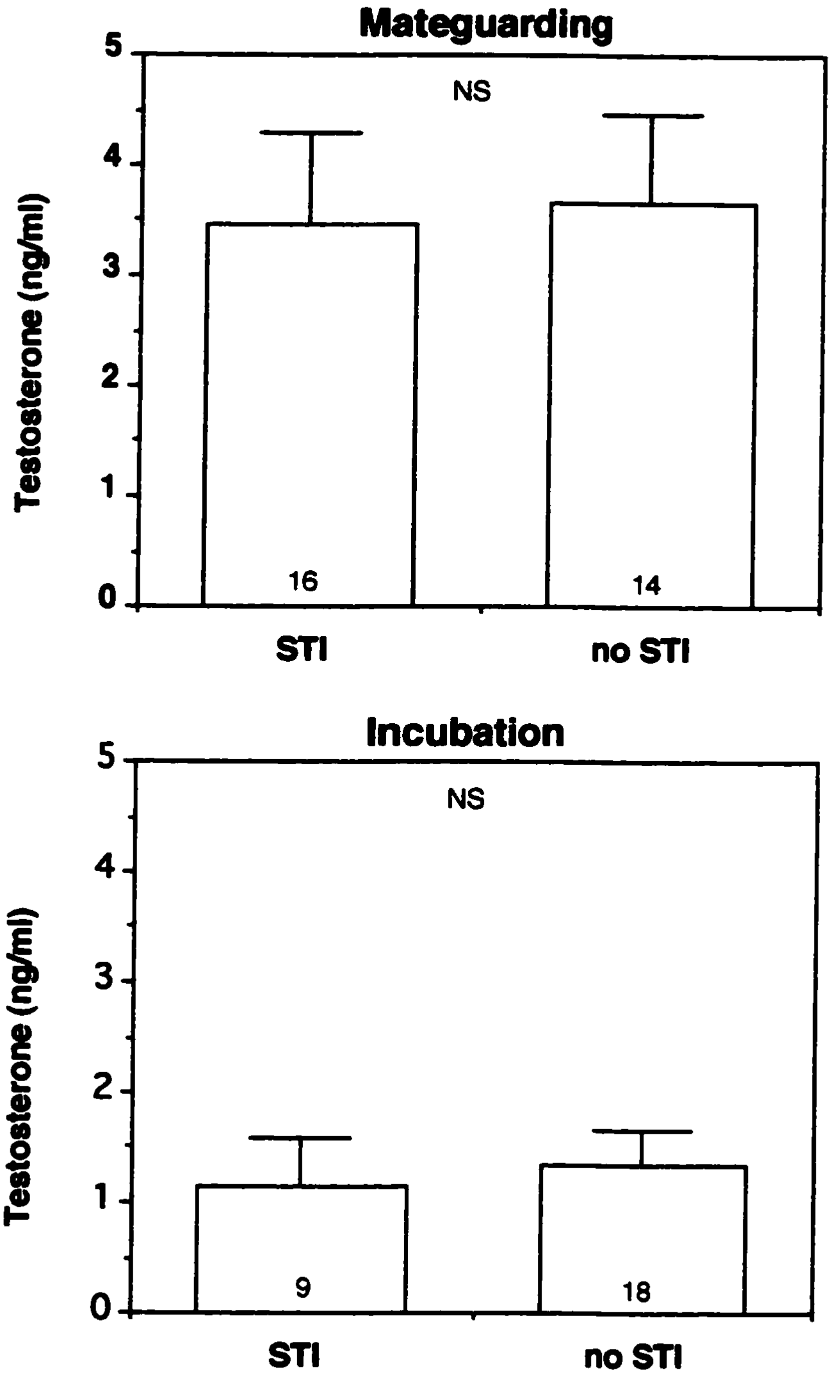

FIGURE 3.3. Plasma testosterone levels in male Lapland longspurs that had just experienced a simulated territorial intrusion ("STI") or had not experienced a simulated territorial intrusion ("no STI"). Birds were tested during mate-guarding (top) or during incubation (bottom). Data are shown as mean ± SEM, with sample sizes at bottom of bars. NS = not significantly different.

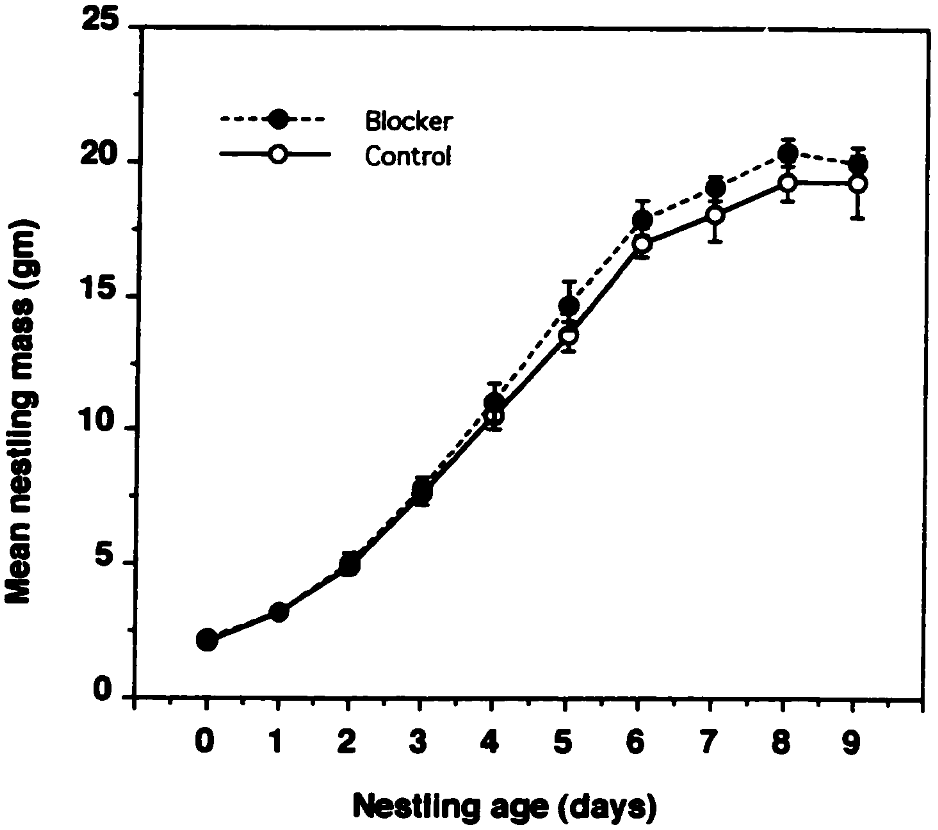

FIGURE 3.4. Mean nestling mass in nests of blocker-implanted and control-implanted males. Data are shown as mean ± SEM. Day 0 = day that first nestling was found. Sample sizes were 8 blocker nests and 10 control nests.

Table 3.1. Tendency of blocker-implanted or control Lapland longspur males to be "found" or "not found" on their territories when observers attempted a scheduled behavioral observation. Simulated territorial intrusions occurred during mate-guarding, while focal observations occurred both during mate-guarding and incubation. Some birds were scheduled for several focal observations; the last section of the table shows whether or not birds were always found for every scheduled focal observation.

	Blocker	Control
Simulated territorial intrusions (mate-guarding)		
Found on territory	11	15
Not found on territory	7	0
Focal observations during laying		
Found on territory	8	8
Not found on territory	2	0
Focal observations during incubation		
Found on territory	6	11
Not found on territory	5	1
Found for every scheduled focal?		
Always found	7	11
Not always found	4	1

Table 3.2. Time until bleeding, corticosterone level, and luteinizing hormone level of blocker-implanted and control Lapland longspur males.

		Mean ± SEM	Range	Median
Time until bleeding (min)				
Blocker	n=9	9.111 ± 2.855	3 - 30	6.00
Control	n=9	8.667 ± 3.167	2 - 30	5.00
Corticosterone (ng/ml)				
Blocker	n=9	21.622 ± 5.711	5.31-53.51	17.82
Control	n=9	17.800 ± 2.895	7.58-32.84	15.74
Luteinizing hormone (ng/ml)				
Blocker	n=5	1.364 ± 0.540	0.15-3.37	1.17
Control	n=5	1.600 ± 0.190	1.01-2.11	1.72

Table 3.3. Body mass, mean fat score, and cloacal protuberance length of blocker-implanted and control-implanted male Lapland longspurs. Results from t-tests (for effect of implant) are shown in the right-hand columns.

	Blocker Mean ± sem (n)	Control Mean ± sem (n)	T	P
Mass				
Before implant	25.776±0.253 (21)	27.485±0.638 (27)	2.253	0.0291
With implant	27.428±0.433 (18)	26.948±0.213 (23)	1.063	0.2945
Mean fat score				
Before implant	1.000 ± 0.158 (21)	1.352 ± 0.218 (27)	1.238	0.2221
With implant	0.833±0.157 (18)	0.913 ± 0.120 (23)	0.411	0.6835
CP length				
Before implant	5.300 ± 0.179 (20)	5.519 ± 0.284 (27)	0.598	0.5526
With implant	6.333 ± 0.229 (18)	6.522 ± 0.165 (23)	0.686	0.4970

Table 3.4. Hatching success, fledging success and cause of nest failure in nests of T-implanted and control male Lapland longspurs. Proportions are #pairs of all pairs in that category who had nests. Means are shown ± SEM with sample size in parentheses. P-values for proportions are from Fisher exact tests, while P-values for means are from t-tests. Sample sizes vary due to late discovery of some nests and depredation or late hatch of other nests.

	Blocker	Control	P
Clutch size & nest success			
Mean clutch size per pair	4.625 ± 0.263 (8)	4.333 ± 0.182 (30)	0.4458
Mean #hatchlings per pair	4.250 ± 0.620 (8)	2.967 ± 0.367 (30)	0.1088
Mean #fledglings per pair	3.00 ± 0.787 (7)	1.586 ± 0.346 (29)	0.4173
At least 1 egg hatched	7 of 8 pairs	24 of 29 pairs	0.6487
At least one fledgling produced	6 of 7 pairs	15 of 26 pairs	0.2027
Occurrence & causes of nest failure			
Nest depredated	1 of 8 pairs	9 of 27 pairs	0.3905
Nest abandoned	1 of 8 pairs	3 of 27 pairs	0.5692
Other nestling death	3 of 7 pairs	7 of 24 pairs	0.6518

Table 3.5. Results of focal observations on male Lapland longspurs with blocker or control implants. Observations were done during mate-guarding (when the female was laying eggs) or during incubation. Sample sizes: n = 8 for each group during incubation; n = 6 for each group during incubation. Z-values and P-values from Mann-Whitney tests are shown for the seven measures with continuous data; X^2 values and P-values (from Fisher exact tests) are shown for the three measures with categorical data.

	Blocker		Control			
	Median	(range)	Median	(range)	Z, X^2	P
I. MATE-GUARDING						
Songs/hr	21.865	(0.0-85.7)	29.192	(0.0-133.5)	0.213	.8312
Song flights/hr	3.572	(0.0-10.0)	8.267	(0.0-34.5)	0.959	.3374
Spontaneous flights	7.286	(0.0-25.0)	5.54	(0.0-21.0)	0.158	.8746
%time singing	33.462	(0.0-61.9)	35.227	(0.0-85.0)	0.213	.8312
%time near female	70.833	(0.0-100.0)	37.539	(0.0-100.0)	0.055	.9563
%time near male	6.227	(0.0-33.3)	0.0	(0.0-15.0)	1.646	.0998
%time foraging	73.433	(14.3-90.5)	65.75	(0.0-100.0)	0.000	>.99
Preening	3 of 8 preened		0 of 8 preened		3.692	.2000
Following female	4 of 8 followed		4 of 8 followed		0.0	>.99
Aggression	5 of 8 aggressive		3 of 8 aggressive		1.0	.6193
II. INCUBATION						
Songs/hr	30.000	(0.0-123.8)	15.00	(0.0-67.5)	0.458	.6470
Song flights/hr	2.376	(0.0-30.0)	5.25	(0.0-24.0)	0.163	.8703
Spontaneous flights	19.071	(6.7-30.0)	13.5	(0.0-32.9)	0.481	.6304
%time singing	26.667	(0.0-87.5)	30.0	(0.0-70.0)	0.196	.8446
%time near female	0.000	(0.0-39.13)	7.5	(0.0-40.0)	1.048	.2947
%time near male	2.174	(0.0-100)	0.0	(0.0-0.0)	1.892	.0585
%time foraging	79.286	(0.0-100)	52.5	(5.0-75.0)	0.262	.2615
Preening	1 of 6 preened		2 of 6 preened		0.444	>.99
Following female	1 of 6 followed		1 of 6 followed		0.0	>.99
Aggression	1 of 6 aggressive		0 of 6 aggressive		1.091	>.99

CHAPTER 4. Endocrine Influences on Parental Care During a Short Breeding Season: Testosterone and Male Parental Care in Lapland Longspurs

INTRODUCTION

Many male birds show a seasonal pattern of testosterone (T) secretion in which plasma T increases in early spring, reaches a peak during territory acquisition and courting of females, and then declines to near baseline levels after the young hatch. The decline is most sudden in species in which males contribute substantially to parental care, and is more gradual in species in which males contribute only slightly or not at all to parental care (reviewed in Beletsky *et al.*, 1995). Subsequent nesting attempts may sometimes be accompanied by additional peaks in T. These fluctuating levels of T presumably indicate changing benefits and costs of high T throughout the breeding season. The possible benefits of high T in male birds have been widely studied (reviewed in Balthazart, 1983, Wingfield & Farner, 1993). For instance, T is involved in development of some secondary sex characters, mate attraction (via increased song, courtship, and sexual activity), and territory defense (via increased song, aggression, and use of space) (Witschi, 1961; Balthazart, 1983; Harding, 1983). The possible costs of high T have been less studied, but may include interference with parental care, lowered immune function, increased mortality, and interference with molt (Dufty, 1989b; Wingfield *et al.*, 1990; Beletsky *et al.*, 1995).

The potential conflict of high T with parental care is particularly interesting, since in this case the T level of a male bird may represent a trade-off between investment in a future brood (via mate acquisition and territory defense, both typically stimulated by T) and investment in the current brood (via parental care, typically inhibited by T). Numerous species show correlations between the seasonal pattern of T secretion, the amount of parental care exhibited by the male, and the opportunities the male may have for siring future broods (Beletsky *et al.*, 1995). For example, males of socially monogamous species with biparental care show a particularly steep decline in plasma T when their eggs hatch. These birds often can respond to certain stimuli -- e.g., presence of a rival male -- with a temporary surge of high plasma T, but in the absence of such stimuli, T levels during the parental stage are quite low (Wingfield *et al.*, 1990). In contrast, polygynous males with biparental care also show a decline of T when the eggs hatch, but the decline is usually more gradual. Finally, males with no parental care tend to maintain high levels of T for most of the breeding season (Wingfield *et al.*, 1994; Beletsky *et al.*, 1995).

Several experimental studies show that monogamous male birds given T implants visit their nests less often than control males, and the young of T-implanted males often grow more slowly than young of controls (e.g. Beletsky *et al.*, 1995; Hegner & Wingfield, 1987; Saino & Møller, 1995; Ketterson *et al.*, 1996). Interestingly, the T-implanted males' reproductive success does not necessarily suffer; T-implanted males may obtain more extra-pair copulations than controls, and furthermore, females in some species can increase their parental care to compensate entirely for the reduced care from their T-implanted mates (Ketterson *et al.*, 1996). Nevertheless, these experiments consistently indicate that high T during the parental phase tends to cause male birds to decrease their investment in the current brood.

The experimental studies mentioned above have primarily focused on populations that have relatively long breeding seasons, and are multiple-brooded; that is, pairs can raise more than one clutch of young per year. In such populations, males may gain a relatively large benefit from remaining responsive to high T late in the breeding season, even at the cost of reduced parental care. This is because high T may enable them to prepare for subsequent broods or to gain extra-pair copulations with nearby fertile females. However, the costs and benefits of high T may be different for males in single-brooded populations with short breeding seasons. Once these single-brooded males reach the parental phase of their first brood, there may be no chance of subsequent broods, and there may be few to no fertile females available for extra-pair copulations. We hypothesize, therefore, that males of single-brooded populations should have some mechanism(s) for ensuring that high T does not interfere with parental care. One possibility is that these males may completely shut down T production and secretion, regardless of external stimuli (such as invading males), so that the male simply never experiences high T during the parental phase. A second possibility (not mutually exclusive with the first) is that males may be unresponsive to high T, such that high T simply does not affect parental care, song rate, and other behavior patterns in the "typical" way that it does in multiple-brooded species.

In this study, we addressed the second possibility described above. We predicted that socially monogamous males with short breeding seasons may have reduced responsiveness to exogenous T during the parental phase. If this is so, T implants would not cause the typical strong reduction in male parental care that has been seen in socially monogamous males with longer breeding seasons. To test this hypothesis, we implanted male Lapland longspurs, *Calcarius lapponicus*, with either T or control (empty) implants. This single-brooded species has a short six-week breeding season at our field site in northern Alaska (Williamson, 1968a; Hunt *et al.*, 1997). We then monitored the males' parental care, measuring both number of nest visits and number of feeding movements made to the young, and we also monitored nestling growth and fledging success of the young. Finally, we also monitored female parental care to quantify the extent of female compensation if male parental care was reduced.

METHODS

Study Population & General Methods

We studied Lapland longspurs at Toolik Field Station, Alaska (68° 38'N, 149° 38" W), during May and June of 1992 and 1994. We captured birds in Potter traps baited with seeds. Each bird was banded with an aluminum U.S. Fish & Wildlife Service band and a unique combination of color bands for subsequent observation and identification.

We checked general condition of each bird by measuring body mass, fat stores, and cloacal protuberance (CP) length of males. Observers were unaware of the experimental status of each bird while measuring these features. We measured body mass to the nearest 0.1 g. We ranked fat stores from 0 to 5, where 0 = no fat, and 5 = bulging fat deposits (Wingfield & Farner, 1978b). Furcular fat and abdominal fat were ranked separately and then averaged to give a mean fat score for each bird. Cloacal protuberances (an androgen-dependent copulatory organ of males) were measured to the nearest mm with a small ruler placed at the antero-ventral surface of the cloacal protuberance.

Testosterone and Control Implants

Implants consisted of 12-mm or 20-mm lengths of silastic tubing (i.d. 1.47mm, o.d. 1.96mm; Dow Corning), either packed with crystalline testosterone or left empty as a control, and sealed with Silastic medical adhesive. We anesthetized birds in the field with a light dose of an inhalant anesthetic, methoxy-fluorane (Metofane; Pitman-Moore), inserted implants under the skin, and sealed the incision site with a veterinary skin bond (Nexaband; Tripoint Medical). Each time a bird was captured, we checked the implants for proper placement and healing of the incision site. We removed the implants at the end of the season whenever possible.

