INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA

UMI®
800-521-0600

EFFECTS OF ELECTRIC AND MAGNETIC FIELDS ON SELECTED PHYSIOLOGICAL AND REPRODUCTIVE PARAMETERS OF AMERICAN KESTRELS.

Kimberly J. Fernie

Faculty of Agriculture and Environmental Sciences
Macdonald Campus of McGill University, Montreal

June 1998

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment for the degree Doctor of Philosophy

Suggested short title:

Effects of Electric and Magnetic Fields on American kestrels.

I dedicate this thesis to my family and friends who have been so supportive of me.

ACKNOWLEDGEMENTS

There are many people I would like to thank for their support and assistance over the past four years. I would like to thank my supervisor, Dr. David Bird, for his friendship and support over the past four years. Dave was very kind in providing many kestrels, associated materials and financial support for my work, and his experience with kestrels for research has been helpful on many occasions. He has been very generous in taking me and his other graduate students to many conferences which provided valuable contacts, friends and good memories. Dave and Toni are very kind, generous people who always opened their home.

Dr. Paul Laguë was enormously patient while I was (and still am) learning about reproductive physiology, especially when preparing for my comprehensive exam. Dr. Laguë provided a control facility for this project, and assisted with the design of the hormone/egg sampling. Dr. Elliot Block helped me when I got 'lost' in the details of my thesis - your insight was very much appreciated when I 'couldn't see the forest for the trees' - and was fundamental in securing the EMF chamber for my work. Hydro Quebec, particularly Dr. Duc Nguyen, were extremely helpful in providing answers, access to, and technical support with the EMF chamber at any time, and EMF matters in general. Dr. Denis Petitclerc, through Agriculture Canada, and his assistant, Jasmin Brochu, were very kind in completing the laboratory analysis of the plasma melatonin samples from the kestrels. Dr. Pierre Dutilleul and Russ Dawson were very helpful with the statistical analysis of this study, and making "statistics fun!". Dr. Gary Bortolotti of the University of Saskatchewan has acted as a mentor, and suggested I investigate free-ranging kestrels nesting under EMF conditions. I am indebted to Dr. Juan Jose Negro who was instrumental in starting the EMF project with the kestrels, helpful in my initial funding applications for the work, and introduced me to Gary.

Many organizations were kind enough to provide financial support for this project and myself, including McGill University (Special 2-Year Hydro Quebec McGill Major Fellowship, Blair Post-Graduate Fellowship, Research and Travel grants), the Department of Natural Resource Sciences, the John K. Cooper Foundation (research award), the American Ornithological Union (1996 Student Travel Award), the International Raptor Research

Foundation (Travel Award 1996, 1997 Student Presentation Award, 2nd place), the Wilson Ornithological Society (1996 award), the P.Q.S.P.B. (1995, 1996 awards), the International Osprey Foundation (1995 Endowment Fund), the Cooper Ornithological Society (1994 2-Year Membership Award), and the Orville Erickson Memorial Scholarship (1994).

I would like to thank the many people who helped me with the daily maintenance of the birds, particularly Nancy Leonard and Peter Thomas.

Finally, I would like to thank Ian Ritchie for his help with the birds, building cages, suggesting small changes which vastly improved the project and my life, for his friendship, and for being so patient and supportive through it all. My mom and dad, Robyn and Stephen were wonderful in providing their friendship, guidance, and advice through the joys, trials and tribulations of "phding." My grandmother provided the inspiration to do 'something different' and the financial support to do so. I could always count on Jim for advice, support, and friendship. Fearless, Ceile and Toby have also been very important to me. The Ritchies, Eli, Patrick and Paul have all made a big difference in my life in the past four years - thank you for helping to make Ste. Anne's my 'home away from home.'

TABLE OF CONTENTS

List of Tables

List of Figures

"Candidates have the option of including, as part of the thesis, the text of one or more papers submitted or to be submitted for publication, or the clearly duplicated text of one or more published papers. These texts must be bound as an integral part of the thesis.

If this option is chosen, connecting texts that provide logical bridges between the different papers are mandatory. The thesis must be written in such a way that it is more than a mere collection of manuscripts; in other words, results of a series of papers must be integrated.

The thesis must still conform to all other requirements of the "Guidelines for Thesis Preparation." The thesis must include: A table of contents, an abstract in English and French, an introduction which clearly states the rationale and objectives of the study, a comprehensive review of the literature, a final conclusion and summary, and a thorough bibliography or reference list.

Additional material must be provided where appropriate (e.g. in appendices) and in sufficient detail to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.

In the case of manuscripts co-authored by the candidate and others, the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent. Supervisors must attest to the accuracy of such statements at the doctoral oral defense. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of all the authors of the co-authored papers."

CONTRIBUTIONS OF CO-AUTHORS

Four manuscripts are now submitted to refereed journals, with two other manuscripts to follow. The four submitted manuscripts include:

1. Effects of electric and magnetic fields on melatonin in reproducing American kestrels;
2. Implications of electric and magnetic fields for behaviour of free-ranging and captive American kestrels;
3. Effects of electric and magnetic fields on the reproductive success of American kestrels;
4. Effects of electric and magnetic fields on body mass and feed-intake of American kestrels.

The data collection, analysis, and manuscript preparation were conducted independently by the senior author. Dr. Bird provided the American kestrels and associated support materials for this project. All co-authors provided editorial comments on final drafts.

The first manuscript on melatonin is co-authored by myself, D.M. Bird, D.L. Nguyen, D. Petitclerc, and E. Block. Dr. Nguyen provided technical support with the EMF facilities, Dr. Petitclerc analyzed the melatonin samples in his laboratory, and Dr. Block suggested the initial investigation of electric and magnetic field effects on melatonin in kestrels.

The second manuscript on behaviour is co-authored by myself, Nancy Leonard, and D.M. Bird. Data collection and analysis of the wild kestrel study was completed by myself. Under the supervision of myself and Dr. Bird, Nancy Leonard was largely responsible for the data collection and analysis of the captive behaviour experiment.

The third paper concerning effects of electric and magnetic fields on reproductive success is co-authored by myself, Russell Dawson, D.M. Bird, and Dr. P. Laguë. Russ Dawson provided assistance with the statistical analysis of some reproductive clutch parameters. Dr. Laguë provided the control facilities for the project, and gave guidance with the experimental design concerning blood sampling for hormones and egg collection.

The fourth paper, effects of electric and magnetic fields on body mass and feed intake of kestrels, is co-authored by myself and D.M. Bird.