In 1992, we implanted males with two 20-mm implants each (effective total length 36 mm), which were placed one on each side between the wing and knee. To minimize problems of loss of these long implants, in 1994 we implanted males with three 12-mm implants (effective total length 30 mm) on the back between the wings. Implants were primarily administered from 8 June to 13 June, which was the middle of incubation for all nests in this study. The exceptions were two control birds that were implanted early in the season, on 15 May 1994 and 17 May 1994; due to inclement weather, we were unable to implant additional control birds later in 1994.

Silastic testosterone implants are known to begin releasing T within a few hours of insertion, and continue to release T at a slowly declining rate for approximately 60 days (e.g. Wingfield, 1984b; Ketterson *et al.*, 1991). The mean time between insertion of implants (typically during incubation, which lasts 11-12 days) and the first observations of parental care (when nestlings were 2-3 days old) was 13.44 ± 1.18 days for T males, and was 18.13 ± 3.40 days for empty-implanted males. Nestlings reached fledging age within 30 days of implant insertion for all T males.

Final sample sizes for males that had known nests were 16 T males, 14 empty-implant males, and 14 unimplanted males. These numbers do not include birds that disappeared, that did not have nests, or that had to be excluded from the study for unusual reasons. These categories occurred as follows. In 1992 we implanted 9 T males and 13 empty-implanted males, and nests of an additional 5 unimplanted males were monitored for fledging success. One T pair was excluded from analysis because the incubating female was injured during capture and was then held in captivity for two weeks while she recovered. Two empty-implant males were not found after implanting. The remaining 1992 males (8 T males, 11 empty-implant males, and 5 unimplanted males) all had known nests. In 1994, we implanted 14 T males and 8 empty-implant males, and nests of an additional 9 unimplanted males were monitored for fledging success. Three T males were excluded from the study due to poor implant placement which interfered with walking (Hunt *et al.*, 1997); these three T males were recaptured and their implants removed. Three additional T males and three empty-implanted males were not seen after implanting, and we could not find females or nests of two other empty-implanted males. The remaining 1994 males (8 T males, 3 empty-implanted males, and 9 unimplanted males) all had known nests.

We refer to males given testosterone implants as "T males", their nests as "T nests", and their mates as "T females". In several analyses, we combine empty-implanted and unimplanted males in a single group of "control males", and call their nests "control nests" and their mates "control females". (Note that the females themselves received no implants.) We found no differences between the empty-implanted and unimplanted groups in any of the variables analyzed, and previous studies indicate that empty-implanted males and unimplanted males do not differ detectably in hormone levels or behavior (e.g. Hunt *et al.* in press).

Blood Sampling & Radioimmunoassay

We bled each trapped bird immediately upon removal from the trap, except for any birds that had been bled within the previous week. We obtained blood samples by puncturing the alar vein and collecting approximately 250-300 ul of blood into heparinized microhematocrit tubes, which were stored temporarily in small field coolers filled with ice packs. Blood was centrifuged and plasma pipetted off within six hours. We then stored the plasma at -20°C, shipped it on dry ice to the University of Washington, and stored it again at -20°C until assaying. We measured plasma levels of T in males with radioimmunoassay, after extraction of steroids with dichloromethane and partial purification on diatomaceous earth/glycol micro columns (Ball & Wingfield, 1987). Percentage of steroids recovered was calculated via addition of 20 ul of radiolabelled T to each sample. Intra- and inter-assay variations for this assay have been reported previously (Hunt *et al.*, 1997). Note that we did not obtain baseline corticosterone data for our males due to the 5-20 min delay involved in checking the traps.

Parental Care

We videotaped 10 T nests and 10 control nests for quantification of parental behavior. The 10 videotaped T nests included all the T nests that survived to day 2 after hatch (seven T nests from 1992 and three T nests from 1994). The videotaped control nests were chosen to match the T nests as closely as possible for clutch size and lay date; we videotaped 5 nests of empty-implanted males from 1992, 3 nests of empty-implanted males from 1994, and 2 nests of unimplanted males from 1994. For videotaping, we used battery-powered VHS videocameras mounted on tripods and placed 1-2 m from the nest. Nests were taped twice, once when nestlings were 2-3 days old and once when they were 4-5 days old. (Lapland longspurs fledge when they are 8-11 days old.) The first videotapes of one control nest and one T nest were not usable due to camera problems. Dates of videotaping were 20 June to 30 June in both years.

We allowed birds a minimum of two hours to acclimate to the camera's presence before taping began, after which an observer returned to the nest briefly to start the videocamera. To exclude disturbance effects of the observer turning on the camera, we did not begin scoring the behavior on the videotape until the tape showed the first adult returning to the nest. (The first adult, usually the female, typically returned within 5-10 minutes of initiation of videotaping.) Each videotape was approximately two hours long, with slight variations due to battery power. Videotapes were scored by two observers unaware of the experimental status of the birds, and inter-observer reliability was near 100%. Nest video data were tallied and checked with a customized computer program that calculated durations and total times of activity bouts for both parents of each nest, and checked all data for any missing or improbable time intervals (J. Muhasky, 1996, using Oracle Media Objects v1.1 for Macintosh).

Females and males were each scored for number of visits to the nest per hour and number of feeding movements ("feeds") to the young per hour. A feeding movement consists of an adult bird lowering its head into the nest, presumably to deliver a parcel of food to one nestling. We scored number of visits and number of feeds separately because we had previously noticed that some females visit frequently but perform few feeds per visit, while some males visit infrequently but perform numerous feeds per visit. In such situations, scoring only the number of visits may underestimate the males' contribution to feeding of nestlings.

We also scored videotapes for percentage of time that females spent at the nest (out of total videotape time). While at the nest, female Lapland longspurs divide their time among brooding, standing, and rearranging nest material (Chapter 5), but in this study we did not examine the relative amount of time allocated to those behaviors.

Ketterson *et al.*, 1996 has suggested that male dark-eyed juncos (*Junco hyemalis*) with T implants may visit their nests less predictably than control males. That is, even if T and control males have equal overall frequency of nest visits, they may differ in the regularity of timing of those visits. To test this possibility in Lapland longspurs, we measured the regularity of the timing of each male's visits to the nest when nestlings were 4-5 days old. For each male, we recorded the elapsed time between consecutive visits, and then calculated the coefficient of variance (CV) of these intervals (i.e., the higher the CV, the less regular the male's timing of nest visits). Males with fewer than four visits were excluded from analysis. We used a t-test to compare the CV's of T males to those of control males. (Analysis of similar data from nests at days 2-3 was not possible, because only one T male visited his nest more than once at this stage.)

The videotapes revealed that nestlings of one 1994 control nest were fed by three adults: the resident female, the resident empty-implanted male, and a neighbor male who had been given implants of testosterone blockers in a concurrent study. All three adults appeared to be visiting and feeding at normal rates. Because the total feeding rate was unusually high, this nest was excluded from analyses of parental care, nestling growth, and nest success.

Nestling Growth

We checked all nests daily, counting the number of eggs and nestlings in each nest and measuring each nestling's body mass to the nearest 0.01g with a portable digital scale. We then averaged the body mass of all nestlings for each nest on each day. Some nests had a nestling that was much smaller than its siblings. We quantified the size differential of the smallest nestling for each nest by dividing the lightest chick's mass by the heaviest chick's mass on day 4 after hatch (where day 1 = the first day that a nestling is found in the nest). We chose day 4, the middle of the nestling period, because size differences were apparent on this day but few nests had been depredated. In addition, we noted any deaths of individual chicks that were not due to predation or nest abandonment.

We obtained at least some nestling mass data for 11 T pairs and 19 control pairs. However, many nests were depredated during the nestling stage, often within 1-3 days after hatching, and few nests survived to fledging. Therefore we conducted three analyses of our nestling mass data: (1) t-tests on all nest data from day 1 to day 9 with the Bonferroni correction for multiple tests ($\alpha = .05/9 = 0.0056$); (2) a repeated-measures ANOVA for only those nests that survived to day 7; and (3) another repeated-measures ANOVA for only those nests that survived to fledging.

Nest Success

We were able to calculate hatching and fledging success for 19 T pairs and 32 control pairs (15 empty-implanted and 17 unimplanted). We measured the percent of nests in which at least one egg hatched, percent of those nests with hatchlings that produced at least one fledgling, the number of fledglings produced per male, and the probability of depredation. Lapland longspur chicks often leave the nest when they are 10 or 11 days old, but may leave as early as 7-8 days if disturbed (Williamson, 1968a,b; KH, unpublished data). We assumed that chicks fledged successfully if they disappeared from the nest on or after day 8, with the nest remaining intact and with any younger chicks and unhatched eggs still present in the nest. We assumed that chicks had been taken by a predator if they disappeared before day 8, and/or if the nest was torn apart and younger chicks and unhatched eggs had also disappeared. Common predators of Lapland longspur chicks at our field site include arctic ground squirrels (*Citellus parryi*), red fox (*Vulpes fulva*), common raven (*Corvus corax*), and long-tailed jaeger (*Stercorarius longicaudus*).

We also monitored the tendency of females to abandon their nests. In some other species, females mated to T-implanted males increase their level of maternal care, presumably to compensate for decreased paternal care (Hegner & Wingfield, 1987; Saino & Møller, 1995). Such compensatory care might stress the female, which might in turn make her more likely to abandon the nest during inclement conditions. We considered nests to be abandoned if eggs were cold for several days and/or nestlings were dead, the female was not seen near the nest, and if a blade of grass placed across the nest remained undisturbed for several days.

Weather conditions varied during the two years of this study. In 1992, weather was generally mild during incubation and feeding of young. In 1994, the weather at our study site was unusually cold, and there were repeated snowstorms during incubation and feeding of young.

Overwinter Survival

To evaluate overwinter survival, we counted the numbers of breeding females and males from 1992 who were seen or captured in 1993. These data undoubtedly underestimate the actual number of birds which survived. Nevertheless, such data are useful for comparisons between two groups.

Timing of Female Fertility

Since sexual behavior of males is often strongly affected by patterns in female fertility, we examined the timing of female fertility in Lapland longspurs at our study site. In birds, the period of female fertility typically begins up to a week before laying starts, and ends 24 hours before the last egg is laid (Montgomerie, 1988; Birkhead, 1996). Sexual activity often occurs only during this period. For example, in the Smith's longspur (*Calcarius pictus*), copulations begin three to five days before clutch initiation and cease approximately 24 hours before the last egg is laid. Based on this information from a close relative of the Lapland longspur, we calculated the period of fertility for every female Lapland on our study site in 1992 and 1994. We then calculated the percentage of fertile females that were fertile on any given date, out of the total number of females observed. We considered a female Lapland to be fertile from five days before laying of her first egg, up to and including the day before laying of her last egg. If a female renested after loss of her first nest, she was considered to have an additional period of fertility beginning on the day the first nest was lost, until the day before the last egg of the replacement clutch was laid.

Statistical Analysis

We used nonparametric tests for most hormonal and behavioral data, as these data generally did not have normal distributions. In these cases, two-group comparisons were carried out with Mann-Whitney tests, multi-group comparisons with Kruskal-Wallis tests, and paired data (e.g., hormone levels before and after implant, male & female behavior within a pair) with Wilcoxon signed rank tests. We used parametric tests for data such as body mass and clutch size that were normally distributed with equal variances between groups. For these data, two-group comparisons were carried out with Student's t-test, and multi-group comparisons with ANOVA (with post-hoc analyses using Fisher's Protected Least Significant Difference). Mean nestling mass was analyzed with repeated-measures ANOVA and with t-tests using the Bonferroni correction, as described above. Differences in proportions between two groups were analyzed with Fisher exact tests. All tests were conducted with Statview v4.1 for the Macintosh, with the significance level set at $\alpha = 0.05$. We used one-tailed tests for those analyses in which we were highly confident of the predicted direction of any experimental effect. These analyses were plasma T level and CP length of T vs. control birds after implanting, and parental care of T vs. control birds. All other tests were two-tailed.

RESULTS

Plasma Testosterone, Mass, Fat, and CP Length

T implants effectively raised plasma T levels. This result has been previously reported for a larger sample of control and T-implanted Lapland longspur males, which included the birds in this study (Hunt *et al.*, 1997). The effectiveness of the T implants is also significant in the smaller sample of birds whose nests were videotaped for this study and for whom we have plasma samples (Table 4.1). Before implanting, control birds and T birds had nearly identical plasma T levels ($U = 32.0$, $p = 0.9578$), but after receiving implants, T birds had significantly higher plasma T levels than controls ($U = 56.0$, $p = 0.0006$). Control birds showed a significant decline in T levels (before vs. after implanting; $Z = 2.023$, $p = 0.0431$), which is typical for male Lapland longspurs entering the parental phase. In contrast, T males showed a significant increase in T (Z = 2.240, p = 0.0251) to levels that are typical of the display/courtship phase early in the season.

T-implanted males also had longer CPs and greater body mass (Table 4.1). Before receiving implants, T and control males had similar CP lengths (U = 38.5, p = 0.4333). After receiving implants, T males had significantly longer CPs than control males (U = 61.0, p = 0.0095). Again, these results are consistent with a more extensive analysis which showed that T implants can prevent the seasonal decline in length of the CP of Lapland longspurs (Hunt *et al.*, 1997). Finally, body mass and fat stores of T and control males were similar before receiving implants (mass, t = 0.248, p = 0.8080; fat, U = 34.0, p = 0.7808), but after receiving implants, T males had significantly greater body mass than control males (t = 2.234, p = 0.0411), and showed a nonsignificant trend toward having greater fat stores (U = 52.5, p = 0.0917).

The elapsed time between implanting and subsequent physiological measurements (of T, mass, fat, and CP) was 15.857 ± 2.721 days for all males combined. Control and T males did not differ significantly in date of implanting, date of recapture, or elapsed time between implanting and recapture (data not shown).

Parental Care

When nestlings were 2-3 days old, T males visited significantly less often than control males (U = 55, p = 0.0239; Fig. 4.1), and gave fewer feeds (U = 51.5, p = 0.0475; Fig. 4.1). These patterns appeared due to a tendency of many T males not to visit the nest at all during the two-hour videotaping period. Only two of nine T males visited the nest, while seven of nine control males visited (Fisher exact test, p = 0.0284). Of the two T males that did visit their nests, one visited often and fed frequently (at rates similar to the female), and the other visited just once in two hours, the only male to do so. Plasma T levels of these two males are not known. Though control males did tend to visit their nests, they visited significantly less often than their females (Z = 2.240, p = 0.0251). However, control males performed as many feeding movements per hour as their females (Z = 0.169, p = 0.8658). T females visited and fed more often than control females (female visits/hr: U = 54, p = 0.0352; female feeds/hr: U = 44, p = 0.0321), probably due to compensation for decreased care by the T males. T females and control females spent similar amounts of time at the nest (T females, mean % time at nest = 57.98 ± 5.5, n = 9; control females, mean % time at nest = 64.65 ± 2.9, n = 8; U = 44.0, p = 0.2207). The combined feeding effort of T pairs was very similar to that of control pairs for both visits and feeds; T pairs slightly outperformed control pairs on both measures when males and females were combined, and the differences were not statistically significant (visits, Z = .289, p = 0.3864; feeds, Z = .579, p = 0.2814).

When nestlings were 4-5 days old, in general, males of both groups dramatically increased their nest visits and feeding rates, while females did not (Fig. 4.1). T males began to visit their nests at rates similar to control males (U = 59, p = 0.1265), though T males still performed fewer feeds per hour than control males (U = 78, p = 0.0036). Nevertheless, T males were now apparently performing enough feeds per hour that T females showed a trend toward reducing their feeding rates (T females, days 2-3 vs. days 4-5; Z = 1.690, p = 0.0910). Control females, in contrast, did not show any trend toward reduced feeding rates as nestlings grew (Z = 0.700, p = 0.4838). Within control pairs, males and females visited and fed at roughly equal rates (visits: Z = 0, p > .99; feeds: Z = .652, p = 0.5147). Within T pairs, females visited more often than males (Z = .2803, p = 0.0051), but females and males fed with similar frequency (Z = 1.376, p = 0.1688). T and control females had similar visits per hour (U = 58.0, p = 0.1443), feeds per hour (U = 49.0, p = 0.3720), and percent time at the nest (T females, mean = 45.49 ± 4.0; control females, mean = 48.34 ± 6.0; U = 51.0, p = 0.3121). The combined feeding effort of T pairs was again similar to that of control pairs, though there was a nonsignificant trend for control pairs to feed more (visits, Z = .082, p = 0.4675; feeds, Z = 1.388, p = 0.0826).

There were no significant differences between T and control nests in number of nestlings, total nestling mass, age of nestlings at videotaping, date of videotaping, or time of day of videotaping (p>.50 for all comparisons; data not shown).

Nestling Growth

Nestling growth in T nests and control nests was similar overall (Fig. 4.2). All nestlings had nearly identical growth in days 1-3, despite the T males' tendency not to visit the nest during this time. Nestlings of T males showed a slight trend toward decreased mass in days 5-7, just after the second videotaping. This trend vanished by fledging age, and fledgling mass was nearly identical in T and control nests. Multiple t-tests with the Bonferroni correction revealed no significant differences between T and control nests for any day; p-values were lowest for days 5 and 7 (day 5, p = 0.0649; day 7, p = 0.0666; Bonferroni-corrected $\alpha = 0.0056$). Repeated-measures ANOVA of those nests that survived until at least day 7 revealed a near-significant trend toward decreased mass in T nests (p = 0.0735, 8 T nests and 11 control nests). However, analyses of nests that survived to day 8 or to fledging revealed no significant differences in nestling mass (p = 0.1454 for the 8 T nests and 7 control nests that survived to day 8; p = 0.4584 for the 5 T nests and 7 control nests that fledged).

For comparison, Figure 4.2 also shows mean nestling mass from three nests at which the females had no male assistance.

Nest Success

T implants did not detectably affect nest success (Table 4.2). Mean number of fledglings produced per male was similar in the two groups (p = 0.9462). The probability that a male's first nest would produce at least one hatchling was 62.5% (10 of 16) for T males and 72.4% (21 of 29) for control nests (Fisher exact test, p = 0.5194). We obtained similar results when renests were considered as well: 68.8% of T males produced a hatchling in the first nest or a subsequent renest, compared to 82.8% of control males (p = 0.4550). Fledgling production was also similar for just those males whose nests survived to hatching: 80% (8 of 10) of T males with hatchlings produced a fledgling, compared to 67% (14 of 21) of control males (p = 0.6770). T nests also were similar to control nests in the size differential between smallest and largest nestlings, the probability of a single nestling dying while in the nest, the probability of the female abandoning the nest, and the probability of predation (Table 4.2).

Overwinter Survival

T implants did not detectably affect overwinter survival of either sex, though sample sizes are small. Since T may interfere with molt if T implants are not removed, we first examined only those T males whose implants were removed before molt began in 1992. Of these males, 43% (3 of 7) returned the next year, compared to 50% (10 of 20) of the control males (Fisher exact test, p>0.9999). We obtained similar results if we included the two T males whose implants were not removed, neither of whom returned the following year (Fisher exact test, p = 0.7225). Female sample sizes were smaller because not all females were banded. 20% (1 of 5) of the banded T females and 45% (5 of 11) of the banded control females were seen in 1993 (Fisher exact test, p = 0.6341). These survival rates are all similar. However, it is important to realize that the power of these tests is low with these sample sizes. A power analysis indicated that a small difference in survival, such as a 10-20% reduction, would likely not be detected as statistically significant unless the sample size was over 100 birds.

Timing of Female Fertility

The period of female fertility was brief and synchronized in both years (Fig. 4.3). Most females were fertile between 31 May and 10 June, with a pronounced peak in female fertility in the first week of June. Virtually no females were fertile after the median date of hatching in either year (Fig. 4.3). In 1994, a few females began nesting before the main peak in fertility, during a period of unusually warm weather in mid-May (Fig. 4.3). However, snowstorms occurred from 25 May 1994 to 1 June 1994, and all of these early nests failed. It is possible that some other females may have had early nests in 1994 that failed before we were able to find them.

DISCUSSION

Testosterone implants in male Lapland longspurs caused a transient decrease in male parental care. T-implanted males visited the nest very rarely when nestlings were 2-3 days old, while T females increased their visits and feeds. These results add to the growing body of literature indicating that one of the costs of high T for socially monogamous males is a reduction in paternal care (Hegner & Wingfield, 1987; Ketterson & Nolan, 1992; Saino & Møller, 1995). However, paternal care of the T-implanted males in this study recovered to near-normal levels as the nestlings grew older, with a normal visit rate and only a slightly depressed feeding rate. In response, T females showed a trend to reduce their compensatory feeding, and began visiting and feeding at rates very similar to control females. This recovery of parental care as nestlings grow has not been seen in other studies of T-implanted birds. Final fledgling weights and nest success of T males were not different from control males.

Interestingly, analysis of feeds often produced different results than analysis of number of nest visits. For instance, control males with young (day 2-3) nestlings visited less than their females, but provided as many feeds. Many studies of parental care assess nest performance solely by number of nest visits. Our results indicate that in some cases, the number of nest visits may not reveal important patterns in parental care. This may be particularly important when comparing sexes, since male birds often visit the nest only when bringing food, but females typically have additional reasons to return to the nest (e.g. brooding). For example, in this study, we occasionally observed females returning to the nest without bringing any food at all, but we never observed a male coming to the nest without food. We suggest that studies of parental care in passerines should include, whenever possible, some estimate of the amount of food delivered, and should not rely entirely on number of nest visits.

Our results raise several questions: 1) What is the mechanism of conflict between high T and parental care? 2) Why did parental care recover in this study as nestlings grew older? and ultimately, 3) What might be the selective forces that maintain the classic "monogamous male" pattern of T secretion, with low T during the parental phase, in Lapland longspurs and in other species?

Mechanism of Conflict Between High T and Parental Care

High T may interfere with parental care via such factors as neglect of the young, neglect of nest defense, or direct aggression toward the young (Wingfield & Moore, 1987; Oring *et al.*, 1989 ;Ketterson *et al.*, 1996). In this study, we never observed a male attacking the young. In a related study, male Lapland longspurs with high-T implants increased their singing rates but were not more aggressive to intruders (Hunt *et al.*, 1997). This suggests that the reduced parental care of high-T males with young nestlings may be due to neglect of the young, caused by the males' devoting less time to parental care and more time to other activities (such as singing). However, we do not have focal observation data to test this hypothesis for the individuals in this study. In other species, nest defense by the male may also be important (e.g. dark-eyed junco, Ketterson *et al.*, 1996). Our anecdotal observations of Lapland longspurs suggest that males may help defend the nest by acting as sentries during early incubation, but they cease sentry behavior after mid-incubation. In this study, T and control males had an identical 33% risk of nest predation, suggesting that high T does not affect nest defense.

Why Did Parental Care Recover as Nestlings Grew?

Male Lapland longspurs with T implants returned to near-normal levels of parental care, relative to control males, as nestlings grew older. In other studies that have tracked changes in parental care of T-implanted males, the males' visit rate either did not improve or actually declined over time, relative to controls. For example, in dark-eyed juncos, T male's parental care relative to control males declined as nestlings age, and T females compensated more and more compared to control females (Ketterson *et al.*, 1992). A similar pattern was seen in house sparrows (*Passer domesticus*); in this species, males typically reduce their number of nest visits as nestlings grow, but T-implanted males reduced their nest visits faster than control males (Hegner & Wingfield, 1987). T males completely ceased feeding by day 12, while control males were still contributing 20% of total nest visits (Hegner & Wingfield, 1987). We did not see this pattern in Lapland longspurs. Instead, the T males increased their visits and feeds to near-normal rates, and the females' extent of compensation (e.g. T female feeding compared to control female feeding) actually declined. In other words, the high T became less effective over time at disrupting parental care. This pattern has not been documented before. We believe that this recovery in paternal care is unlikely to be due to a decline in release of T from the implants, for two reasons. First, the hormone data, where available, shows that T males have high T (over 8 ng/ml) through the end of the breeding season, and the implants still had visible crystalline hormone at the end of the study (Hunt *et al.*, 1997; Hunt, unpublished data). Second, it seems unlikely that the hormone released from the implants would decrease significantly over just two days, the elapsed time between the first and second videotapes.

Generally, a male's commitment to parental care should be greater if he has a low probability of encountering other fertile females, and/or if the female is unable to compensate for lack of his assistance at the nest (Kempenaers & Sheldon, 1997; Westneat & Sargent, 1996). Both of these variables may change during a season, and males may alter their commitment to the nest in response. For example, a male bird may monitor nestling cues, such as begging intensity (e.g. Whittingham & Robertson, 1993), and may not invest much time or energy in feeding nestlings until his assistance is required for nestling survival. If the number of available fertile females, the need for territory defense, and/or the nestlings' need for male assistance change in predictable ways, this may result in selection for a certain seasonal pattern of parental care, which in turn may select for a certain seasonal pattern of T secretion and/or responsiveness to T.

These factors do change predictably during the Lapland longspurs' breeding season. First, the availability of fertile females is strongly curtailed by the very short breeding season of Lapland longspurs, as indicated in Figure 4.3. In contrast to dark-eyed juncos and house sparrows, which typically produce several broods per season (Hegner & Wingfield, 1987; Ketterson *et al.*, 1996), Lapland longspurs are single-brooded. Furthermore, Lapland longspurs tend to breed very synchronously (Fig. 4.3; Williamson, 1968b). Once the eggs hatch, there is almost no chance that a male will encounter another fertile female. This eliminates one of the major potential benefits of high T for a male -- increased attractiveness to other potential mates. Another potential benefit of high T is enhanced territorial defense and/or resource defense, but Lapland males cease to defend their territories in mid-incubation when invertebrate food resources become abundant (Tryon & MacLean, 1980), and after incubation starts they generally do not respond to high T with increased territorial aggression (Hunt *et al.*, 1997). Finally, the need for male assistance at Lapland longspur nests may increase predictably as nestlings age. Though the data are sparse, the results from the three nests with no males (Fig. 4.2) suggest that female Lapland longspurs can probably compensate for lack of male assistance until day 4 or 5, after which nestling mass and nestling survival fall below normal. (Note that male-removal experiments would be required to verify that male care is necessary for normal nestling growth in Laplands). Parental care after fledging may be particularly important in Lapland longspurs, since Lapland longspur parents split their broods, with each adult taking charge of 1-3 fledglings (McLaughlin & Montgomerie, 1985). Reduced paternal care at this time would probably be catastrophic for those fledglings that were under the male's care.

Taken together, the short season and increasing need for male assistance may have selected for males that exhibit a greater commitment to parental care as nestlings age. Such increasing commitment to parental care during the first week of nestling growth is, in itself, not particularly surprising; it is known to occur in unimplanted males of this and of other species (e.g. Breitwisch *et al.*, 1986; Conrad & Robertson, 1993). However, Laplands are unusual in that the increasing commitment to parental care occurs even in males with T implants. That is, not only do male Laplands have low circulating levels of T throughout the period of feeding young, but, in addition, males appear to lose sensitivity to T as nestlings age. A related possibility is that male Laplands may be particularly responsive to behavioral cues from nestlings (e.g. begging), regardless of T level. Perhaps this decreased sensitivity to T is a sort of fail-safe; with both low circulating T and low sensitivity to T (and/or increased responsiveness to cues from nestlings), male Laplands may doubly ensure their ability to provide high parental care. It is worth noting here that in other species, males that generally have low T during the parental phase may still experience brief spikes of higher T. This may occur, for instance, when a male is challenged by an intruder (Wingfield *et al.* 1987). In the Lapland longspur, reduction of sensitivity to T may ensure that even such brief T spikes would not interfere with parental care.

Selective Forces on the Pattern of T Secretion

Studies on other avian species have shown great variation in the effect of parental-phase T implants on the male's reproductive success (Ketterson *et al.*, 1996). Major causes of this variation include: whether high T reduces male parental care; if it does, whether the females then compensate for reduced care from the male; whether T affects genetic parentage at the primary or other nests; and whether T has hidden costs (e.g. lowered overwinter survival). These benefits and costs of high T may in turn be affected by such factors as length of the breeding season, as discussed above, and male-male density. By examining these patterns further, we can elucidate some of the underlying forces of natural selection and sexual selection that presumably have driven the evolution of seasonal patterns in circulating hormone levels. In general, we expect that the typical seasonal pattern of T seen in monogamous male birds -- high T during the sexual phase, and low T during the parental phase -- should prove to be the most fit pattern (Ketterson *et al.*, 1996). Here, we will not attempt a full review of the literature or of all possible costs and benefits of T (see Wingfield & Farner, 1993 and Ketterson *et al.*, 1996), but will instead concentrate on comparison of results of experimentally elevated T on paternal care. Generally, the results can be divided into three groups: 1) T implants reduce male care, with no or inadequate female compensation; 2) T implants reduce male care, with full female compensation; and 3) T implants do not reduce male care.

In the first group, which includes most species studied to date, experimentally elevated T reduces paternal care, and females do not compensate fully. As a result, the T males may fledge fewer or smaller chicks than control males, and high T carries an obvious cost for both parents. This is the result we would expect to see if the natural pattern of T secretion over the breeding season is indeed the pattern that maximizes fledging success at the male's primary nest. Examples include the house sparrow (Hegner & Wingfield, 1987); pied flycatcher (*Ficedula hypoleuca*; Silverin, 1980); yellow-headed blackbird (*Xanthocephalus xanthocephalus*; Beletsky *et al.*, 1995); reed warbler (*Acrocephalus scirpaceus*, Dittami *et al.*, 1991); and the spotted sandpiper (*Actitus macularia*, Oring *et al.*, 1989; note that females normally do not assist in parental care in this species). However, most of these studies did not track all possible effects of T (such as over-winter survival), concentrating more or less exclusively on parental care.

In the second category, T implants result in reduced paternal care, but females can and do compensate fully. Thus, the T-implanted males fledge normal numbers of chicks with normal body mass. This is usually an unexpected result, as it seems to indicate that the "normal" seasonal pattern of T secretion is not necessarily the most fit pattern. The best example of this group, and the most complete study to date on the effects of exogenous T in a passerine, is the dark-eyed junco (reviewed in Ketterson *et al.*, 1996). T-implanted juncos have lowered nest visit rates, but females fully compensate with increased visit rates, and nestlings grow and fledge normally. T-implanted males appear to gain an extra benefit of increased attractiveness to other females, and in addition defend larger territories and spend less time at the nest. The resulting complicated mix of costs and benefits (at the male's primary nest, at other nests, and at future nests) appears to result in T males and control males having nearly identical fitness. Lapland longspurs with day 2-3 nestlings also appear to fall in this group, with reduced parental care from T-implanted males, full compensation by females, normal nestling growth, and normal fledging success (this study).

Finally, it is theoretically possible for T implants to have no appreciable effect on male paternal care, such that nestlings and females are unaffected. This, too, would be an unexpected result. For instance, male Lapland longspurs with day 4-5 nestlings showed only a slight effect of T on paternal care, which did not noticeably affect nestlings and required little, if any, compensation by females. We are aware of only one experimental avian study that found no effect at all of T on paternal care: implants of T blockers do not decrease parental care in male bobwhite quail (*Colinus virginianus*; Vleck & Dobrott, 1993). Note, however, that T implants were not performed, and that males were tested with foster young. There is one non-avian study that may also fit in this category. Male coqui frogs, *Eleutherodactylus coqui*, have a seasonal T profile similar to that of most birds, with high T during the sexual phase and low T during the parental phase, and are analogous to bobwhite quail in that males protect and brood young but do not feed them. T implants do not reduce parental care in male coqui frogs (Townsend *et al.*, 1991).

We return now to the issue of what selective forces are responsible for the near-ubiquity of the common T profile of monogamous birds, with low T during the parental phase. Species such as dark-eyed juncos and Lapland longspurs, in which high T apparently does not decrease fitness, raise a question: why do males of these species have low T during parental phase, if a high-T phenotype has nearly identical fitness? One obvious answer is that these studies may not have detected some costs of high T. For example, most studies track nestling survival only until fledging, but survival of T-implanted young may be decreased after fledging. Additionally, it is possible that young of T-implanted males may have decreased survival only during "bad" years, and not during "good" or "average" years. Finally, many field studies cannot attain the very large sample sizes (n's over 50) that are required to detect small differences between experimental groups (Thomas & Juanes, 1996). However, even the dark-eyed junco study, a long-term study with quite large sample sizes in which young were monitored after fledging, has revealed no differences in fitness between control and high-T males.

One further possibility is that sexual selection by females may play a role in maintaining high male parental care, with low circulating T and/or low sensitivity to T during the parental phase. For instance, suppose that in Lapland longspurs, females can compensate fully for lack of male care, but also incur some cost (e.g. reduced fat stores, poor overwinter survival) in doing so. In such a situation, females should attempt to choose males who demonstrate somehow that they will have low T levels (and high parental care) during the parental phase. For example, a female might mate with a male whom she had observed in the previous year to exhibit high parental care. We should also bear in mind that the sexes may be in conflict about the "desired" T level of males. If high T benefits the male but harms the female, we would expect females to attempt to choose males with low T, while, in turn, males should attempt to have higher T than the females would prefer. It would be particularly interesting to see if female choice on T profile -- if it occurs at all -- might vary with the ability of the female to compensate for lack of paternal care.

Ultimately, in those species in which T reduces male parental care, the temporal pattern of T secretion should be determined by such factors as: the potential for the male to sire further offspring during the rest of the breeding season (low if breeding season is short; high if nest predation is common); the need for the male to continue defending his territory (high if male-male aggression is common; high if resources are scarce); ability of the female to compensate (low if brood is split after fledging; low if male plays an essential role in nest defense); cost to the female's future reproductive efforts if she does compensate; and other costs to the male (e.g. overwinter survival). Female choice on male T profile may theoretically occur in some species. We suspect that males of species with short breeding seasons, and in which females cannot compensate for loss of male assistance, may show not only low T but also low responsiveness to T implants. The evolutionary causes and consequences of common patterns of hormone secretion may be further explored with studies of birds with short breeding seasons, and birds with obligate paternal care.

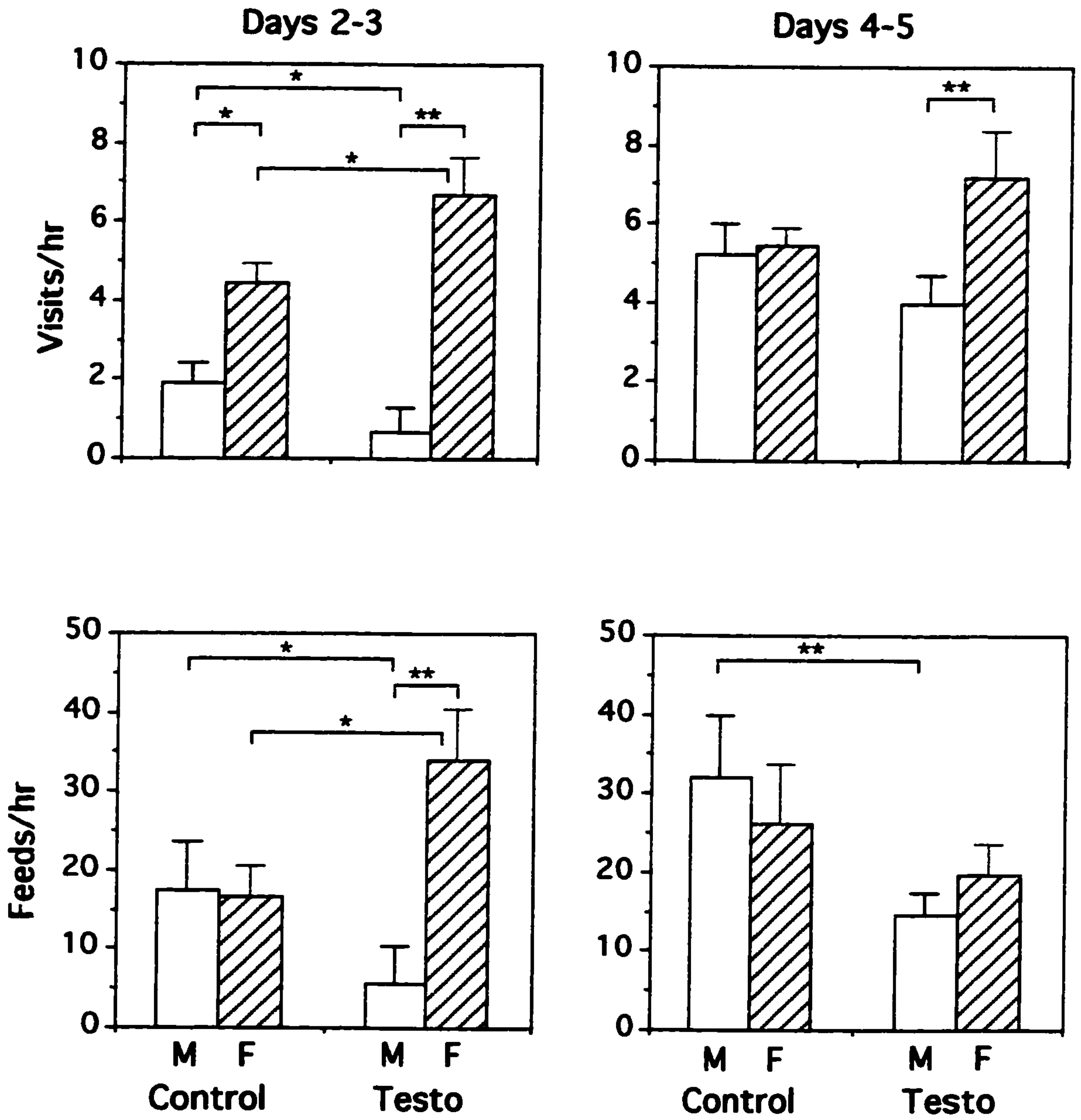

FIGURE 4.1. Parental care of Lapland longspur pairs in which the male had an empty or no implant ("Control") or a testosterone implant ("Testo"). The left panels show nest visits per hour (top) and feeding movements per hour (bottom) when nestlings were 2-3 days old; the right panels show nest visits and feeding movements when nestlings were 4-5 days old. M = males (open bars); F = females (hatched bars). Data shown are mean ± sem; * indicates $P<.05$, and ** indicates $P<.01$. Sample sizes = 10 control nests and 10 testosterone nests.