Two other manuscripts are in preparation: effects of electric and magnetic fields on growth of young kestrels, and on hematocrit, total protein, and carotenoid levels.

STATEMENT OF ORIGINALITY

In fulfilling the requirements for the degree, Doctor of Philosophy, the research must show originality. This research makes the following original contributions to the scientific literature:

1. exposing American kestrels, adults, eggs, embryos, and young to electric and magnetic fields (EMFs);
2. determining the effects of EMFs on plasma melatonin concentrations in reproducing birds and fledgling birds;
3. determining plasma melatonin concentrations in a diurnal raptorial species;
4. discovering kestrels perceive EMFs as light as indicated by melatonin results;
5. determining the amount of time a wild falconiiform species is exposed to EMFs during the reproductive season;
6. evaluating the effects of EMFs on reproductive behaviour of birds;
7. evaluating the direct effects of EMF exposure on the reproductive success of an avian species, including:
    i) using adult birds to produce eggs under EMF conditions to investigate EMF effects;
    ii) using adult birds, rather than artificial incubators, to investigate EMF effects on embryonic development;
    iii) examining EMF effects on hatching and fledging success, and growth of birds;
8. determining EMF effects on body mass (adults, young) and feed intake of kestrels;
9. showing there is a sexually-dimorphic response or sensitivity to EMF exposure;
10. suggesting EMFs affect avian species during high resting metabolic rate periods.

THESIS ABSTRACT

Birds nest under electric and magnetic fields (EMFs) generated by transmission lines, which may affect their reproductive success and/or melatonin governing their circadian and circannual cycles. Over two years, captive kestrels were used to determine whether EMFs affect their plasma melatonin concentrations and their reproductive success. EMFs were equivalent to that which wild kestrels are exposed to while nesting under 735 kV transmission lines, and daily exposure used in the captive study (88 - 98% time budget) was potentially equivalent to that of wild kestrels (90% ♀, 80% ♂). Captive kestrels were housed in control or EMF conditions to determine short-term (one season; S-EMF) and longer-term EMF (two seasons; L-EMF) effects.

Plasma melatonin in adult EMF males was suppressed at 42 d and elevated at 70 d of EMF exposure compared to controls. Melatonin levels in EMF males at mid-season were similar to controls at season's end, suggesting a seasonal phase-shift. Melatonin was suppressed in L-EMF fledgling birds but not in adult females or males (1995) at 70 d. Plasma melatonin, higher in adult males than females at 70 d post-pairing, was not directly associated with body mass changes in kestrels.

Captive EMF birds were more active and alert but groomed less often than controls. EMF exposure affected reproductive success of kestrels. Fertility and fledging success were higher, and hatching success lower in S-EMF clutches. Hatching success was higher, but fledging success lower in L-EMF clutches. In S-EMF clutches, mean egg volume and mass were greater, eggs had slightly more albumen but thinner eggshells, and embryos were larger than controls. L-EMF hatchlings were heavier than controls.

The melatonin results for male kestrels indicate that kestrels perceive EMFs as light, thus altering their photoperiod. Photoperiodic manipulations advance molt onset, which is associated with increased body mass in male kestrels. S-EMF males were heavier at 56 d of exposure when molt began, but this was unlikely related to feed intake which was unchanged. EMF exposure had no effect on body mass and pectoral muscle scores of reproducing females. The sexually-dimorphic response in body mass and melatonin concentrations suggests that male kestrels may be more sensitive to EMF exposure than females.

RÉSUMÉ

Les oiseaux nichent sous les lignes électriques qui génèrent des champs électromagnétiques (CÉM) pouvant affecter leur succès de reproduction et/ou les cycles circadiens et circannuels gouvernés par la mélatonine. Sur une période de deux ans, des faucons crécerelles élèvés en captivité ont été utilisés pour déterminer si les CÉM affectaient les concentrations de mélatonine du plasma et le succès reproducteur. Les CÉM étaient équivalents à ceux générés par des lignes de 735 kV sous lesquels des crécerelles nichent. La durée d'exposition à ces CÉM était potentiellement équivalente (88%-98% du budget-temps) à celle observée en nature (femelles 90%, mâles 80%). Les faucons captifs étaient assignés soit comme témoins, soit soumis à des CÉM pour déterminer les effets à court terme (une saison; CÉM-court) et à long terme (2 saisons; CÉM-long).

La mélatonine plasmatique était supprimée chez les mâles adultes à 42 jours d'exposition aux CÉM mais supérieure aux témoins à 70 jours d'exposition. Les taux de mélatonine des mâles sous CÉM à mi-saison étaient équivalents à ceux des témoins en fin de saison, suggérant la présence d'un changement de phase saisonnier. La mélatonine était supprimée chez les oisillons après l'envol sous traitement CÉM-long, mais pas chez les mâles et les femelles adultes (1995) exposés 70 jours. La mélatonine plasmatique, plus élevée chez les mâles que chez les femelles 70 jours après la formation des paires, n'était pas directement associée avec les changements de poids observés.

Les oiseaux captifs exposés à des CÉM étaient plus actifs et plus alertes que les individus témoins, mais se nettoyaient les plumes moins souvent. L'exposition aux CÉM a affecté le succès de reproduction des faucons. Pour les couvées sous CÉM-court, la fertilité et le nombre d'oisillons à l'envol étaient plus élevés, mais le taux d'éclosion plus bas que chez les témoins. Pour les couvées sous CÉM-long, le taux d'éclosion était plus élevé, mais le nombre d'oisillons à l'envol plus bas. Pour les couvées CÉM-court, le volume et le poids moyens des oeufs étaient plus élevé, les oeufs contenaient un peu plus d'albumen mais une coquille plus mince, et les embryons étaient plus gros que chez les témoins. Les oisillons éclos sous CÉM-long étaient plus lourds que les témoins.

Les données concernant la mélatonine indiquent que les crécerelles mâles perçoivent les CÉM comme la lumière, ceux-ci affectant la photopériode. Chez les mâles, la manipulation de la photopériode avance la mue, associée à un accroissement du poids. Les mâles sous CÉM-court étaient plus lourds à 56 jours d'exposition quand la mue a commencé, sans relation apparente avec la prise de nourriture qui n'avait pas changé. L'exposition aux CÉM n'a pas affecté le poids corporel et le volume des muscles pectoraux des femelles reproductrices. La différence de réponse selon le sexe, au niveau du poids corporel et des concentrations de mélatonine, suggère que les mâles seraient plus sensibles que les femelles à une exposition aux CÉM.

The circannual and circadian rhythms of avian species are governed by photoperiod. Circannual rhythms involve events which occur on an annual basis, while circadian rhythms are events and patterns occurring within an approximate 24 h period (solar day). The lengthening and shortening of the photoperiod throughout the year governs physiological and behavioural mechanisms that determine the timing and duration of circannual events – reproduction, molting, and migration. Photoperiod also determines circadian physiology and behaviour, including locomotor activity, feeding, and sleeping. Consequently, circannual and circadian rhythms act as biological clocks to ensure the survival of the bird, coordinating its behaviour and interactions with the environment, and with inter- and intra-specific individuals.

The main physiological mechanisms for circannual rhythms remain unknown, either being distinct clocks, or more likely resulting from interactions of various neuronal, neuroendocrine and endocrine functions (Chabot and Menaker 1992, Adachi *et al.* 1995, Dawson 1997, among others). In contrast, the major components of the circadian pacemaker are the pineal organ and suprachiasmatic nuclei of the hypothalamus (Gwinner 1996). Environmental light stimulates the avian hypothalamus, signalling the synthesis of melatonin in the pineal gland. Melatonin synthesis shows clear rhythmicity: increasing and peaking in amplitude during dark periods while being suppressed during light periods. This rhythmicity likely acts on the suprachiasmatic nucleus as part of the overall circadian pacemaking system (Cassone 1990, Gwinner *et al.* 1994, Heigl and Gwinner 1995), governing circadian events critical to survival (e.g., body temperature, feeding). However, melatonin is also involved in the circannual events of molting (Gupta *et al.* 1987) and migration (Schneider *et al.* 1994a,b, Schneider 1995).

The nocturnal synthesis, release, and amplitude of melatonin have been suppressed in some mammalian species by ultraviolet wavelengths (Reiter 1992, 1993), changes in the direction of the geomagnetic field (Olcese *et al.* 1985, Reiter 1992), pulsed magnetic fields (e.g., Kato *et al.* 1993, 1994a,b, Yellon 1994), and alternating electric and magnetic fields (EMFs; Reiter 1985). Changes in the duration of the nocturnal melatonin peak have more

importance in determining physiological actions than changes in the amplitude of melatonin (Reiter 1992).

Research Rationale

Electric and magnetic fields (EMFs) are generated by electrical transmission lines, with the magnetic field, the horizontal component, measured in microteslas (μT), and the electric field, the vertical component, measured in kilovolts/metre (kV/m). Globally, birds use electrical transmission lines and associated structures for nesting, roosting, and hunting purposes throughout the year. Raptorial species are encouraged to nest on electrical structures through the installation of artificial nest platforms, and American kestrels (*Falco sparverius*) commonly nest in boxes placed on hydro poles. Consequently, raptors are exposed to EMFs throughout extended and repeated breeding seasons, with unknown implications (Steenhof *et al.* 1993).

By nesting under EMF conditions, melatonin concentrations in birds may be suppressed by EMFs. EMFs may be perceived as light, which could affect photoperiodic conditions. The suppression of melatonin and alteration of photoperiods would have implications for avian circannual and circadian rhythms. Changes in an artificial magnetic field reduced nocturnal synthesis of melatonin in migrating Pied flycatchers (*Ficedula hypoleuca*, Schneider *et al.* 1994a). Furthermore, reproductive success of Tree swallows (*Tachycineta bicolor*) was reduced under power lines, but this could not be directly attributed to EMF exposure (Doherty and Grubb 1996).

Clearly, melatonin is governed by photoperiodic conditions, is critical to avian circannual and circadian rhythms, and has been equivocally suppressed by EMFs in mammalian species. It is unknown whether EMFs suppress melatonin in reproducing birds and whether EMF exposure directly affects reproductive success. Consequently, I conducted a two-year reproductive study using captive American kestrels to test the hypotheses that electric and magnetic fields from powerlines suppress melatonin concentrations in birds, and that EMF exposure affects reproductive success of kestrels. Free-ranging kestrels were used to determine the amount of EMF exposure wild reproducing kestrels would experience.

Research Objectives

Specifically, I determined whether EMF exposure of reproducing American kestrels affects plasma melatonin concentrations, which would indicate that kestrels perceive EMFs as light with implications for the seasonal melatonin profile of the birds. Second, I determined the amount of time free-ranging reproducing kestrels are exposed to EMFs to provide a relevant timeframe for the laboratory study which determined the effects of EMF exposure on the reproductive behaviour of captive kestrels. Third, I examined the influence of EMF exposure on overall reproductive success of captive American kestrels. Finally, I studied the effects of EMF exposure on body mass of captive reproducing kestrels, as well as on food intake of wintering kestrels.

Most, if not all, birds are photoperiodic (Cockrem 1995). Variations in daylength govern avian physiology concerning circannual cycles of reproduction, molting, and migration, as well as circadian rhythms of locomotor activity, feeding, and sleeping. Circannual events follow an annual pattern, while circadian events occur on an approximate 24 h or solar day basis. Infradian rhythms operate on a basis of more than 24 h, while ultradian rhythms have a basis of less than 24 h. This literature review will focus on circannual and circadian rhythms in avian species.

Avian circannual and circadian rhythms act as biological clocks providing the major basis for timing of physiological and behavioural events (Gwinner 1975, 1996), and allowing successful environmental, inter- and intra-specific interactions. These clocks are essential to establishing the timing and duration of circannual events which are separated for most birds, because of conflicting physiological and heavy energetic demands, and seasonal variations in resources. Variations in daylength are the primary external cue or zeitgeber for circannual cycles of mid- to high-latitude species (Gwinner 1977), with rainfall and resource availability as primary zeitgebers for lower latitude species (Wingfield *et al.* 1992). Biological clocks also regulate basic daily functions. Circadian cycles, based on light:dark periods, are intrinsic and entrained by zeitgebers too. Under most conditions, light is the dominant zeitgeber, with food availability serving as a weak zeitgeber (Hau and Gwinner 1992, 1996, 1997).