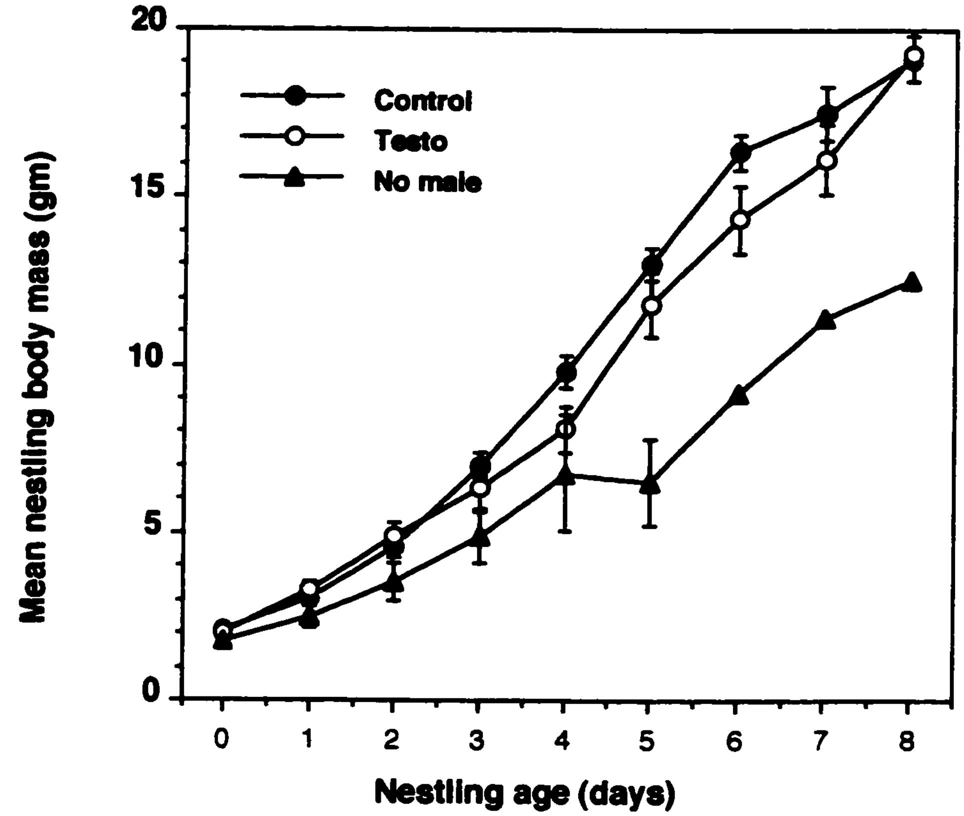

FIGURE 4.2. Mean nestling growth (± SEM) for Lapland longspur nests at which males were control-implanted (n=15) or testosterone-implanted (n=9). Data are also shown for three nests at which no male was present.

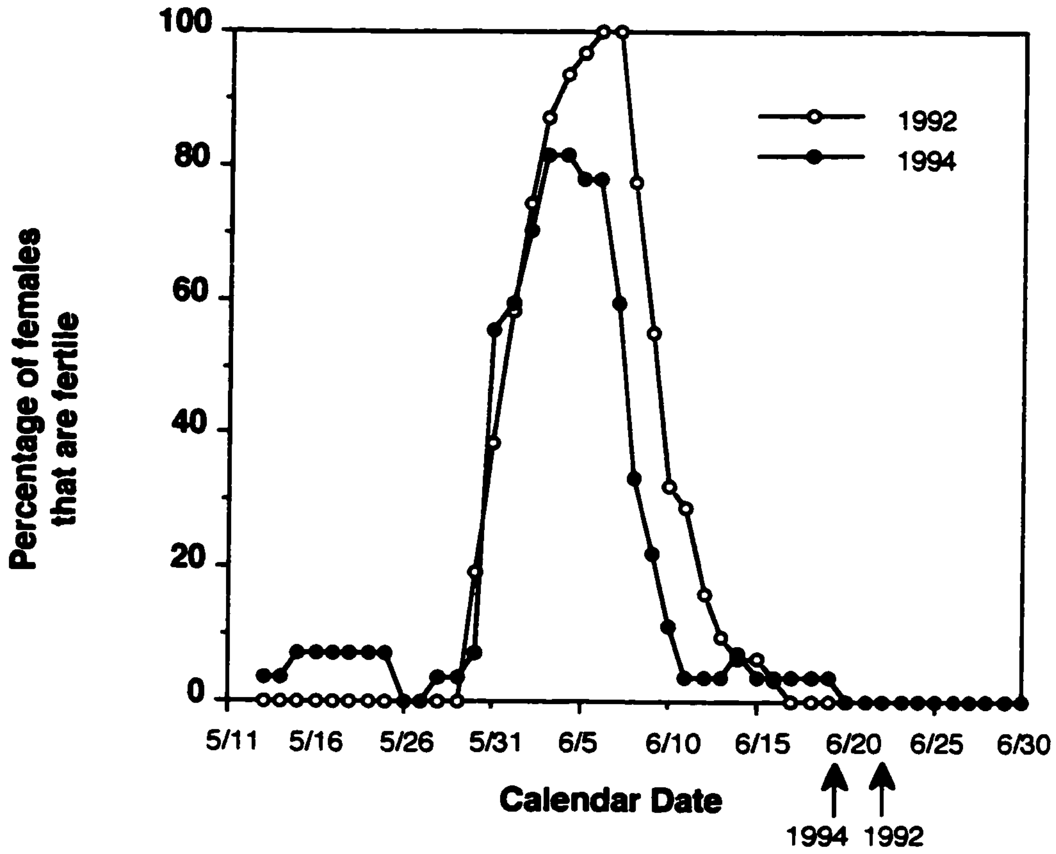

FIGURE 4.3. Timing of the fertile period of female Lapland longspurs at Toolik Lake in 1992 and 1994. The figure shows the percentage of all females of known breeding status who were fertile on the dates shown. Arrows show the median date of hatching for each year. Sample sizes were 31 females in 1992 and 27 females in 1994.

Table 4.1. Testosterone, body mass, fat stores, and cloacal protuberance length of control and T-implanted male Lapland longspurs. U-values are from Mann-Whitney U tests, and t-values are from t-tests.

	Control		T-implanted			
	Mean ± sem (n)	Median	Mean ± sem (n)	Median	U,t	P
Testosterone (ng/ml)						
Before implant	4.536 ± 1.277 (7)	4.29	4.977 ± 1.397 (9)	4.19	U=32.0	0.9578
With implant	0.426 ± 1.397 (7)	0.35	23.479 ± 5.261 (8)	20.67	U=56.0	0.0006
Body mass (g)						
Before implant	26.950 ± 0.672 (6)	27.2	27.100 ± 0.223 (9)	27.1	t=0.248	0.8080
With implant	27.078 ± 0.453 (9)	26.6	28.438 ± 0.395 (8)	28.1	t=2.234	0.0411
Fat stores						
Before implant	0.714 ± 0.101 (7)	0.5	0.889 ± 0.247 (9)	0.5	U=34.0	0.7808
With implant	1.222 ± 0.222 (9)	1.5	1.688 ± 0.210 (8)	2.0	U=52.5	0.0917
CP length (mm)						
Before implant	6.286 ± 0.421 (7)	6.0	6.667 ± 0.236 (9)	7.0	U=38.5	0.4333
With implant	5.250 ± 0.366 (8)	5.0	7.625 ± 0.324 (8)	8.0	U=61.0	0.0095

Table 4.2. Hatching success, fledging success and cause of nest failure in nests of T-implanted and control male Lapland longspurs. Proportions are #pairs of all pairs in that category who had nests. Means are shown ± SEM with sample size in parentheses. Sample sizes vary due to late discovery of some nests and depredation or late hatch of other nests. P-values for proportions are from Fisher exact tests, and P-values for means are from t-tests. Chick mass differential = body mass of smallest chick divided by body mass of largest chick; data from day 4. Data are not included from one T nest and six C nests that hatched late and could not be followed to fledging.

	Control	T-implanted	P
Of pairs with nests:			
Eggs depredated	4 of 29 pairs	3 of 16 pairs	0.6860
Eggs or nestlings depredated	9 of 27 pairs	5 of 15 pairs	>0.99
At least 1 egg hatched (1st nest)	21 of 29 pairs	10 of 16 pairs	0.5194
At least 1 egg hatched (1st or renest)	24 of 29 pairs	11 of 16 pairs	0.4550
At least one fledgling produced	15 of 26 pairs	8 of 15 pairs	>0.99
Nest abandoned, snowstorm	3 of 12 pairs	3 of 8 pairs	0.6424
Nest abandoned, any cause	3 of 27 pairs	4 of 16 pairs	0.3944
Mean clutch size per pair	4.379 ± 0.182 (29)	4.812 ± 0.188 (16)	0.1319
Mean #hatchlings per pair	3.069 ± 0.364 (29)	2.500 ± 0.563 (16)	0.3819
Mean #fledglings per pair	1.586 ± 0.346 (29)	1.625 ± 0.446 (16)	0.9462
Of pairs with nests that hatched:			
Nestlings depredated	5 of 21 pairs	1 of 10 pairs	0.6342
Other nestling death	7 of 24 pairs	5 of 10 pairs	0.2713
At least one fledgling produced	14 of 21 pairs	8 of 10 pairs	0.6770
Chick mass differential^{d}	0.577 ± .042 (19)	0.544 ± .051 (10)	0.6422

CHAPTER 5. Endocrine Influences on Parental Care During a Short Breeding Season: Estradiol and Female Nest Behavior in Lapland Longspurs

INTRODUCTION

The timing of reproduction and of reproductive behaviors is critical for animals that breed in high-latitude environments (Immelmann, 1971; Pielou, 1994). Arctic birds must initiate sexual behavior immediately upon arrival at the breeding grounds, and then must soon switch to parental behavior (Carey, 1988). Sexual behavior may be reinitiated if the first nest is lost early, enabling pairs to produce a new clutch. In the Arctic, however, renesting ability is lost after a few weeks. In seasonally breeding vertebrates, the initation and termination of such sexual and parental behaviors is usually strongly affected by reproductive hormones, particularly the sex steroids (Balthazart, 1983; Harding, 1983). Circulating levels of sex steroids in both males and females are usually high during the sexual phase, and then decrease sharply when birds enter the parental phase (e.g. Wingfield & Farner, 1993. Consequences of the decline in testosterone on male sexual behavior and paternal care are well-studied in temperate-latitude species (Wingfield *et al.*, 1990; Beletsky *et al.*, 1995). However, little is known about control of the initiation and termination of sexual and/or parental behavior of high-latitude species, in which the timing of these behaviors is particularly crucial.

In temperate-latitude species, the reduction of steroids (and corresponding sexual behaviors) in either sex during the parental stage results in an obvious cost. By reducing sexual behaviors the birds may be giving up reproductive opportunites, such as rapid initiation of renests if the current nest is lost, extra-pair copulations for males, and intraspecific parasitism for females (e.g. Ketterson & Nolan, 1992). Thus, it has been theorized that there must be a benefit to the sharp reduction in sexual hormones and sexual behavior, specifically, that sex steroids may be incompatible with parental behavior. If this is so, reducing levels of sex steroids should result in improved care of current offspring that more than compensates for the loss of potential additional offspring. Research in this area has focussed almost entirely on testosterone's effect on male parental behavior in multiple-brooded species. Numerous studies have shown that high testosterone does cause reduced parental care in males of these species (e.g. Hegner & Wingfield, 1987; Ketterson *et al.*, 1996; Chapter 4).

Similar reasoning may apply to females, in which the ovarian steroids estradiol and progesterone are typically elevated during the sexual phase and then decline to low levels during incubation. Some of the major physiological roles of estradiol are to promote growth of the oviduct and the ovary, and synthesis of yolk by the liver. In addition, estradiol plays an essential role in activation of various sexual behaviors, such as copulation solicitation and nest-building, and these behaviors can in turn affect sexual behavior and testosterone levels in the female's mate (e.g. Moore, 1982; Wingfield *et al.*, 1989). It is possible that the sharp reduction in these sex steroids after laying in females may be as critical as similar reductions in testosterone in males, and that disruption of these patterns may have deleterious effects on lifetime reproductive success. For example, a reduction in estrogen may result in costs such as the loss of opportunities for renesting, while prolonged high levels of estrogen might disrupt parental care and result in decreased survival of offspring. However, there have been almost no experimental studies that focus on the potential costs and benefits of high sex steroid levels in females in the wild.

Birds that have a very short breeding season, such as arctic birds, may have different costs and benefits associated with patterns of sex steroid secretion than do birds of temperate latitudes. In particular, cessation of sexual behaviors after the first nest has been started may carry almost no cost for arctic birds, since further reproduction is highly unlikely to be successful. Thus, the observed decline in sex steroids after the sexual phase may be unrelated to parental care, and may instead be due to the low potential for successful reproduction late in season. If this is so, experimentally increased levels of sex steroids in either sex may not interfere with parental behavior. Additionally, arctic birds may have different methods of control of sexual behaviors. For example, males of temperate-zone species often show increases in testosterone and sexual behavior in response to sexual solicitation from females (Balthazart, 1983), but we predict that arctic birds would not be responsive to such behavioral cues after the first nest has been started.

We have conducted a five-year study on the normal hormone-behavior patterns and the effects of experimentally elevated steroid hormones on sexual and parental behavior in male and female Lapland longspurs (*Calcarius lapponicus*). The Lapland longspur is a single-brooded, socially monogamous passerine that breeds on the arctic tundra. It is notable for its short breeding season, brief hormonal peaks, and brief expression of sexual behavior, which we have described previously (Hunt *et al.*, 1995; Hunt *et al.*, 1997). Here we report the effect of an experimentally altered pattern of estradiol in females on nest behavior, nest success, and survival of free-living Lapland longspur pairs during one year of our study. We focus on three questions: First, does elevated estradiol allow increased sexual activity, including initiation of new nests, at an inappropriately late time in the breeding season? Second, does elevated estradiol interfere with parental behavior? Third, does estradiol level of female Laplands affect the behavior or testosterone levels of their mates?

METHODS

Study Population & General Methods

We studied Lapland longspurs at Toolik Field Station, Alaska (68° 38'N, 149° 38" W), during May and June of 1993. We gave subcutaneous implants of 17-β estradiol (E_2) to 15 female Lapland longspurs, empty implants to nine females, and no implants to 14 females (see below for details). The unimplanted females were otherwise treated identically to implanted females, e.g. unimplanted and implanted females were captured and handled with similar frequency. All females had known nests, and all but one had known mates. One E_2 female, band #63766, appeared not to have assistance from any male after she completed her clutch.

Empty-implanted and unimplanted females behaved very similarly (see Results), and thus we refer to them collectively as "control females". Males paired to E_2-implanted females are "E_2 males", and males paired to control females are "control males" (note that the males themselves did not receive implants). Similarly, nests of E_2 females are "E_2 nests", and nests of control females are "control nests".

Birds were captured in Potter traps baited with seeds or at mist nets placed by nests. Each bird was banded with an aluminum U.S. Fish & Wildlife Service band and a unique combination of color bands for subsequent visual identification and observation. We bled each bird when appropriate and checked the bird's hormone implants (if any). No birds were bled more than once a week.

We checked general condition of each bird by measuring body mass to the nearest gram, and assessing fat stores, brood patch development of females, and cloacal protuberance length of males. Observers were unaware of the experimental status of each bird while measuring these features. We ranked fat stores from 0 to 5, where 0 = no fat, and 5 = bulging fat deposits (see Wingfield & Farner, 1978b). Furcular fat and abdominal fat were ranked separately and then averaged to give a mean fat score for each bird. We also ranked brood patches on a scale of 0-5, with 0 = undeveloped brood patch, with abundant downy feathers and smooth skin; 1 = loss of some feathers from brood patch but no edema; 2 = no feathers on brood patch and slight edema; 3 = peak of brood patch development, with no feathers, pronounced edema, and warm, puffy skin; 4 = no feathers and edema disappearing, leaving wrinkled skin; and 5 = refeathering of brood patch (modified from Wingfield & Farner, 1978b). Cloacal protuberances (CP, an androgen-dependent copulatory organ of males) were measured to the nearest mm with a small ruler placed at the antero-ventral surface of the cloacal protuberance. For analyses of mass, fat, and brood patch, control birds were selected to match E_2 birds for reproductive stage and date. Owing to the brevity of the breeding season (which limited the time available for capturing males), full sample sizes of control males for the CP comparison required more than one year of study. Final sample sizes for the CP analysis were six estradiol males and nineteen control males (six from 1993 and 13 from 1994).

Estradiol Implants

Females were implanted between 28 May 1993 and 4 June 1993. In most cases this was the first or second day of incubation, though two control birds were implanted on the fifth day of incubation. We chose to insert implants just after laying, rather than before, so that the surge of exogenous estradiol would not disrupt ovarian development or egg formation.

The subcutaneous E_2 implants consisted of crystalline estradiol (Sigma, St. Louis, Missouri) packed into 12-mm lengths of silastic tubing sealed with Silastic medical adhesive (i.d. 1.47mm, o.d. 1.96mm; Dow Corning: Midland, Michigan). We anesthetized birds in the field with a light dose of an inhalant anesthetic, methoxy-fluorane (Metofane, Pitman-Moore, Mundelein, Illinois), and inserted one implant beneath the skin on the bird's left side, between the wing and knee (for details see Hunt *et al.*, 1997). The incision site was closed with a veterinary skin bond (Nexaband, Tripoint Medical: Raleigh, North Carolina). We checked the implants each time a bird was captured, and removed them at the end of the season whenever possible. Note that measurement of circulating levels of E_2 allowed us to assess the effectiveness of the E_2 implants, and that placement of empty implants into control birds allowed us to control for the possible stressful effects of the implant procedure.

The mean elapsed time between insertion of the implant into the female and subsequent observations was 5.6 days for observations of male territorial behavior, and 15.4 days for videotaping of nest behavior of males and females. Previous studies (e.g.

Wingfield, 1984b; Chandler *et al.*, 1997) have shown that steroid implants begin to release measurable levels of steroid hormones within a few hours, and then continue to release steroids at a slowly declining rate for approximately 60 days.

Blood Sampling & Radioimmunoassay

We obtained blood samples by puncturing the alar vein and collecting approximately 250-300 ul of blood into heparinized microhematocrit tubes. The tubes were stored temporarily in small field coolers filled with ice packs, and were centrifuged within six hours. We then stored the plasma at -20°C and later shipped it on dry ice to the University of Washington for radioimmunoassay. We measured plasma levels of E_2 and P in females, and of T in males, by radioimmunoassay after partial purification of steroid extracts on diatomaceous earth/glycol micro columns. We did not assay samples for corticosterone because the 5-20 minute delay involved in checking the traps would likely result in highly variable corticosterone levels due to capture stress. All samples from females were analyzed in the same assay, while samples from males were analyzed in two assays. The testosterone assay has been described in detail by Ball & Wingfield (1986). Inter- and intra-assay variations for male testosterone are reported in (Hunt *et al.*, 1997).

Estradiol and progesterone (P) were assayed with a modification of the techniques described in Wingfield & Farner (1975) and Wingfield *et al.* (1991) as follows. Samples were equilibrated overnight with approximately 2000 cpm each of tritiated progesterone and tritiated 17-β estradiol, and were then extracted with 4 ml ethyl ether and dried under nitrogen gas. Dried extracts were then redissolved in 0.5 ml of 10% ethyl acetate in iso-octane and were transferred to the top of diatomaceous earth microcolumns. The columns used were "short columns" (modified from Wingfield & Farner, 1975), consisting of a 0.3-ml diatomaceous earth:water (3:1, v/v) glycol trap with a 0.6-ml diatomaceous earth:propylene glycol:ethylene glycol (6:1.5:1.5 w/v/v) stationary phase. (These "short columns" are less susceptible to drying out than traditional long columns, and may be used when it is not necessary to separate estradiol from corticosterone.) Fractions were eluted in order of polarity with increasing concentrations of ethyl acetate in iso-octane. The first two fractions were 2 ml of pure iso-octane, which elutes most P, followed by 1.5 ml of 10% ethyl acetate in iso-octane, which elutes dihydrotestosterone and the remainder of P. These two fractions were collected into the same tube and saved for P radioimmunoassay. The third fraction, 2.0 ml of 20% ethyl acetate in iso-octane, contained testosterone and was discarded. The fourth fraction, 2.5 ml of 40% ethyl acetate in iso-octane, contained E_2 (and some corticosterone), and was collected and saved for E_2 radioimmunoassay. The P and E_2 fractions were dried under nitrogen gas, redissolved in assay buffer, and allowed to equilibrate overnight at 4C. Fractions were assayed with RIA, using standard curves over the range of 4-1000 pg for P, and 2-500 pg for E_2. Bound and unbound hormones were separated by adding 0.5 ml of dextran-coated charcoal for 12 min followed by centrifugation at 2000 rpm for 10 min. The supernatant (containing bound hormone) was decanted into scintillation vials and mixed with 4.5 ml of Ultima Gold scintillation fluid (Packard Instrument Co., Meriden, CT, USA). Samples were counted for 10 min in a Beckman LS 3801 liquid scintillation counter.

Incubation Length & Nestling Growth

We found the nests of all fifteen E_2 females, nine empty-implanted females, and fourteen unimplanted females. All nests were checked daily as soon as they were found. On each day, we counted the number of eggs and nestlings present in each nest, and measured every nestling's body mass daily to the nearest 0.01 g, using a portable digital scale.

The duration of incubation could be exactly determined only for thirteen nests which were discovered during laying and which survived at least to hatching. Six of these nests belonged to E_2 females, and the remainder to empty-implanted (5) or unimplanted (2) females. Incubation length was calculated by subtracting the date of laying of the second-to-last egg from the date that the first nestling was found. Note that we considered incubation to begin on the day on which the last egg is laid, and we considered the last day of incubation to be the day before a nestling was found in the nest. An additional six nests (4 E_2 and 2 unimplanted) were discovered during laying and survived an incubation of at least 11 days, which is the mean duration of incubation of Lapland longspurs at Toolik (K. Hunt, unpublished data), but were then depredated before hatching. For nests which were discovered after laying, we used an average incubation length of 11 days to estimate the dates of laying.

Nestling weights were averaged for each nest on each day. The resulting mean nestling weights were compared between E_2 and C nests with repeated-measures ANOVA. Due to a high predation rate throughout the nestling stage, we conducted two separate analyses. The first analysis included data from day 0 to day 4 from all nests that survived until day 4. The second included data from day 0 to day 7 from only those nests that survived until fledging. We excluded nestling growth data from the E_2 female who apparently did not have a mate, since her nestlings showed a markedly reduced growth rate.

Parental Care

We videotaped nine E_2 nests and ten control nests for two hours each, when nestlings were four or five days old. We chose this age because nestlings at this time grow rapidly and require frequent feeding, but are not yet old enough to fledge prematurely due to nest disturbance (Chapter 4). We chose control nests to match E_2 nests as closely as possible for clutch size and nestling age (Table 5.1). All nests were videotaped between 12 June and 19 June, 1993. Because E_2 nests tended to hatch slightly later than control nests (see Results), E_2 nests were videotaped on slightly later dates than control nests (Table 5.1). Again, we excluded data from the female who did not have a mate.

We videotaped nests with battery-powered VHS videocameras mounted on tripods and placed 1-2 m from the nest. We allowed birds a minimum of two hours to acclimate to the camera's presence before taping began, by placing a videocamera or a dummy (a black box mounted on a tripod) 1-2 m from the nest. The purpose of the dummy was to habituate birds to the presence of a camera-shaped object at times when the real cameras were in use at other nests. After the minimum two hours had passed, an observer returned briefly to turn on the camera, and then immediately left the area. Since the birds' behavior during the first few minutes was undoubtedly affected by the brief presence of the observer, behavior on the videotapes was scored starting when the first adult bird (usually the female) returned to the nest. This was typically 5-10 minutes after videotaping began. Male and female longspurs appeared to acclimate well to the presence of the videocamera or dummy, and typically gave no alarm calls during the two-hour taping after the observer left. Videotapes were scored by two observers unaware of which females had estradiol implants. Inter-observer reliability was near 100%. Videotapes were viewed on a VCR with a real-time counter, allowing a detailed time-activity analysis of female and male behavior at the nest. Nest video data were tallied and checked with a customized Macintosh computer program (J. Muhasky, 1996, using Oracle Media Objects v.1.1) that calculated durations and total times of activity bouts for both parents of each nest, and checked all data for any missing or improbable time intervals.

Females and males were each scored for percent time at the nest, number of visits per hour, and number of feeding movements ("feeds") per hour. A feed is a discrete movement of the parent's head downward toward the young, presumably to give a parcel of food to one nestling. Thus, during one nest visit a parent usually performs several feeds. Females were also scored for number of digs per hour, which is a female-specific behavior that appears to be related to nest maintenance. During a typical dig, a female stands up and looks down at the young, sticks her bill deep into the nest while leaning sharply head-down, and vibrates her whole body rapidly for 1-2 seconds. Usually several digs are performed in succession. We also subdivided each female's time at the nest into time spent feeding young, time spent brooding young, time spent standing (including digging), and time spent interacting with male. Brief adjustments in a female's position that lasted less than five seconds, and that did not involve a dig, were not counted. When females left their nests, we noted any apparent reason for the departure. We also noted the nature of any interactions between male and female, particularly regarding the female's response if the male arrived while the female was in the nest.

Renesting

As soon as a female was known to have lost a nest, we monitored that female approximately one hour per day for signs of renesting. As Lapland longspur nests can be difficult to find, we searched extensively for renests before concluding that a female had not renested. Our search area for each female encompassed her mate's original territory, plus a circle of approximately 200m diameter centered on the site of the first nest, plus any areas where the female was frequently seen. A female was considered not to have re-nested if she satisfied all of these criteria: 1) After loss of the first nest, she was never seen carrying nesting material or acting as if she had a nest nearby (giving alarm calls and coming close to the observer, or appearing suddenly and preening), and her mate did not act as if a nest were nearby. 2) No birds were flushed when a single observer walked back-and-forth across the whole search area in a tight zig-zag pattern, passing within 1m of every point. 3) No birds were flushed when ropes were dragged across the whole search area. 4) If the female was captured after the nest was lost, she did not have a well-developed brood patch.

Nest Success

We assumed that chicks that disappeared on or after day 8, with the nest remaining intact, fledged successfully. Chicks that disappeared before day 8, and chicks that disappeared on or after day 8 but with the nest torn apart, were assumed to have been taken by a predator. Lapland longspur chicks usually leave the nest when they are 10 or 11 days old, but may leave as early as 7-8 days if disturbed by a predator or a scientist (Williamson, 1968b; K. Hunt, unpublished observations). Common predators of Lapland longspur chicks at our field site include arctic ground squirrels (*Citellus parryi*), red fox (*Vulpes fulva*), common raven (*Corvus corax*), and long-tailed jaeger (*Stercorarius longicaudus*).

Male Territorial Behavior

If E_2 females show increased levels of sexual solicitation, their mates might respond with increased territorial aggression, as has been shown for the song sparrow, *Melospiza melodia* (Runfeldt & Wingfield, 1985) and white-crowned sparrow, *Zonotrichia leucophrys pugetensis* (Moore, 1983). Therefore, we studied aggression of E_2 and control males with simulated territorial intrusions. Male Lapland longspurs are known to respond strongly to simulated intrusions when they are mate-guarding their females (Chapter 3). This technique has been described in detail elsewhere (Hunt *et al.*, 1995; Hunt *et al.*, 1997); briefly, we placed a live caged decoy 20m from the nest of each male with playback of conspecific song, and recorded the resident male's responses for the next ten minutes. For this study, we scored each male as "aggressive" or "not aggressive", where aggressiveness is defined as the occurrence of attacks and/or threat posture, as in Hunt *et al.*, 1997. We were not able to observe males for song rate due to time constraints. Note that male Lapland longspurs typically do not respond to simulated territorial intrusions with song, and in general song is not correlated with measures of aggression in this species (Hunt *et al.*, 1995).

Overwinter Survival

To evaluate overwinter survival of E_2 and control females and their mates, we counted the numbers of breeding females and males from 1993 who were subsequently seen or captured in 1994. These re-sightings are undoubtedly an underestimate of over-winter survival, but nevertheless it is clearly useful to compare re-sighting percentages of experimental and control groups.

Statistical Analysis

Data from empty-implanted and unimplanted females were grouped as a single "control female" group, after testing for any differences between the two groups (none were found; Table 5.2). Similarly, males with empty-implanted mates were grouped with males with unimplanted mates as a single "control male" group, after testing for any differences (again, none were found; Table 5.2).

We used nonparametric tests for most hormonal and behavioral data, as these data generally did not have normal distributions. For these data, two-group comparisons were carried out with Mann-Whitney tests; multi-group comparisons with Kruskal-Wallis tests; and paired data (e.g., hormone levels before versus after implant; behavior of male versus female) with Wilcoxon signed rank tests. Some data, such as body mass and clutch size, were normally distributed with equal variances between groups, and thus were analyzed with parametric tests. For these data, two-group comparisons were carried out with Student's t-test, and multi-group comparisons with ANOVA (with post-hoc analyses using Fisher's Protected Least Significant Difference). Mean nestling mass was analyzed with repeated-measures ANOVA as described above. Differences in proportions between two groups were analyzed with Fisher exact tests. All tests were conducted with Statview v4.1 for the Macintosh, with the significance level set at $\alpha = 0.05$, and all tests were two-tailed.

RESULTS

Hormones & Body Condition of Females

E_2 females had higher E_2 than control females during late incubation and feeding of nestlings, indicating that the implants were functioning as expected (Fig. 5.1; Mann-Whitney test, U = 120, Z = 3.96, $p<0.0001$). Average circulating E_2 in the E_2-implanted females was within physiologic levels and is comparable to normal peaks in E_2 that occur during laying in passerine birds (e.g. Silverin *et al.*, 1986, Wingfield & Farner, 1993). Progesterone levels during this time were not significantly different in E_2 females and control females, though there was a trend for E_2 females to have higher progesterone (control mean = 1.18 ± 0.194 ng/ml, n = 10; E_2 mean = 1.63 ± 0.265 ng/ml, n = 12; Mann-Whitney test, U = 85, Z = 1.648, p = 0.0993).

Earlier in the season, just before insertion of the implants, control females and E_2 females had similar E_2 and P levels (mean estradiol: control females = 0.609 ± .334, E_2 females = 0.298 ± .055, Mann-Whitney test, U = 62.5, Z = 1.123, p = 0.2613; mean progesterone: control females = 1.874 ± .573, E_2 females = 1.806 ± .383; Mann-Whitney test, U = 49, Z = .077, p = 0.9385; all samples from laying or the day after laying). This indicates that the E_2 females had normal hormone levels before receiving their implants.

E_2 females had greater body mass than control females (Fig. 5.2, upper panel; $t_{16} = 3.661$, $p = 0.0021$), and showed a nonsignificant trend toward increased mean fat score compared to control females (Fig. 5.2, lower panel; $t_{16} = .1612$, $p = 0.1266$). There were no differences in brood patch development. All females caught while incubating or feeding young nestlings (four E_2 females & eight control females) had fully developed brood patches, with pronounced edema and no breast feathers. Females caught while feeding older nestlings (four E_2 females & six control females) or after loss of a nest (three E_2 females & three control females) had regressing brood patches, with refeathering and loss of edema.

Testosterone and Body Condition of Males

During the parental stages of incubation and nestling-feeding, males mated to E_2 females had cloacal protuberances that were significantly longer than those of control males (Fig. 5.3, lower panel; Mann-Whitney test, $U = 93.5$, $Z = 2.400$, $p = 0.0164$). However, E_2 males did not have significantly increased testosterone levels compared to control males, though there was a trend in that direction (Fig. 5.3, upper panel; $U = 41.5$, $Z = 1.718$, $p = 0.0858$). The sample size of the E_2 group in the testosterone comparison was small, as we were able to capture only six E_2 males for blood sampling while their females had E_2 implants. Five of these six E_2 males had measurable testosterone levels, and two had testosterone over 1 ng/ml. Four of nine control males captured on the same dates had measurable testosterone levels, and one had testosterone over 1 ng/ml. Comparison of birds with measurable vs. non-measurable testosterone in the two groups was not significant (Fisher exact test, $p = 0.2867$). In general, testosterone levels of both groups were low, below 1.0 ng/ml, and appeared similar to parental-stage testosterone levels of unimplanted Lapland longspurs studied in other years (Hunt *et al.*, 1995).

Incubation Length

Eggs in E_2 nests hatched after longer incubation periods than eggs in C nests (Figs. 5.4 and 5.5; Mann-Whitney test, $U = 26.0$, $Z = 2.133$, $p = 0.0329$). This is probably due to differences in female incubation behavior rather than to differences in egg physiology, since most eggs were laid before the females received implants. Because female behavior at the nest may be affected by the experience of receiving an implant at the beginning of incubation, this analysis included only nests of females who received E_2 or empty implants and for whom incubation length was exactly known ("E_2, exact" and "Empty-imp, exact" nests in Fig. 5.4), and did not include two nests of unimplanted females for whom incubation length was also exactly known ("Unimp, exact" nests in Fig. 5.4, upper panel). If those two nests are included, the statistical p-value decreases ($p = 0.0267$). We also did not include nests that survived for at least 11 or more days of incubation but were then depredated before hatching. This eliminated two nests of unimplanted females that survived a minimum of 11 days until predation, and four nests of estradiol females that survived a minimum of 11 days (one nest) or 12 days (three nests) until predation ("Unimp, minimum" and "E_2, minimum" nests in Fig. 5.4). If any or all of these nests are included in the analysis, the difference between E_2 and control nests becomes more pronounced and the p-value decreases (when all nests included, $p = 0.01$).

Nestling Growth

Nestlings of E_2 females grew normally (Fig. 5.6). There was no effect of implant on mean nestling mass, either in the analysis of days 0-4 for all available nests (nine E_2 nests and eight control nests; $F = .130$, $p = 0.7196$) or in the analysis of days 0-7 for successful nests only (five E_2 nests and five control nests; $F = 0.001$, $p = 0.9812$). There was no interaction of day with implant in either analysis.

Parental Care

E_2 and control parents had very similar patterns of parental behavior at the nest. There were no significant differences for either sex in time at the nest, number of visits per hour, or number of feeding movements per hour, and females had a similar number of digs per hour (Fig. 5.7; statistical results shown in Table 5.3). While at the nest, E_2 and control females spent similar proportions of time brooding, standing, and feeding (Fig. 5.8, Table 5.3). In both groups, females spent significantly more time at the nest than males (Fig. 5.7; E_2 pairs, $Z = 2.666$, $p = 0.0077$; control pairs, $Z = 3.823$, $p = 0.0001$), and females visited the nest more often than males, though this was significant only in control pairs (Fig. 5.7; E_2 pairs, $Z = 0.652$, $p = 0.5147$; control pairs, $Z = 2.293$, $p = 0.0218$). In both groups, males and females provided similar numbers of feeds per hour (Fig. 5.7; E_2 pairs, $Z = 0.059$, $p = 0.9528$; control pairs, $Z = 1.481$, $p = 0.1386$).

However, E_2 and control females differed markedly in their response to the male. In general, when a Lapland longspur male arrives at the nest while the female is on the nest, the female responds in one of two ways, either leaving the nest promptly (thus allowing the male to feed the nestlings), or remaining on the nest and begging from the male. A begging female typically turns her head to face the male, rapidly opens and closes her bill, utters a high-pitched cheeping sound, and sometimes flutters her wings. In this study, control females usually left the nest promptly when the male arrived, but E_2 females tended to stay on the nest and beg from the male (Fig. 5.9). Of the nine E_2 females, eight were observed to beg at least once from the male, and the ninth exhibited the unique response of remaining at the nest without begging. Of the nine control females, only two were observed to beg; the other seven control females always left the nest promptly whenever the male arrived. The occurrence of begging was significantly higher in E_2 than in control females (Fisher exact test, $p = 0.0152$). The mean probability of staying at the nest when the male arrived was also significantly higher in E_2 females (69%) than in C females (10%; Mann-Whitney test, $U = 76$, $Z = 3.247$, $p = 0.0012$).

Renesting

E_2 females did not show an increased probability of renesting compared to control females, and the probability of renesting was highly dependent on date for both groups. 11 estradiol females and 13 control or unimplanted females lost their first nests, and dates of nest loss spanned May 25 to June 23 (Table 5.4). Females of both groups were likely to renest if the first nest was lost on or before June 9, and did not renest if the first nest was lost after June 9.