The locations of the main physiological mechanisms for circannual rhythms remain unknown, but may be distinct clocks, or, more likely (Dawson 1997) result from interactions of various neuronal, neuroendocrine and endocrine functions (Chabot and Menaker 1992, Adachi *et al.* 1995, among others). In contrast, the major components of the circadian pacemaker are the pineal organ and suprachiasmatic nuclei of the anterior hypothalamus (Gwinner 1996). Measurement of daylength involves environmental light stimulating neural receptors in the hypothalamus. Hypothalamic neural signals stimulate synthesis of melatonin in the pineal gland. The melatonin rhythm acts on another oscillator, likely the suprachiasmatic nucleus, as part of the overall circadian pacemaking system (Heigl and

Gwinner 1995), but differences between species are evident in the regulating role of the pineal and other pacemakers (Janik *et al.* 1992). Retinal melatonin is synthesized and acts as a circadian pacemaker (Adachi *et al.* 1995, Krause and Dubocovich 1997).

Melatonin secretion is a significant component of the avian circadian pacemaking system (Cassone 1990, Gwinner *et al.* 1994). Its periodic secretion, high in dark conditions but suppressed by light conditions, is required for the internal synchronization of different components of the circadian system, and influences numerous functions critical to the survival of vertebrates. In avian species, melatonin is involved in circadian regulation of body temperature (Pang *et al.* 1991), locomotor activity and feeding patterns (Schneider 1995), growth and development of young (Lamošová *et al.* 1997), and in circannual events involving seasonal metabolism (Zeman *et al.* 1993, Ramachandran *et al.* 1996), plumage colour changes (Gupta *et al.* 1987) at molting which are important to mate selection (Hill 1990, Sætre *et al.* 1994, Sundberg 1995), and migration (Schneider *et al.* 1994a, Schneider 1995).

Reproduction

The circannual cycle of reproduction is primarily a function of seasonal variations in daylength, although not through melatonin (Siopes and El Halawani 1989, Pang *et al.* 1991, Dey and Maitra 1995, Ramachandran *et al.* 1996, Wilson 1991). The endogenous circannual time program determines the reproductive period of a bird, not its reproductive performance (Gwinner *et al.* 1995).

Each year, the timing and duration of the reproductive season varies among and within species, populations, and individuals, and is tied to seasonal changes through external or proximate factors (e.g., habitat, vegetation, temperature, rainfall). Variations in daylength are the most important proximate factor for reproduction (Farner and Follet 1979, Wingfield *et al.* 1992), and correspond to marked seasonal photogonadal responses (Kumar and Kumar 1991). Ultimate factors (e.g., food supply, nest site availability) provide additional physiological controls for timing of clutch production to ensure that conditions are optimal for survival of young (Lack 1968, Perrins 1970, Wingfield *et al.* 1992).

Avian reproduction consists of regular changes in photoperiodic response between photosensitivity and photorefraction (Cockrem 1995), which are reflected by development

and regression of reproductive organs and behaviour controlled by neuroendocrine and endocrine systems (Wingfield *et al.* 1992). The breeding season starts during the short days of autumn when birds become photosensitive. Short days, when the photophase is shorter than the scotophase, are essential to successful avian reproduction by allowing birds to regain photosensitivity (Dawson and Goldsmith 1997). Regaining photosenstivity is gradual and dependent on the length and duration of photoperiods (Dawson 1991, Kumar and Kumar 1992).

Reproduction and Neuroendocrine, Endocrine Systems

Initial photosensitivity, or the regaining of photosensitivity, occurs in the hypothalamus, with daylength controlling the timing, synthesis, and secretion of gonodotropin-releasing hormone (GnRH), a neurohormone (Dawson and Goldsmith 1997). Increases in hypothalamic GnRH and plasma leutenizing hormone (LH), occur only when birds are photostimulated after a lag of 30 short days or more (Follett and Pearce-Kelly 1990, Wilson 1990, 1992, Dawson and Goldsmith 1997). This gradual change from a photorefractory to photosensitive state may be characterized by a spontaneous increase in hypothalamic GnRH, pituitary LH content, testicular mass, and in-vitro testosterone release (Bluhm *et al.* 1991).

Once light stimulates hypothalamic receptors of neurosecretory cells, GnRH is released, travels to the anterior pituitary, and induces synthesis and release of LH and follicle stimulating hormone (FSH). The increasing secretion of LH shows clear rhythmicity and is weakly circadian in nature (Follett *et al.* 1992). LH and FSH directly affect gonadal and follicular activity, with the increase in LH secretion and rapid gonadal development largely dependent on increases in photoperiod length (Ravikumar and Tewary 1991, Silverin *et al.* 1993). LH and steroid hormones are regulated by positive and negative feedback systems (Etches 1996).

Photorefraction

As birds enter the incubation phase, daylength continues to increase, and birds are continuously exposed to long days until they lose photosensitivity and become photorefractive. The breeding season is finite and cannot be extended (Kumar and Kumar

1991). An endogenous circadian rhythm is involved in the establishment, maintenance, and termination of photorefraction (Ravikumar and Tewary 1990). Photorefraction, testicular regression, and prenuptial molt develop and are associated with changes in the circadian system, particularly in entrainment properties (Pohl 1994). Gonadal regression in late summer is programmed during the increasing photoperiods of spring and early summer, likely the second and third week after photostimulation begins (Wilson 1997), before photoinduced testicular growth ends (Wilson and Reinert 1995).

Species differ in the timing of photorefractoriness. Species with short breeding seasons (e.g., owls, herons) which end at or shortly after the summer solstice, generally become absolutely photorefractory – breeding ceases while daylength is long and still increasing. Prolonged exposure to long days in these species results in spontaneous regression of the reproductive system. Once regression has occurred, birds cannot respond to further increases in daylength (Kumar and Kumar 1991, Dawson 1998). Conversely, other species with longer breeding seasons (e.g., quail, April - August) are relatively photorefractory – a decrease in daylength is required to induce gonadal regression, but a reduction in GnRH does not occur. Absolute and relative photorefractoriness are unlikely to be extremes of a single underlying photoperiodic control mechanism (Dawson 1998).

Photorefraction is promoted by a reduction in hypothalamic GnRH synthesis (Dawson *et al.* 1985, Bluhm *et al.* 1991), rather than inhibition of its release (Parry *et al.* 1997). Circulating LH is reduced to nonbreeding levels through decreasing steroid levels (Dawson 1997), and declining daylength accompanied by low ambient temperatures (Wada *et al.* 1990). The seasonal photoinduced increase in prolactin (PRL) accelerates gonadal regression during the onset of photorefractoriness, but does not itself cause photorefraction (Dawson and Sharp 1998). Thyroxine does not affect testicular regression (Wilson and Reinert 1995), but thyroid hormones are required for photorefraction (Wilson 1997, Dawson 1998 and references therein).