Nest Success

E_2 and control females had similar nest success. There were no differences in clutch size, number of hatchlings, number of unhatched eggs, number of fledglings, or proportion of nests that produced at least one fledgling (Table 5.5).

Male Territorial Behavior

E_2 and control males had similar patterns of territorial behavior in response to a simulated territorial intrusion. Both groups had variable but generally low levels of territorial aggression. Aggressive behavior (threat posture and/or attacks) was seen in one of six E_2 males (17%), and in four of eight control males (50%). This difference is not statistically significant (Fisher exact test, $p = 0.3007$).

Anecdotal observations indicated that E_2 males also did not show any elevation in mate-guarding or "nest sentry" behavior. These behaviors are common in unimplanted males during egg-laying and early incubation, and are very noticable due to the males' tendency to give frequent calls when engaged in mate-guarding or nest sentry behavior. Neither E_2 males nor control males were observed to guard their females or act as nest sentries after mid-incubation.

Overwinter Survival

There were no differences in female or male resightings in 1995 (Fisher exact tests; $p>.99$ for females, $p = 0.2925$ for males). Six of the 15 E_2 females (40%) were resighted in 1995, compared to eight of 18 control females (44%). Seven of the 13 E_2 males (54%) were resighted, compared to 16 of 22 control males (73%).

DISCUSSION

E_2 implants given to nesting Lapland longspur females resulted in an increased duration of incubation, increased mass, and an increased tendency to beg from the male, but apparently did not interfere with any aspects of parental care. We did not detect any increase in sexual activity due to E_2 implants. Males showed some indications of hormonal response to their mate's E_2 levels, as reflected in the length of the males' cloacal protuberances.

The lengthened incubation period of E_2 females could be due to decreased proportion of time spent incubating and/or to decreased effectiveness of incubation. We believe decreased effectiveness of incubation is unlikely, since the brood patches of E_2 females remained well developed. E_2 can decrease nest attentiveness in females of some other species (e.g. Lehrman, 1958). The apparent decrease in nest attentiveness seen in this study may indicate that females were spending more time doing some conflicting activity, such as foraging, soliciting copulations from the male, or perhaps standing in the nest instead of sitting. Further investigation of this question will require focal observations and nest videotapes of incubating females.

The increased mass of E_2 females is probably partially due to maintenance of a well-developed ovary and oviduct. One of the major physiologic effects of E_2 is to stimulate growth of the oviduct, and exogenous E_2 administered after the reproductive tract has developed can prevent the normal regression of both ovary and oviduct (Gilbert, 1979). In similar-sized passerines, the mass of the developed ovary and oviduct is typically about 1.0-2.0 g, regressing to approximately 0.01-0.02 g by the end of the breeding season (e.g. Dufty, 1989a; Gilbert, 1979; Wolfson, 1953). On average, the E_2 females in this study weighed approximately 3.5g more than control females. Thus, increased ovary and oviduct mass probably account for some, but not all, of the increase in body mass. E_2 may also increase body mass in female birds by increasing liver mass, increasing deposition of calcium in the long bones, and possibly increasing fat stores (Marshall, 1961; Lofts & Murton, 1973). In a study on pied flycatchers (*Ficedula hypoleuca*), Silverin (1991) found that E_2-implanted females had significantly higher fat stores than controls. Our data show a nonsignificant trend toward increased fat in E_2 females, but due to high variation this analysis had low statistical power, and thus a greater sample size would be needed to conclusively test this question. If E_2 does increase fat stores in Lapland longspurs, the mechanism may involve increased foraging, decreased activity, or consumption of food obtained while begging from the male.

E_2 females did not show any clear indication of increased sexual or courtship behaviors, though they did show an increased tendency to beg from the male (see below). They were not observed soliciting from or copulating with their mates, events that are usually observed early in the breeding season in about one-third of pairs on our study site. Females of several temperate-zone species will solicit frequently from males when given E_2 implants, whether in the breeding season or in the non-breeding season (e.g. song sparrow, Wingfield & Monk., 1994; white-crowned sparrow, Moore, 1984). We did not observe females systematically for copulation solicitation, however, and it is possible that E_2 females did solicit from males occasionally. We did, however, search systematically for renests, and we are confident that E_2 females did not build nests and lay eggs later than control females. We consider it unlikely that exogenous E_2 prevents females from nesting by shutting down GnRH or gonadotropin release through negative feedback, because E_2 females who lost their nests early were able to initiate new nests. Similarly, E_2 implants given to female white-crowned sparrows and pied flycatchers before laying did not interfere with laying of the first clutch of the season (Moore, 1982; Silverin, 1991).

The effect of E_2 on begging behavior was entirely unexpected, since Lapland longspur females are not known to beg from males during courtship, and males typically do not feed females during incubation (Williamson, 1968a,b; Lyon & Montgomerie, 1987). We have conducted numerous observations of Lapland longspur pairs during and prior to egg-laying, and have witnessed many instances of sexual solicitation and copulation, but have never before observed females begging from males. In this study, males usually fed the females that begged, though some males seemed reluctant to feed the female and seemed to be looking for the nestlings. Begging females whose males did not actively feed them often reached up and plucked food from the males' bills. After receiving food from the male, females usually stood up immediately and fed the received food to the nestlings. Frequently the male remained alongside the female at this time and fed his remaining stock of food to the nestlings. Interestingly, Lyon & Montgomerie (1987) did observe occasional instances of male Lapland longspurs feeding females at the nest during incubation, though they considered this behavior rare enough that they classified Lapland longspurs as non-incubation-feeders. We suspect that begging may be a natural, though usually infrequent, component of female incubation and brooding behavior in Lapland longspurs. If this is so, E_2 may play a hitherto unknown role in promoting this aspect of nest behavior.

Our data suggest that E_2 males may have been responding hormonally to behavioral cues from their E_2-implanted females. E_2 males had longer cloacal protuberances, and cloacal protuberance (CP) length is highly androgen-sensitive (Salt, 1954; Lofts & Murton, 1973; Hunt *et al.*, 1997). However, E_2 males did not have significantly greater testosterone levels than control males, though there was a slight trend in that direction. In general, testosterone levels of both groups were low, and were well below the testosterone levels seen earlier in the breeding season (Hunt *et al.*, 1995, Hunt *et al.*, 1997). The difference in CP length may be due to an earlier difference in testosterone level, since changes in CP length can lag slightly behind changes in testosterone level (Salt, 1954). Alternatively, our sample sizes may have been too small to detect a slight, but real, difference in testosterone.

In many other bird species, males show increases in testosterone levels, sexual behavior, and territorial behavior when exposed to sexually receptive females, (reviewed in Balthazart, 1983; Wingfield *et al.*, 1994). For example, in white-crowned sparrows (subspecies *pugetensis*) and song sparrows, males mated to E_2-implanted females exhibited increased territorial behavior, and had testosterone levels during incubation that were two to four times higher than testosterone levels of controls, and were similar to the testosterone peaks seen during courtship (Moore, 1982; Wingfield *et al.*, 1989). In the pied flycatcher (*Ficedula hypoleuca*), males showed no hormonal changes in response to E_2-implanted mates at any point during the breeding season, but did show increased territorial and mate-guarding behavior (Silverin, 1991). In at least one species, the song sparrow, there are indications that these hormonal and behavioral responses to E_2-treated females wane as the season progresses. In Wingfield *et al.*'s 1989 study, testosterone levels of the male song sparrows with E_2-treated mates fell below 1.0 ng/ml in late June and early July, and were no longer significantly different from control males (though a small sample of five E_2 males showed a rebound to high testosterone levels of about 2.0 ng/ml in July). In a subsequent study, male song sparrows whose females received E_2 implants late in the breeding season showed prolonged territorial behavior into the fall, a delayed molt schedule, and significantly elevated testosterone from late July to October (Runfeldt & Wingfield, 1985). However, the testosterone levels of the E_2 males were only about 50% higher than those of controls, and in general the T levels of both groups were low (below 1.0 ng/ml), well below the T peak seen earlier in the breeding season Runfeldt & Wingfield, 1985). Finally, when E_2 implants were given to female song sparrows in the fall after cessation of breeding activity, the males associated with those females showed no changes in testosterone level until the following February, despite clear indications of sexual receptivity in the females throughout the fall (Wingfield & Monk., 1994). Taken together, these studies indicate a pattern in which male song sparrows seem to respond most strongly to E_2-implanted mates in the early breeding season, less strongly in the late breeding season, and not at all in the autumn (though note the song sparrows studied in autumn were of a different subspecies than the previous studies).

The male Lapland longspurs in this study, examined during incubation and early feeding, showed a relatively weak hormonal response to E_2-implanted females, much weaker than the white-crowned sparrows and songs sparrows tested at similar stages (Moore, 1982; Wingfield *et al.*, 1989). In this respect, the male Laplands are similar to male pied flycatchers, which showed no hormonal changes in response to E_2-treated mates even in the early breeding season (Silverin, 1991). This weak response could be due to a lack of behavioral cues from the females. However, in theory a male Lapland longspur could have detected high E_2 in a female by her increased begging behavior. Another possibility is that the weak response of male Laplands could be related to their breeding habitat. Lapland longspurs and pied flycatchers both have a short breeding season and are single-brooded, whereas song sparrows and white-crowned sparrows (subspecies *pugetensis*) have longer breeding seasons and may raise multiple broods. Thus, Lapland longspurs and pied flycatchers during June may be in a situation analogous to that of song sparrows during autumn - in both cases, the birds' chances of initiating a new clutch are nearly zero. However, the pied flycatchers in Silverin's (1991) study did show significant increases in territorial and mate-guarding behavior in response to E_2-implanted females, despite their short breeding season, whereas the Lapland longspur males in this study showed no increases in territorial aggression. This difference may be related to the very different patterns in territorial behavior in the two species. Pied flycatchers and defend their territories throughout the breeding season (Silverin, 1991), whereas the Lapland longspurs defend their territories only for a few days at the very beginning of the breeding season, and even then have amorphous and fluid territorial boundaries (Hunt *et al.*, 1995). The abundant nesting sites and food resources of the tundra may make continued territorial behavior unnecessarily costly for Lapland longspurs, and thus, territorial behavior may be "turned off" as the breeding season progresses. To test these ideas, we are currently investigating patterns of territorial behavior in other grassland-breeding birds with breeding seasons of different lengths.

Elevated E_2 in females did not interfere with parental behavior of either sex. Both males and females showed strikingly similar patterns of parental behavior, and E_2 pairs fledged normal numbers of healthy fledglings. This result is in contrast to the numerous studies of elevated testosterone in males of temperate-zone species, which generally results in decreased parental behavior of those males (e.g. dark-eyed junco, *Junco hyemalis*, Ketterson *et al.*, 1996; pied flycatcher, Silverin, 1980; house sparrow, *Passer domesticus*, Hegner & Wingfield, 1987). It is not known whether the lack of conflict of E_2 with parental behavior is typical of female passerines, since few studies have directly measured the effect of E_2 on parental behavior in free-living females. However, the information available is in agreement with our results. Female reed warblers (*Acrocephalus scirpaceus*) with E_2 implants were found to visit their nests at normal rates compared to control females (Dittami *et al.*, 1991). The mates of the E_2 females also fed at normal rates, and the E_2 nestlings grew normally. However, there were only five E_2 females in this study (Dittami *et al.*, 1991). Anecdotal reports of white-crowned sparrows (Moore, 1982) and song sparrows (Wingfield *et al.*, 1989) also indicate that females with E_2 implants fed their young normally.

Concluding Remarks

In summary, we found no apparent cost of prolonged high E_2 levels in female Lapland longspurs or their mates. E_2 females weighed more than controls, had normal reproductive success, and had normal overwinter survival. E_2 did alter a few aspects of female behavior, notably incubation and begging behavior. However, neither sex exhibited an elevation in the expected sexual, courtship, or territorial behaviors. For example, the E_2 females showed no indications of elevated sexual solicitaton or increased nest-building, and their mates showed no changes in territorial aggression or testosterone level. Lapland longspur females may have very few behaviors that are E_2-dependent. Alternatively, they may have sexual behaviors that are E_2-dependent early in the season, but that then become irreversibly turned off, and thus E_2-independent, later in the season. Finally, it must be emphasized that this study examined only a single sex steroid, 17-β estradiol. Lapland longspurs might respond behaviorally to progesterone, or to a combination of estradiol with progesterone (see Balthazart, 1983).

The generally weak response to E_2 seen in this study parallels the results from previous studies of the effects of exogenous testosterone in male Lapland longspurs. Those studies indicate that male Lapland longspurs show decreased responsiveness to testosterone after the first few weeks of the breeding season, as compared to temperate-zone species (Hunt *et al.*, 1997; also see Chapters 2 and 4). Though males show pronounced territorial aggression and mate-guarding behavior in the first and second week on the breeding grounds, and though testosterone appears involved in these behaviors during those early weeks (Hunt *et al.*, 1995; Hunt *et al.*, 1997; also see Chapter 3), both territorial aggression and mate-guarding cease by mid-incubation. After that time, treatment with exogenous testosterone is insufficient to activate territorial aggression or aggressive behavior. Thus, both sexes of Lapland longspurs show only weak behavioral responses to prolonged high levels of reproductive steroids, unlike most temperate-zone species. The extremely brief breeding season of the Arctic may have imposed strong selection on Lapland longspurs for a sharply curtailed period of sexual behavior, with an abrupt transition to parental behavior that cannot be reversed simply by behavioral cues from mates or by an elevation of a single sex hormone. In addition, the open nesting habitat and abundant arthropod food resources may have selected for generally reduced territorial behavior. Interestingly, late re-nesting and occasional double-clutching does occur in the southernmost breeding populations of Lapland longspurs (e.g. on the Aleutian Islands). It would be interesting to see if individuals in these populations would respond more strongly to exogenous sex steroids with behavioral changes.

Finally, we must consider the puzzle raised by the apparent lack of cost of high E_2 in females. If high E_2 late in the season carries no cost, and may even carry a benefit (e.g. increased fat stores in some species), then why do most females have low E_2 late in the season? A similar puzzle is known from dark-eyed juncos (Ketterson *et al.*, 1996), where males apparently do not incur a net cost in lifetime reproduction when given exogenous testosterone. Such results force us to question the assumption that the commonly observed phenotypes are necessarily better than all possible alternatives. If an experimentally induced phenotype, such as elevated sex steroids late in the season, does not incur an observable cost and may even confer a benefit, why is the phenotype not more common? One obvious possibility is that the elevated hormone levels may have caused costs that we were unable to detect (e.g. decreased survival of fledglings, or slight decreases in overwinter survival of adults). Ketterson (1996) suggests that in general, slight deviations from common hormonal patterns generally are not costly, and that only extreme deviations may be truly at a disadvantage. A related possibility is that slight deviations may not be costly during a favorable year, but may be quite costly during a harsh year. This has been seen, for example, in long-term studies of bill size of Darwin's finches in drought versus non-drought years (Grant & Grant, 1993). It may be that in a particularly harsh arctic spring, Lapland longspurs with unusual prolonged high levels of sex steroids would be at a disadvantage. For example, the slightly inefficient incubation behavior exhibited by E_2 females in this study might have been disastrous if heavy snowstorms had occurred during incubation, as occurred in the year after this study. Such ideas await a long-term study of heritability and fitness costs of various patterns of hormone levels under various ecological conditions.

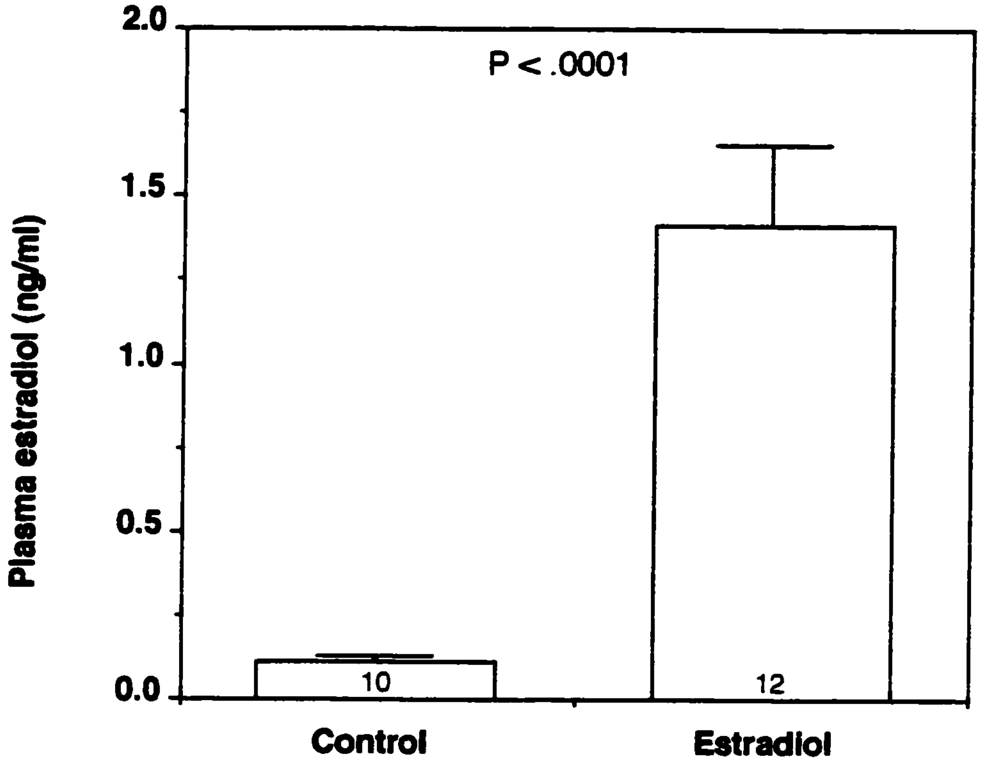

FIGURE 5.1. Plasma estradiol levels in control and estradiol-implanted Lapland longspur females. Data are shown as means ± SEM, with sample sizes at bottom of columns. Significance level from Mann-Whitney test is shown at top.

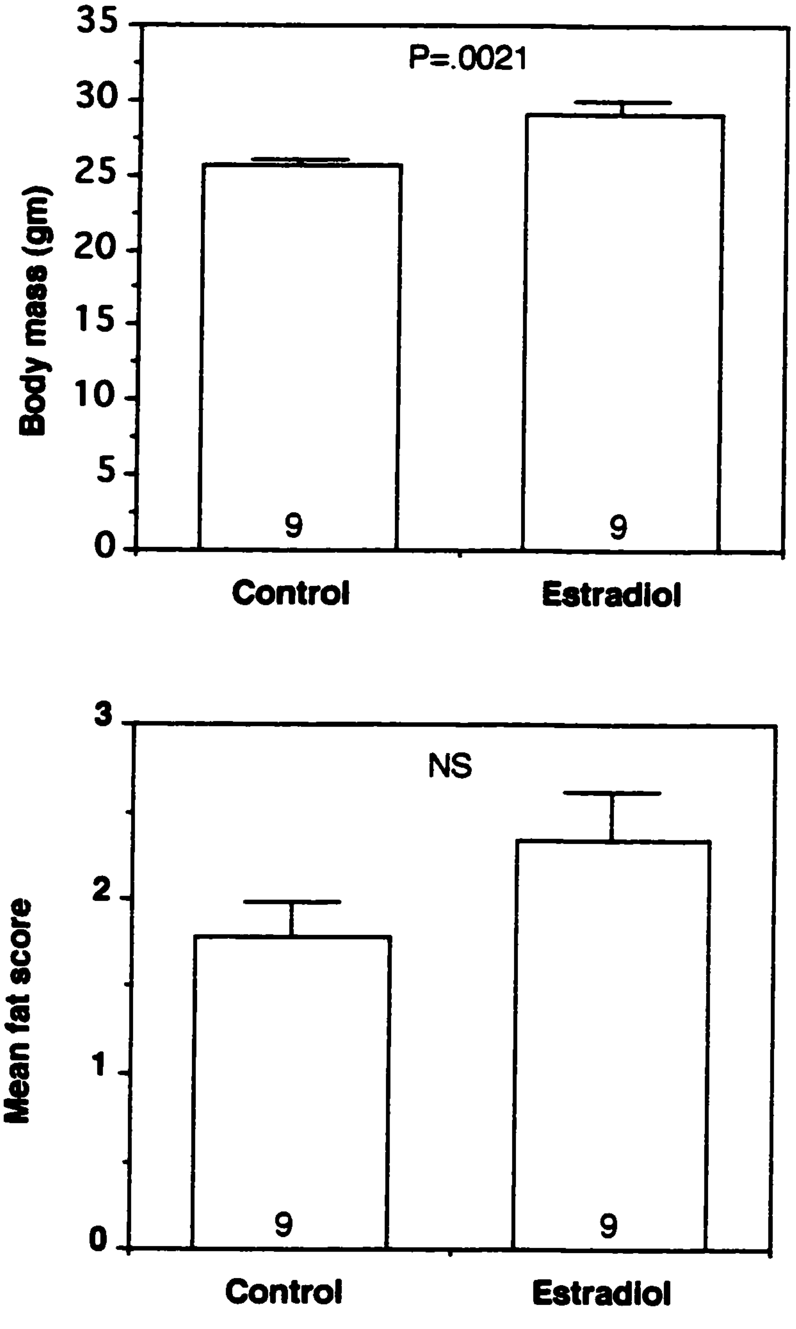

FIGURE 5.2. Body mass (upper panel) and mean fat score (lower panel) of control and estradiol-implanted Lapland longspur females. Data are shown as means ± SEM, with sample sizes at bottom of columns. Significance levels from t-tests are shown at top.

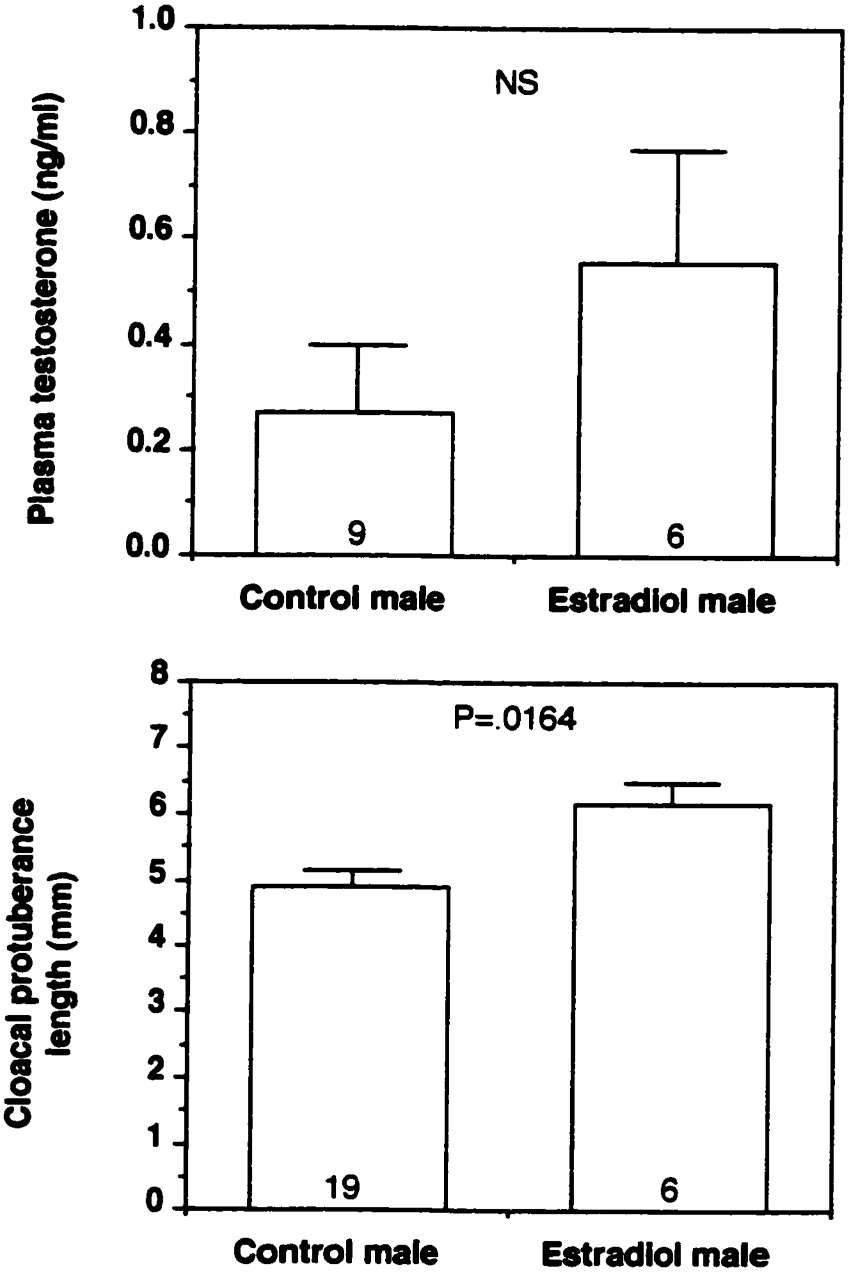

FIGURE 5.3. Plasma testosterone levels (upper panel) and length of the cloacal protuberance, an androgen-dependent copulatory organ (lower panel), in male Lapland longspurs with control or estradiol-implanted mates. Data are shown as means ± SEM, with sample sizes at bottom of columns. Significance levels from Mann-Whitney tests are shown at top.

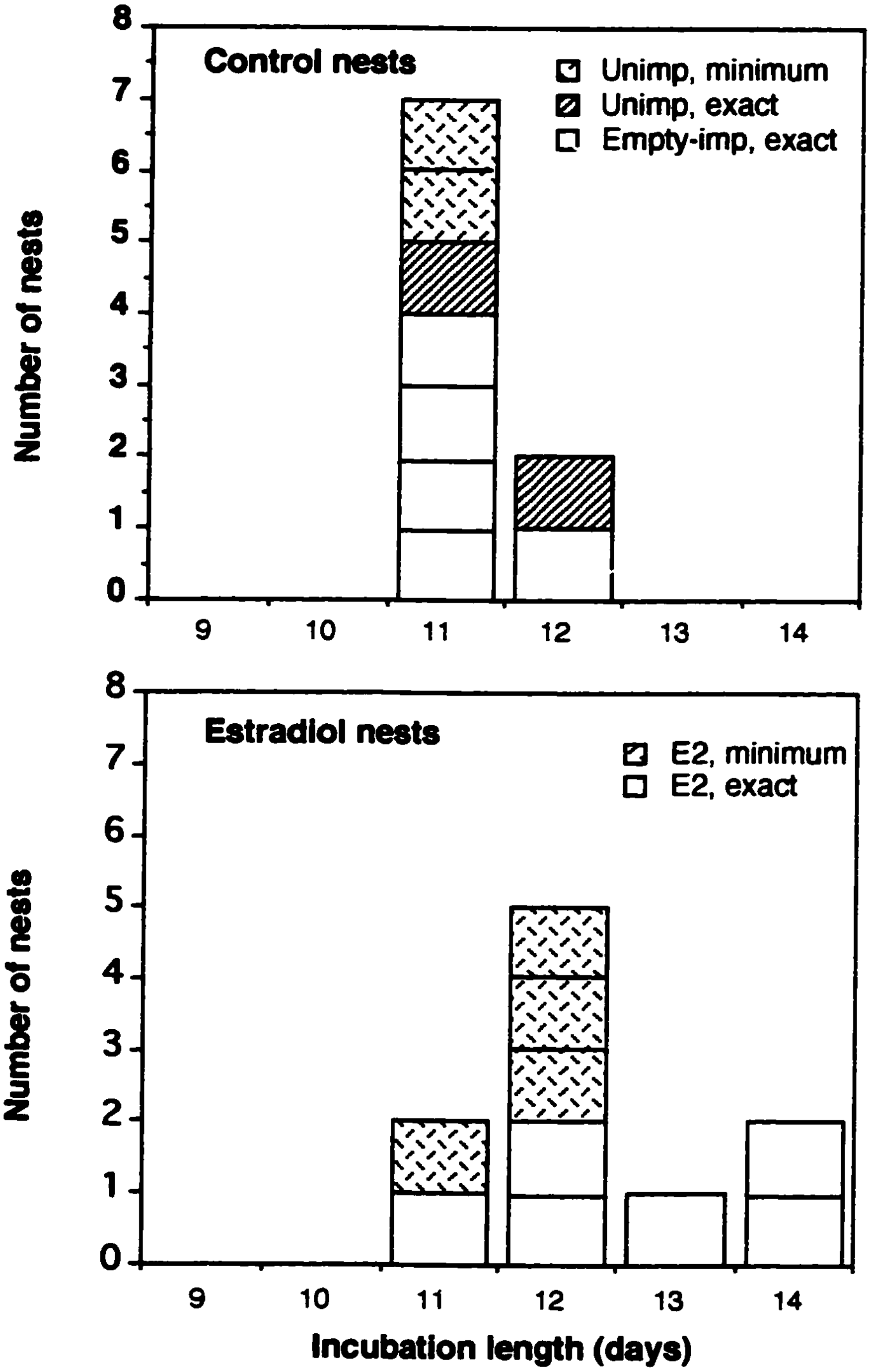

FIGURE 5.4. Effect of estradiol implants on the duration of the incubation period of nests of female Lapland longspurs. The upper panel shows nests of females with empty implants (open boxes) or no implants (cross-hatched and stippled boxes); the lower panel shows nests of females with estradiol implants. Open and cross-hatched boxes represent exactly known incubation lengths from nests that were found during laying, while stippled boxes represent the minimum possible incubation lengths for nests that were found after laying.

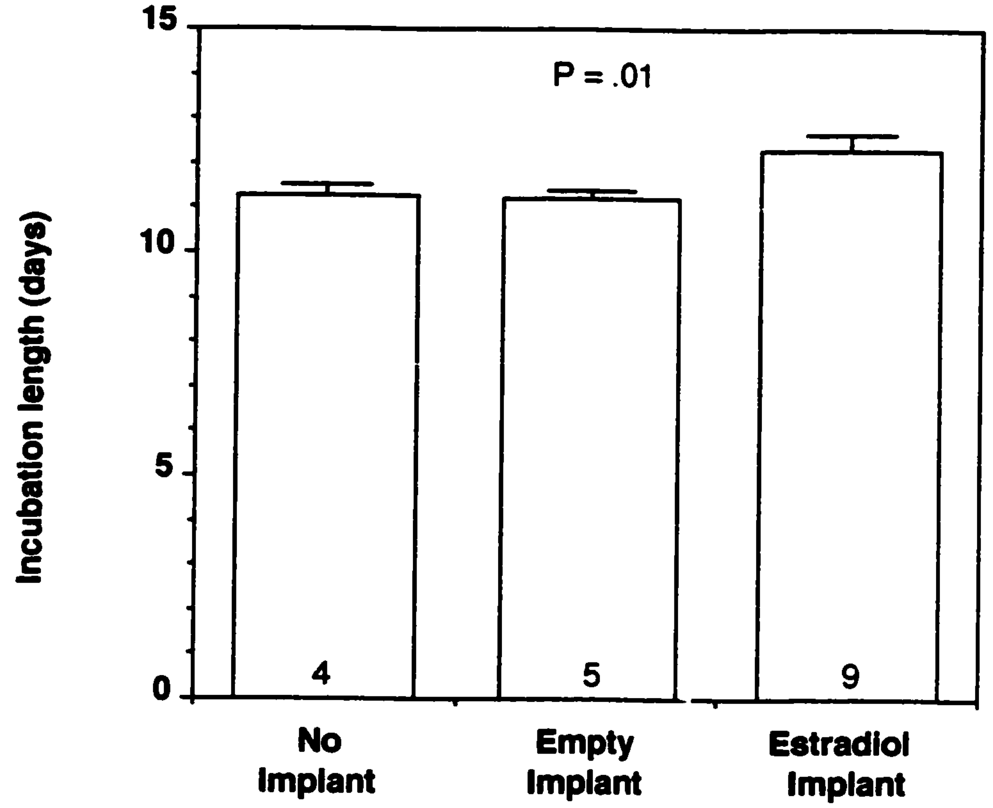

FIGURE 5.5. Mean incubation lengths of females with no implants, empty implants, or estradiol implants. Data are shown as mean ± SEM, with sample sizes at bottom of columns. Significance level is shown at top, from Mann-Whitney test on estradiol versus control (where control = combined empty- and no-implant groups).

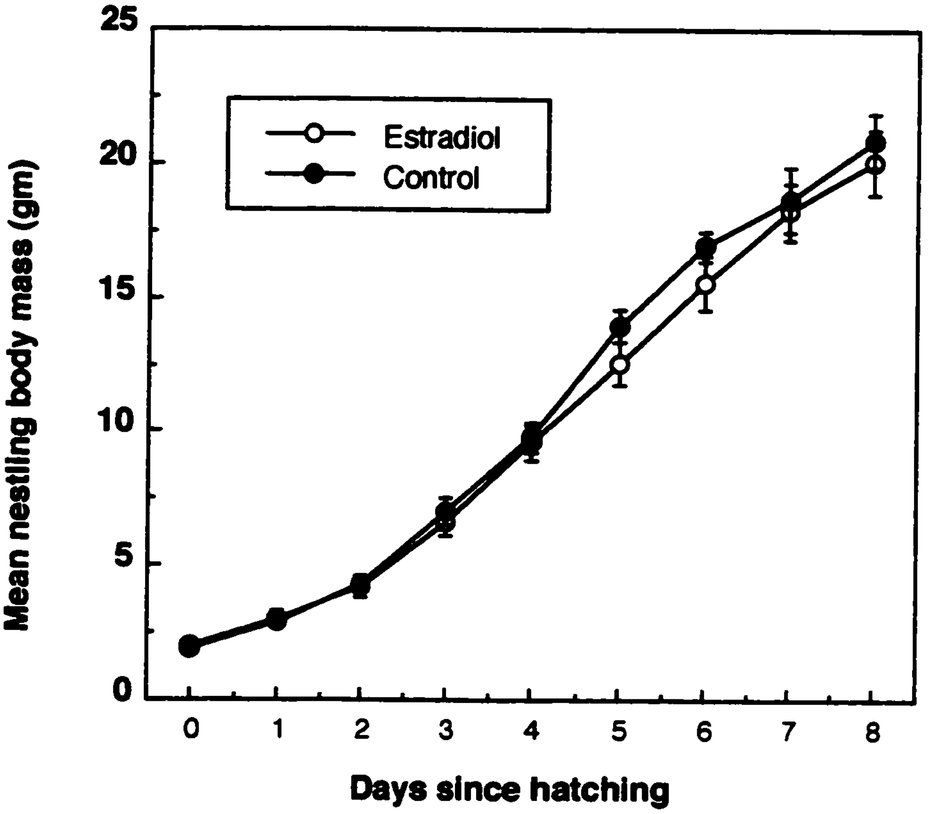

FIGURE 5.6. Nestling growth curves from nests with control or estradiol-implanted females. Data are shown as means ± SEM. Daily nestling masses were averaged to provide a mean nestling mass for each nest for each day. Day 0 = day that the first nestling is found in the nest. Sample sizes = ten estradiol nests and fourteen control nests.

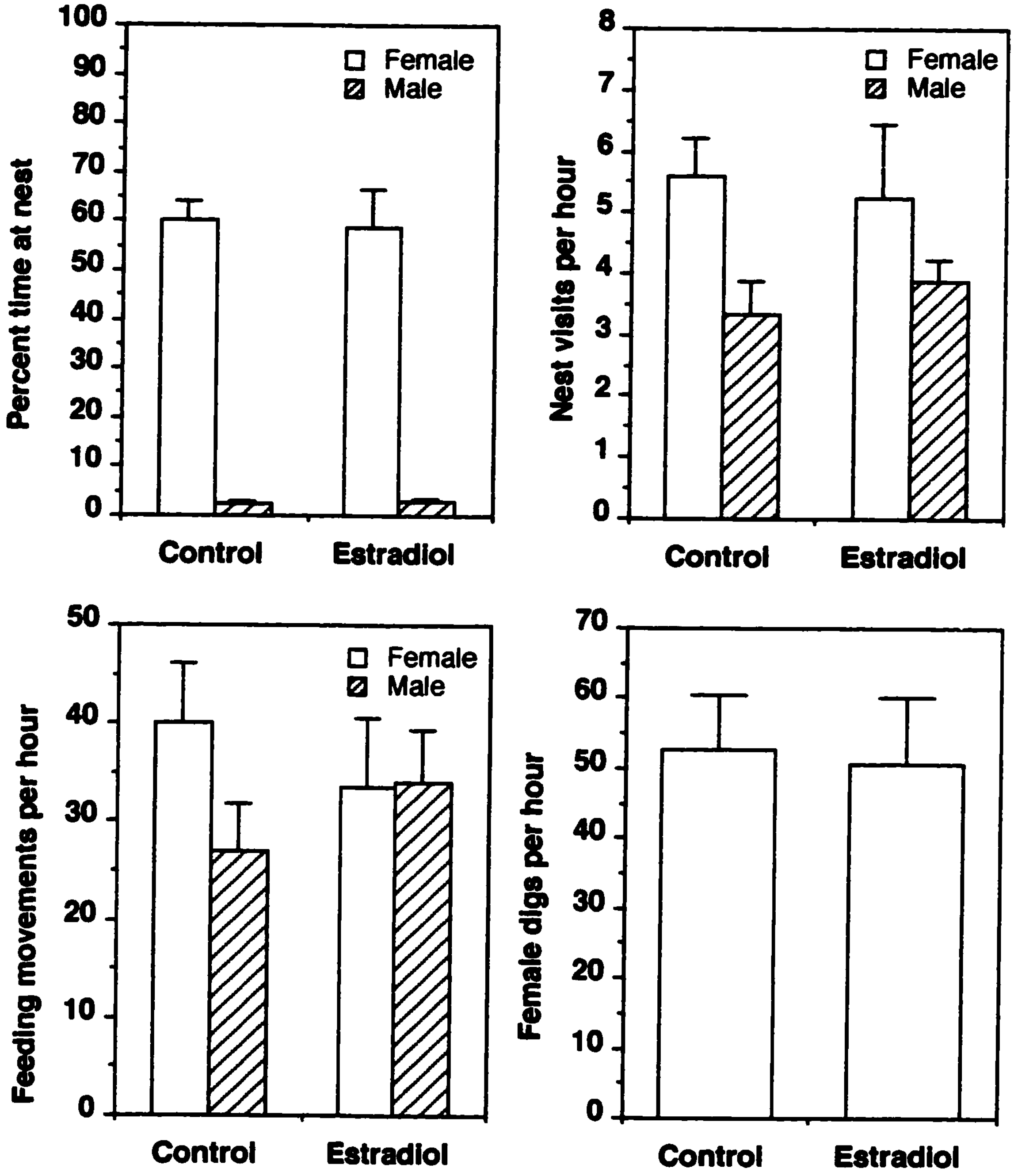

FIGURE 5.7. Parental behavior of control or estradiol-implanted Lapland longspur females and their mates, as measured by two-hour videotapes when nestlings were 4-5 days old. Data are shown as means ± SEM. Sample sizes = ten control nests and nine estradiol nests.