Molting

Photorefraction is tightly linked to postnuptial molt (Wilson and Reinert 1995), which is programmed within four weeks of exposure to long days (Moore *et al.* 1983, Wilson 1997).

Molt is controlled by decreases in daylength. If daylength decreases before molt starts, the onset of molt is advanced but the rate of molt is unaffected. A decrease in daylength near the start of molt increases the rate of moulting (Dawson 1998). The thyroid gland (Wilson 1997), and increasing PRL during the breeding season, are also involved in inducing postnuptial molt (Dawson and Sharp 1998).

As the high energetic costs of molting can conflict with those of reproduction and migration, these circannual events are often separated, with molt beginning at (e.g., European kestrel *Falco tinnunculus*, Meijer 1989) or during (Payne 1972) gonadal regression but preceding migration. Gonadal steroids inhibit the timing of molt (Dawson 1994, 1997, 1998), preventing molting during clutch production when steroids are high, but allowing molting to occur during incubation or gonadal regression when steroids are low. Molting may be halted if it is not completed prior to migration, but the majority of species complete molting before migration.

Migration

Timing of Migration

Variations in photoperiod, weather, and resources determine the timing of migration, but photoperiod is the only environmental factor known to change the time course of migration (Gwinner 1996). The circannual rhythm responds to photoperiod in a functionally adaptive manner: short photoperiods of late summer and autumn advance post-juvenile or pre-basic molt, the onset of migratory restlessness or zugunruhe, and ensure that young from late clutches develop faster so as to leave the breeding grounds on time. Lengthening photoperiods of winter and early spring end autumn migration and ensure that winter molting and spring migration occur at an appropriate time. Lengthening photoperiods past the equator, stop birds which migrated beyond regular wintering grounds in the fall, and ensure that they begin spring migration sooner so as to arrive in their breeding range at an appropriate time (Gwinner 1996). However, the flexibility of migratory timing and distance to photoperiodic changes is species-specific (Gwinner 1989).

Circannual signals of unknown nature (Gwinner 1996) change the circadian system, increasing nocturnal activity or zugunruhe in birds which are normally active during the day

but migrate at night. Zugunruhe is exclusive to migratory birds. Circannual changes in the diurnal pattern of melatonin, particularly a slight reduction in nocturnal melatonin peaks (Gwinner *et al.* 1993), are associated with or causally linked to zugunruhe (Gwinner 1996), but not in a simple or direct manner (Gwinner *et al.* 1993). When zugunruhe is completed, the nocturnal peak of melatonin increases.

Daylength determines the timing of subcutaneous fat stores prior to migration (Wolfson 1942), while body mass and zugunruhe appear rhythmically linked. Caged Garden warblers (*Sylvia borin*) spontaneously and repeatedly underwent changes in body mass and migratory activity. Zugunruhe was slowed down or interrupted when body mass increased, particularly at the onset of the migratory season (Gwinner 1996). While prolactin is not involved in migratory fattening of some species (i.e., Japanese quail, *Coturnix coturnix japonica*, Boswell *et al.* 1995 but see Nicolls *et al.* 1988), the amount of subcutaneous fat stores is linked to body condition of nestling Blue tits (*Parus caeruleus*), with possible implications for survival during migration (Merila and Svensson 1997).The amount of fat stores is also dependent on migration distance. Short-range migrants store moderate fat levels of 13% to 25% of body mass as they regularly refuel while migrating, but long-range, intercontinental migrants can store 30% to 47% for long, nonstop flights (Berthold 1975).

Distance and Direction of Migration

The spatial patterns of migration (i.e., spring and autumn direction, changes during migration, amount and duration of zugunruhe) are also determined by programs linked to circannual cycles, which may be species- or population-specific (Gwinner 1996, Helbig 1996). While an internal clock integrates direction and distance information in determining the final location (Jander 1963), pineal melatonin is critical in determining migratory distance and proper orientation. Melatonin may be involved in expressing the genetically encoded information during migration concerning migratory direction and the geomagnetic field (Schneider *et al.* 1994a,b), particularly during a bird's initial migration (Helbig 1991). Alternatively, melatonin may be involved in the time program governing the specific migratory direction at a given time (Schneider *et al.* 1994a,b). Melatonin is also likely involved in making changes in the direction birds migrate during and within each season, as they are

controlled by circannual rhythms (Gwinner 1996), and are correlated to changes in the earth's magnetic field (Gwinner and Wiltschko 1978, 1980).

The distance migrated is also determined by the temporal program for autumn migration, but it is unknown if the program determines the migration distance, or the temporal framework in which other factors (e.g., body mass, humidity, darkness) determine the distance (Gwinner 1996). Migration distance is also a function of the nightly and seasonal amount of zugunruhe (Holberton 1993, Able 1995), which is greater in terms of nightly amount and seasonal duration in birds migrating longer distances (Gwinner 1986, 1996). Food availability, and hence the nutritional and energetic state of the bird, modifies the daily distances migrated. Energetically depleted birds will stop migrating for short time periods if food is available, but will continue migration if food is unavailable to restore energetic reserves.

Navigation and Orientation

Direction and orientation during spring and autumn migration also depend on the interrelationships of the stars, the geomagnetic field, and sunset factors. These mechanisms and their interrelationships have been well studied in only three species, the European robin (*Erithacus rubecula*), Blackcap (*S. atricapilla*), and the Savannah sparrow (*Passerculus sandwichensis*; Able 1995). Current evidence suggests that in short-term orientation, magnetic cues are more important than stars, but both are overridden by visual information at sunset (Able 1995).

Migratory direction is found through a complex system involving two mechanisms: the magnetic compass, repeatedly recalibrated throughout life (Able and Able 1995), and the star compass which is calibrated by celestial rotation. The magnetic field serves as a reference system for genetically encoded directional information (Bingman 1981, Able 1995), as it is more intense at the magnetic poles (N and S; 60,000 nT) than at the equator (30,000 nT) where it is more regular (Skiles 1985). As observed in other species (Wiltschko and Wiltschko 1991 and references therein), the magnetic compass of European robins (*Erithacus rubecula*) is narrowly tuned to the total intensity of the ambient magnetic field, but adapts to changing intensities within three days (Wiltschko and Wiltschko 1988). Some species

maintain an appropriate direction throughout migration under constant magnetic field conditions (Gwinner and Wiltschko 1978, Helbig *et al.* 1989), whereas other species only maintain a correct direction if they encounter changing magnetic fields as they travel (Able 1995).

The star compass also serves to give directional reference for innate directional information, with stellar orientation developing independently of magnetic fields during ontogeny (Able and Able 1995). While the star compass changes nightly, seasonally, and with geographic longitude, the appearance of the sky is unimportant (Wiltschko *et al.* 1987). Likely, migratory direction is derived from the constant spatial relationship between stars (Emlen 1967a,b). Celestial rotation is crucial for establishing the star compass (Emlen 1970, Wiltschko *et al.* 1987, Able and Able 1990), and possibly calibrating the magnetic compass (Able 1995).

During ontogeny and establishment of the direction of initial migration, celestial rotation and stars have a more important directional role than the magnetic compass (Able 1995), although the directional importance of the stars is modified with time (Beason 1987). During actual migration, the magnetic field becomes increasingly more important, taking precedence as the bird encounters new celestial skies, since it controls celestial cues which maintain flight direction (Wiltschko and Wiltschko 1991).

The view of the setting sun, especially the characteristic pattern of polarized light during the day, are also important orientation cues for night-migrating species which develop independently of magnetic directions during ontogeny (Able and Able 1997 and references therein). Patterns of polarized skylight during the day assess celestial rotation (Able and Able 1993), but the role of sunset factors during migration remains unclear. Their interaction with and importance relative to other cues is species-specific (Wiltschko and Wiltschko 1991 and references therein), and appears more variable than that of celestial and magnetic field cues.

Landscape features and constant winds may be used temporarily to find and maintain the migration course (Wiltschko and Wiltschko 1988, 1991, Able 1995). A navigational map based on olfactory (Papi 1991) and/or magnetic information has been proposed for homing pigeons, with some evidence for migratory species (Able 1995).

The Effect of Photoperiod on Circadian Physiological Parameters

Photoperiodic length and light intensity are also linked to many circadian physiological functions which are critical to the survival of birds. Body mass and composition (fat, protein content) of male broiler chickens were affected by light intensity and photoperiod length (Charles *et al.* 1992), but had no effect on body mass, behaviour, and efficiency of food utilization of female turkeys (Denbow *et al.* 1990). The colour of light, red or blue, and its intensity, affected the behaviour, feeding, activity level, growth, and bone strength of broiler chickens (Prayitno *et al.* 1997). Body temperature rhythms adjust to a wide range of photoperiod lengths, yet the energy balance of pigeons is remarkably constant over many photoperiod lengths (Basco *et al.* 1996). Ultraviolet light may also be utilized by avian species for mate choice (Bennett *et al.* 1996) and hunting purposes (Viltala *et al.* 1995).

Electric and Magnetic Fields and Melatonin

Clearly, variations in photoperiod are critical in ascertaining the timing and duration of the circannual events of reproduction, molting, and migration. Photoperiod also plays a fundamental role in the circadian rhythms of avian species by determining and synchronizing the circadian rhythm of melatonin synthesis in the pineal gland (Reiter 1992), a critical component of the avian pacemaker clock. Melatonin is also synthesized in the chicken retina, and is believed to influence transmission of light signals to the brain (Krause and Dubocovich 1997 and references therein). Melatonin levels peak in dark periods, but are suppressed by light periods. The duration and amplitude of the melatonin rhythm synchronizes circadian and circannual physiological changes within the organism (Reiter 1987, 1993). Variations in the duration of darkness affect the duration of the melatonin peak, which is more important than amplitude changes in determining the physiological action of melatonin in mammals (Reiter 1992).

Ultraviolet wavelengths, changes in the direction of the geomagnetic field (Olcese *et al.* 1985, Reiter 1992), pulsed magnetic fields (e.g., Kato *et al.* 1993, 1994a,b, Yellon 1994), and alternating electric and magnetic fields (EMFs), depress nocturnal melatonin synthesis, release, and amplitude in some mammalian species (Reiter 1985).

By introducing electrical transmission and distribution lines, humans have brought

major sources of EMFs to the environment which exceed the geomagnetic field and geoelectric fields associated with weather conditions (Reiter 1993). Transmission lines and associated structures have proved beneficial and detrimental to avian species, particularly birds of prey or raptors. Transmission towers provide alternative sites for perching, nesting, roosting, and hunting (Steenhof *et al.* 1993), with artificial nest platforms and boxes erected for use by raptors (Olendorff *et al.* 1981, Steenhof *et al.* 1993). However, power lines and structures have electrocuted individuals of many species (Olendorff *et al.* 1981, Ferrer *et al.* 1991, Bevanger 1994, Negro and Ferrer 1995).

The use of electrical transmission structures by birds exposes them to EMFs generated by electrical lines, but with unknown implications (Steenhof *et al.* 1993). Raptors nesting on transmission lines and structures are exposed to EMFs for long periods throughout the breeding season (e.g., > 3 months), and repeatedly if site fidelity is strong (Steenhof *et al.* 1993).

While inconclusive, EMFs may have reduced reproductive success of Tree swallows (*Tachycineta bicolor*; Doherty and Grubb 1996). Effects of EMF exposure on avian fertility and hatching success are ambiguous, but EMFs have clearly affected embryonic development, delaying development of Sea urchins (*Stronglyocentrotus purpuratus*, Cameron *et al.* 1993) and chickens (*Gallus domesticus*, Delgado *et al.* 1982, Ubeda *et al.* 1983, 1994, Juutilainen *et al.* 1987, Martin 1988, but see Maffeo *et al.* 1988, Martin 1992, Coulton and Barker 1991, Veicsteinas *et al.* 1996), and increasing body mass and/or bone length of mice (Kowalczuk *et al.* 1994) and chickens (Rooze and Hisenkamp 1985). EMF laboratory experiments on birds have utilized artificial incubators which may be affected by EMFs, particularly temperature and humidity, thereby having implications for successful embryonic development and hatching (Martin 1992). EMFs from power lines may also affect migration, as altered magnetic fields reduced nocturnal synthesis of melatonin in migrating Pied flycatchers (*Ficedula hypoleuca*, Schneider *et al.* 1994a).

EMFs have equivocally reduced nocturnal melatonin levels in some mammalian species, as EMFs may be perceived as light (Reiter 1992, 1993). It is unknown if EMFs affect melatonin concentrations in avian species during reproduction. Changes in melatonin

rhythmicity have implications for the physiology of the organism (Brainard *et al.* 1986), particularly for avian species as photoperiod and melatonin are closely linked in determining and expressing the timing and/or duration of circannual and circadian rhythms.