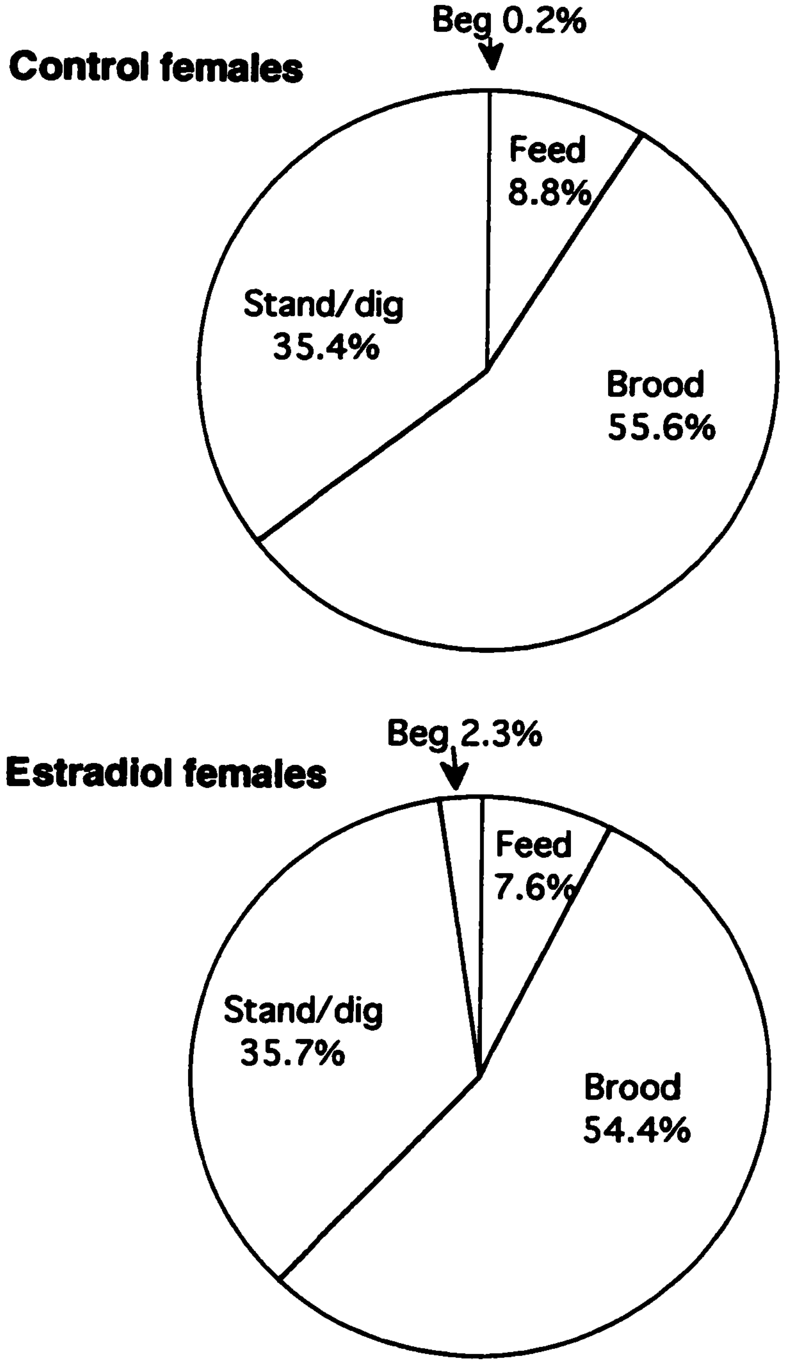

FIGURE 5.8. Time-budget analysis of the nest behavior of of control or estradiol-implanted Lapland longspur females. Data are presented as mean percentage of time that females spent doing each activity, out of the total time that females spent at their nests. Sample sizes = nine control females and nine estradiol females.

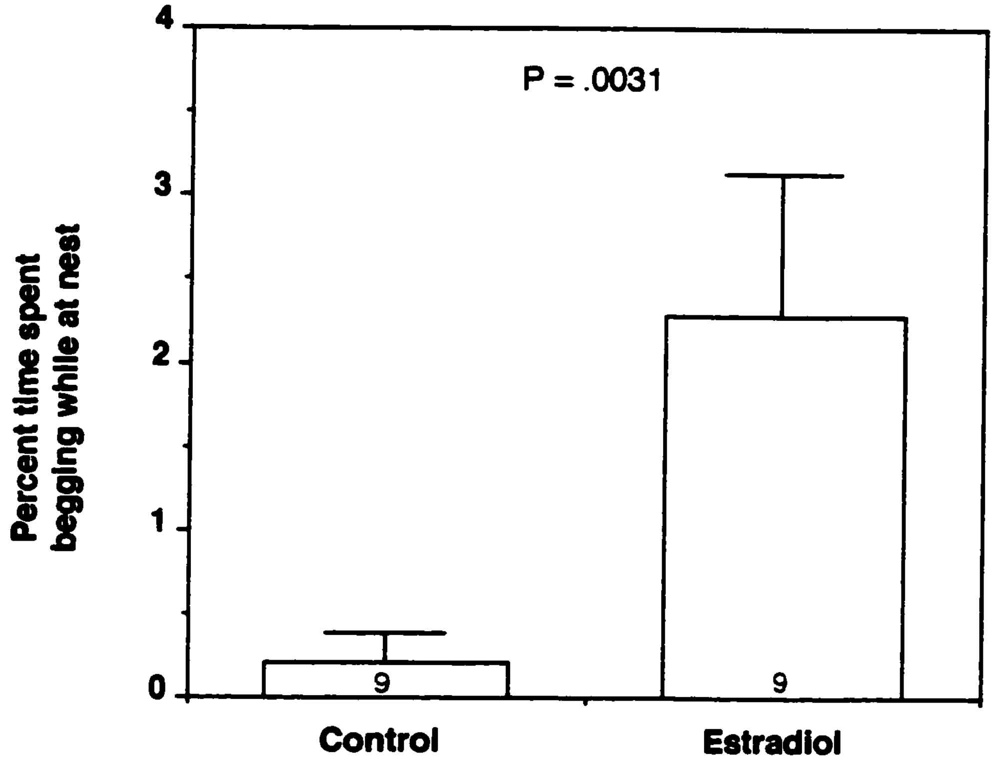

FIGURE 5.9. Percentage of time that control or estradiol-implanted Lapland longspur females spent begging from their mates, out of the total time that females were at their nests. Data are shown as means ± SEM, with sample sizes at bottom of columns. Significance level is shown at top.

Table 5.1. Clutch size and nestling age of Lapland longspur nests that were videotaped for analysis of parental behavior. Estradiol females received a single estradiol implanton the date shown, while control females received an empty implant ($n = 4$) or no implant ($n = 6$; "N/A" in date of implant column). Results for t-tests on effect of implant (estradiol versus control nests) for data in the last two columns were: number of nestlings, $t_{17} = 0.075$, $P = 0.9414$; age when videotaped, $t_{17} = 0.773$, $P = 0.4499$.

Female band#	Nest	Date of implant	Date of videotape	Number of nestlings	Age when videotaped
Estradiol females					
48369	K2	2 June	15 June	3	4
48553	M4	2 June	15 June	5	5
63742	N1	2 June	18 June	4	3
63745	K5	31 May	17 June	2	4
63769	K10	31 May	14 June	4	4
63771	K21	1 June	15 June	2	4
63821	M1	1 June	19 June	2	4
63911	M5	30 May	16 June	4	5
63958	K20	2 June	16 June	5	4
			Mean ± SEM:	3.44 ± 0.41	4.67 ± 0.44
Control females					
48530	K25	30 May	12 June	3	4
63519	M3	31 May	13 June	3	4
63772	K16	1 June	14 June	4	4
63831	K1	30 May	14 June	5	4
39062	N3	N/A	12 June	5	4
48377	K28	N/A	12 June	3	6
63752	K6	N/A	18 June	1	5
63761	M7	N/A	14 June	3	4
UB-K27	K27	N/A	13 June	2	4
UB-K15	K15	N/A	19 June	5	4
			Mean ± SEM:	3.40 ± 0.43	4.3 ± 0.21

Table 5.2. Some comparisons of physiological and behavioral data for pairs of Lapland longspurs in which females had empty implants vs. no implants. Means are shown ± SEM (n). Statistics shown are for Mann-Whitney tests (Z value shown), T-tests (T value shown), repeated-measures ANOVA (F value shown), or Fisher exact tests, as indicated.

	Empty Implant	No Implant	Statistics (P-value)	
Female hormones, physiology, and survival				
Estradiol	0.132 ± 0.024 (6)	0.092 ± 0.009 (4)	Z=1.399	(0.1619)
Progesterone	1.265 ± 0.300 (6)	1.053 ± 0.219 (4)	Z=0.000	(>.9999)
Mass	26.275 ± 0.433 (4)	25.040 ± 0.729 (5)	T_7=1.357	(0.2168)
Mean fat	2.000 ± 0.354 (4)	1.600 ± 0.245 (5)	T_7=0.960	(0.3690)
Survival to 1994	3 of 9	5 of 9	Fisher exact P=.6372	
Male hormones, physiology, and survival				
Testosterone	0.137 ± 0.042 (3)	0.340 ± 0.188 (6)	Z=0.132	(0.8951)
CP length	5.000 ± 0.000 (2)	4.750 ± 0.854 (4)	T_4=0.195	(0.8548)
Mass	29.075 ± 0.522 (4)	28.840 ± 0.677 (5)	T_7=0.263	(0.8003)
Meanfat	1.625 ± 0.239 (4)	1.100 ± 0.332 (5)	T_7=0.525	(0.2625)
Survival to 1994	6 of 8	10 of 14	Fisher exact P>.9999	
Male behavioral response to simulated territorial intrusion				
Aggressive	3 of 5	1 of 3	Fisher exact P>.9999	
Nest data				
Incubation length	11.200 ± 0.200 (5)	11.250 ± 0.250 (4)	T_7=0.158	(0.8786)
Clutch size	4.600 ± 0.306 (10)	4.500 ± 0.139 (14)	T_{22}=0.328	(0.7457)
#hatched eggs	2.556 ± 0.603 (9)	3.077 ± 0.383 (13)	T_{20}=0.767	(0.4520)
#fledglings	1.750 ± 0.726 (8)	1.500 ± 0.557 (12)	T_{18}=0.277	(0.7851)
Renesting	2 of 5	1 of 5	Fisher exact P>.9999	
Nestling growth				
Repeated measures ANOVA on mean nestling mass, days 1-7:			$F_{1,6}$ =0.005	(0.9485)
Behavior at nest				
Female feeds/hr	41.62 ± 10.847 (4)	38.638 ± 7.718 (5)	T_7=2.982	(0.8243)
Female visits/hr	6.433 ± 1.196 (4)	5.018 ± 0.699 (6)	T_8=1.414	(0.3040)
Female digs/hr	52.855 ± 17.39 (4)	52.810 ± 6.026 (5)	T_7=0.003	(0.9979)
Male feeds/hr	18.568 ± 7.975 (4)	33.794 ± 4.502 (5)	T_7=1.757	(0.1224)
Male visits/hr	2.175 ± 0.782 (4)	4.102 ± 0.642 (6)	T_8=1.903	(0.0935)
Female feed%	9.825 ± 4.137 (4)	7.901 ± 2.192 (5)	T_7=0.437	(0.6754)
Female brood%	65.280 ± 9.766 (4)	47.850 ± 14.876 (5)	T_7=0.927	(0.3877)
Female stand%	24.772 ± 6.699 (4)	44.006 ± 13.007 (5)	T_7=1.206	(0.2671)
Female begging	0 of 3	2 of 6	Fisher exact P=0.500	

Table 5.3. Effect of female implant (estradiol versus control) on parental behavior of Lapland longspur pairs. Data are shown as mean ± SEM (n). Statistics are Z-values (P-value in parentheses), from Mann-Whitney tests.

	Estradiol	Control	Statistics
Females			
%Time at nest	58.683 ± 7.832 (9)	60.049 ± 4.168 (10)	Z=0.000 (>.9999)
Visits/hr	5.251 ± 1.192 (9)	5.584 ± 0.638 (10)	Z=0.816 (0.4142)
Feeds/hr	33.539 ± 7.102 (9)	39.963 ± 6.036 (9)	Z=0.751 (0.4529)
Digs/hr	50.713 ± 9.588 (9)	52.830 ± 7.777 (9)	Z=0.132 (0.8946)
Time-budget analysis of female's activities while at nest:			
%time feeding	7.631 ± 2.432 (9)	8.757 ± 2.074 (9)	Z=0.486 (0.6272)
%time brooding	54.440 ± 9.272 (9)	55.597 ± 9.314 (9)	Z=0.221 (0.8253)
%time standing	35.652 ± 7.888 (9)	35.458 ± 8.149 (9)	Z=0.132 (0.8946)
%time begging	2.277 ± 0.851 (9)	0.214 ± 0.178 (9)	Z=2.957 (0.0031) *
Males			
%Time at nest	3.018 ± 0.265 (9)	2.270 ± 0.438 (10)	Z=1.061 (0.2885)
Visits/hr	3.900 ± 0.328 (9)	3.331 ± 0.564 (10)	Z=0.939 (0.3475)
Feeds/hr	27.027 ± 4.836 (9)	33.869 ± 5.370 (9)	Z=0.751 (0.4529)
Combined male + female care			
%Time at nest	60.758 ± 7.746 (9)	62.192 ± 4.120 (10)	Z=0.082 (0.9349)
Visits/hr	9.151 ± 1.280 (9)	8.915 ± 0.821 (10)	Z=0.245 (0.8065)
Feeds/hr	67.408 ± 9.823 (9)	66.991 ± 6.497 (9)	Z=0.309 (0.7573)

Table 5.4. Occurrence of renesting in estradiol-implanted and control Lapland longspur females whose first nests failed on the date indicated.

Band	Date first nest failed	Renest?
Estradiol Females		
63766	May 25	YES
63777	June 5	YES
63770	June 8	YES
63763	June 8	no
63756	June 10	no
48378	June 11	no
63769	June 16	no
63911	June 17	no
63771	June 18	no
63821	June 21	no
63745	June 23	no
Control Females		
63363	June 4	YES
UB-K7	June 4	no
63776	June 6	YES
63869	June 9	YES
48549	June 9	no
63757	June 10	no
63870	June 12	no
UB-K19	June 12	no
UB-K24	June 12	no
39062	June 14	no
63774	June 16	no
UB-K31	June 19	no
63752	June 21	no

Table 5.5. Hatching success, fledging success and cause of nest failure in nests of estradiol-implanted and control female Lapland longspurs. Means are shown ± SEM (n). Sample sizes vary due to late discovery of some nests and depredation or late hatch of other nests. P-values for means are from t-tests; P-values for proportions are from Fisher exact tests. "Unhatched eggs" refers only to eggs that failed to hatch but remained intact in the nest, not to eggs that were lost to predators.

	Estradiol	Control	P
Mean clutch size per pair	4.667 ± 0.126 (15)	4.609 ± 0.137 (23)	0.7944
Mean #hatchlings per pair	3.083 ± 0.434 (12)	2.909 ± 0.341 (22)	0.8248
Mean number of unhatched eggs per pair	1.182 ± 0.325 (11)	1.000 ± 0.291 (18)	0.5502
Mean #fledglings per pair	1.545 ± 0.666 (11)	1.714 ± 0.426 (21)	0.8090
Proportion of pairs that produced at least one fledgling	4 of 11 pairs	10 of 21 pairs	0.7120
Proportion of pairs with hatchlings that produced at least one fledgling	4 of 10 pairs	10 of 18 pairs	0.6946

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BIOGRAPHICAL NOTE

Kathleen Ellen Hunt was born on May 1, 1965, in New Haven, Connecticut. The child of academic parents, she moved to Princeton, New Jersey, and then to Brookline, Massachusetts, where her father Shane taught economics at Boston University and her mother Barbara was an elementary school principal. She graduated from Brookline High School in 1983 with the highest GPA in her class. Kathleen then attended Oberlin College, Ohio, where she majored in biology. In 1985, Kathleen took one year off from college to explore various careers in biology, studying physiology of endangered deer at the National Zoological Park, and working as a veterinary assistant. After this year, Kathleen returned to college determined to forge a career in animal biology. She graduated in 1988 with the Oberlin award for excellence in biology, and during the next two years she studied primate endocrinology at McLean Hospital, and won an internship to study communication of golden lion tamarins at the National Zoo. She also had her first experience with wild birds during this time, at the New England Wildlife Center, where she hand-raised hundreds of nestlings and studied imprinting in blue jays and American robins. After discovering that birds could be as interesting as mammals, if not more so, Kathleen decided to enter graduate school to study the endocrinology and behavior of birds. She was accepted into the Department of Zoology at the University of Washington in 1990, where she joined John Wingfield's lab. She first travelled to Alaska in the spring of 1991, where she began her work on Lapland longspurs. While at U.W., Kathleen has also studied endangered honeycreepers in Hawaii, and prairie longspurs in Montana.

Her publications include:

Hunt, K., Hahn, T.P., and J.C. Wingfield. 1997. Testosterone implants increase song but not aggression in male Lapland longspurs. Animal Behaviour 54:1177-1192.

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