American kestrels

The American kestrel (*Falco sparverius*) may spend part of its circannual cycle, reproduction and molting, and its circadian cycle exposed to EMFs from power lines. This cavity-nesting species readily hunts from transmission lines (K. Fernie, pers. obs.), and nests in boxes placed on hydro poles (Villarroel 1996) in cities, suburbs, and farmland areas of North and South America (Balgooyen 1976, Bird 1988). Male kestrels of eastern Canada arrive first on the breeding territory in late March or early April, having migrated from overwintering grounds south of the Carolinas (Bird 1988). Once pairs have been established, an average clutch of five eggs is laid within two weeks. Incubation is typically 28 d, and chicks fledge 28 d after hatching but remain with the parents near the nest box for approximately 10 d more (Balgooyen 1976). Females are the primary incubator until young are 10 d of age, and males provide food for both the female and young. Females begin to molt during incubation, with males following within two weeks (Porter and Wiemeyer 1972).

The American kestrel has made an excellent model species in previous research, including numerous captive studies investigating behavioral and nutritional ecology, endocrinology, parasitology, physiology, and toxicology (Bird 1982, 1985).

Thesis Hypotheses

Clearly, American kestrels may be exposed to EMFs from electrical transmission lines during reproduction and molting, and consequently make an excellent model species for determining EMF effects on melatonin concentrations and reproductive parameters of raptorial species. Given the importance of melatonin in avian circannual and circadian physiology and behaviour, the equivocal effects of EMFs on melatonin in mammalian species but unknown effects in reproducing avian species, it is important to test the hypothesis that electric and magnetic fields (EMFs) affect the plasma melatonin concentrations in reproducing kestrels. This study will also test the hypothesis that EMFs affect reproductive success of birds. EMF effects on selected behavioural and physiological parameters related to reproduction and development of this species will be investigated.

CONNECTING STATEMENT 1.

Melatonin is an integral part of the system governing circannual and circadian rhythms of avian species. Changes in the duration of nocturnal melatonin synthesis have important physiological implications. Nocturnal melatonin concentrations in mammalian species have been equivocally affected by ultraviolet wavelengths, changes in the geomagnetic field, and by electric and magnetic fields. Alterations in a magnetic field have affected melatonin levels in migrating Pied flycatchers, but it is unknown if EMFs from transmission lines affect plasma melatonin concentrations in reproducing birds.

CHAPTER 2. EFFECTS OF ELECTRIC AND MAGNETIC FIELDS ON MELATONIN IN REPRODUCING AMERICAN KESTRELS.

Abstract

Birds reproduce within electric and magnetic fields (EMFs) of transmission lines. Melatonin influences physiological and behavioural processes critical to survival, and has been equivocally suppressed by EMFs in mammalian species. We determined if EMFs affect plasma melatonin concentrations in reproducing adult and fledgling American kestrels (*Falco sparverius*), and if altered melatonin levels were correlated to changes in body mass caused by EMF exposure as reported previously. Captive kestrels were paired in 1995 and 1996, with half of the 1995 sample exposed again to determine effects of longer-term EMF (L-EMF) exposure. Plasma melatonin in adult males, ranging from 49.65 to 89.71 pg/ml, was affected by EMF exposure, being suppressed at 42 d and elevated at 70 d of EMF exposure. The similarity in melatonin levels between EMF males at 42 d and controls at 70 d likely indicates a seasonal phase-shift of the melatonin profile of male kestrels by EMF exposure. Average plasma melatonin concentrations 70 d after pairing ranged from 18.37 to 71.84 pg/ml in adult females, and 37.61 to 77.90 pg/ml in 35 d old fledgling birds. Plasma melatonin was suppressed in L-EMF fledgling birds only, not in adult females or 1995 adult males at 70 d of EMF exposure. Results from the 1996 males are more powerful than 1995 as multiple sampling accounts for natural heterogeneity. Plasma melatonin of adult males was higher than adult female kestrels, possibly explaining the differential melatonin response of the two sexes at 70 d exposure. Melatonin and body mass are not directly associated in adult or fledgling American kestrels.

Introduction

Melatonin influences numerous functions critical to the survival of vertebrates. In avian species, melatonin is involved in the regulation of body temperature (Pang *et al.* 1991), seasonal metabolism (Zeman *et al.* 1993, Ramachandran *et al.* 1996), locomotor activity and feeding patterns (Schneider 1995), plumage colour changes (Gupta *et al.* 1987) which are important to mate selection (Hill 1990, Sætre *et al.* 1994, Sundberg 1995), growth and development of young (Lamošová *et al.* 1997), and migration (Schneider *et al.* 1994a, Schneider 1995). Yet, melatonin is unlikely to control the reproductive season in avian species (Siopes and El Halawani 1989, Pang *et al.* 1991, Wilson 1991, Dey and Maitra 1995, Ramachandran *et al.* 1996).

Electric and magnetic fields (EMFs) are known to suppress the scotoperiod rise in plasma melatonin levels in some mammalian species. One hypothesis for this occurrence is that EMFs are perceived as light (Reiter 1992, 1993). Birds are exposed to EMFs, particularly raptors, which use transmission towers for roosting and nesting over extended (e.g., >3 months) and repeated (e.g., >3 years) periods (Steenhof *et al.* 1993). Reproductive success of Tree swallows (*Tachycineta bicolor*) was lower under powerlines than in control areas, although this could not be directly attributed to EMFs, but success was similar for Eastern bluebirds (*Sialia sialis*) and House wrens (*Troglodytes aedon*) in control and powerline areas (Doherty and Grubb 1996).

We examined the possible impact of EMFs on plasma melatonin levels in reproducing adult and fledgling American kestrels, using a captive breeding colony. We tested the hypothesis that EMFs will affect plasma melatonin levels in kestrels, regardless of age or sex. Plasma concentrations of melatonin were compared in adult and fledgling males and females, and correlations between melatonin and body mass in kestrels were calculated. Here we include body mass data for adult kestrels which have been reported previously (Fernie and Bird, submitted).

Methods and Materials

Fifty-six reproducing pairs of captive American kestrels were used from the Avian Science and Conservation Centre (ASCC) of McGill University for each year of study. In

1995, 28 pairs were randomly assigned to a control room, and 28 pairs to an EMF exposure chamber. In 1996, new pairs were randomly assigned to each room (13 control, 15 S-EMF pairs) for determining short-term EMF effects (one breeding season), and 13 control pairs and 15 EMF pairs from 1995 were used for a second breeding season to identify possible longer-term EMF effects.

A control room (10 m long, 8 m wide, 3 m high) and an EMF room (15 m long, 10 m wide, 3 m high) were used to house the kestrels. The design of the EMF exposure chamber has been described elsewhere (Nguyen *et al.* 1991). In the control and EMF rooms, humidity, temperature and photoperiod clocks reflected natural conditions (lat. 45°30', long. 73°26') with no significant differences between the two rooms (t-tests, all Ps > 0.05). Each room had a ventilation fan. Average light intensity at the birds' head level was not significantly different with EMFs on or off (lights on; t_{24}=-1.1990 P>0.05; EMF 160 lux, ctl 150 lux). Noise levels, indicative of vibrations (D. Nguyen, pers. comm.), were similar when EMFs were on or off (t_8=0.64 P>0.05).

A magnetic field of 29.35 ± 0.03 μT in the EMF room was created by a current running through coils in the walls, floor, and ceiling of the room. The electric field of 9.67 ± 0.04 kV/m was generated by two plates suspended from the ceiling (Nguyen *et al.* 1991, Burchard *et al.* 1996). The EMFs, controlled by a computer to provide consistent and uniform fields, were equivalent to that which kestrels are exposed to when nesting under 735-kV transmission lines running at peak capacity. The magnetic and electric fields in the control room were 2.02 ± 0.27 μT and 0.03 ± 0.01 kV/m, respectively.

Each pair was housed in a visually-isolated breeding pen (0.75 m x 0.75 m x 1.27 m) made of reinforced corrugated cardboard and covered with nylon netting. A standard wooden nest box (0.3 m x 0.3 m x 0.37 m) was attached to the pen, and a rope perch ran diagonally across the pen. All pens and nest boxes were identical in size in both rooms. Wood shavings provided bedding and nesting material. Metal materials were minimized to reduce disturbance of the electric field and possible shocks to the birds (F. Renaud, pers. comm.), while magnetic fields penetrated all housing materials (D. Nguyen, pers. comm.). Kestrels were provided *ad libitum* day-old whole cockerels and water each day at the same time, with all

feed leftovers removed at feeding.

Kestrel pairs were genetically unrelated within the past seven generations. Each bird had previous breeding experience. All adults were similar in age (2 - 5 y), with no significant differences within sex in age, size (wing chord), body mass, or condition (body mass: wing chord index) at pairing (t-tests, all Ps > 0.05).

Kestrels were paired on 11 May 1995 and 13 May 1996, with exposure of EMF pairs beginning immediately and lasting for 95 d in 1995, and 91 d in 1996, until young were 35 d old, one wk after fledging. The birds were exposed to EMFs for approximately 21 h/d, or 87.5% of a 24 hr period, in 1995, and to approximately 23.5 h/d or 98% of 24 h, in 1996. These exposure periods are comparable to that which free-ranging kestrels are potentially exposed to during the reproductive period (Fernie, in prep., Fernie *et al.*, submitted - a).

To avoid possible behavioural and physiological modifications from blood sampling (Hoysak and Weatherhead 1991), approximately 1.1 ml of blood per bird was drawn from the jugular vein into a 1 ml heparinized syringe with 27 gauge needle, then kept on ice and centrifuged (10 min, 9000 rpm, 16,000 x G, Eppendorf 1451) within two hours. Plasma was stored at -20°C until further analysis. Kestrels were weighed to the nearest 0.1 g (Sartorius scale, model PT600). Sampling of male kestrels occurred between 0800 and 1000 before morning feeding to minimize circadian fluctuations in hormone levels (García-Rodríguez *et al.* 1987, Ferrer 1990), and to minimize variations in body mass due to prior feeding. Females were measured at 1500 to avoid disturbing egg laying which generally occurs late at night (Liou *et al.* 1987, I. Ritchie, pers. comm.).

While mammalian EMF-melatonin research generally involved nocturnal blood sampling through cannulae or under dim red lights, we blood-sampled during the photoperiod (morning) to minimize disturbing ovipositioning. Elevated nocturnal plasma melatonin levels are essential for regulating the time of oviposition in the chicken (*Gallus domesticus*, Liou *et al.* 1987). Given the rapid suppression (e.g., < 15 min, Vakkuri *et al.* 1985) and gradual recovery of nocturnal melatonin levels from light, including red lights (Binkley and Geller 1975, Binkley *et al.* 1977), in domestic and wild avian species (Liou *et al.* 1987, Meyer and Millam 1991, Schneider *et al.* 1994b, Adachi *et al.* 1995), we believed that nocturnal

sampling of kestrels may have suppressed melatonin levels of laying females which would have affected ovipositioning. Blood sampling at 1500 continued after clutch production allowing for comparisons of melatonin profiles throughout the reproductive season.

Males were sampled once every two weeks until 70 d after the experiment began. Female kestrels were measured three times; between laying of the third and fourth eggs ('mid-laying'), 20 days post-laying, and at the end of the experiment (70 d). Plasma samples from males at 14, 42, and 70 d of the experiment, and plasma samples from females at 70 d, were selected for the melatonin analysis. Unfortunately, the 14 and 42 d samples from males in 1995 were lost during the radioimmunoassay process.

Serum was extracted from collected plasma and analyzed for melatonin by the double antibody radioimmunoassay (Webley *et al.* 1985). In each assay tube, 250 µl of serum or standard, 50 µl of tracer (2400 rpm diluted in 1:400 normal sheep sera), 100 µl of the first antibody (1:9000 sheep anti-melatonin), and 200 µl of the second antibody (1:70 donkey anti-sheep antiserum) were added. Tubes were vortexed, incubated at 4°C for 18 h, and centrifuged for 20 min at 3000 rpm (2125xG). To prevent warming of the pellet, tubes were rapidly inverted with a wire mesh to pour off the supernatant, the tube tops blotted, rinsed gently, and blotted again. Tubes were reinverted and dried with the wire mesh removed. With 20 µl of ddH_2O added to each tube, the pellet was dissolved by vigorously shaking each tube. About 3.25 ml of scintillant was added and the tube capped and cooled at 1°C for a minimum of 24 h. A Beta counter was used (10 min per sample). The intra- and inter-assay coefficients of variation were 9.2% and 17.1%, respectively.

Statistical analyses were conducted using S.A.S. software (1985) and performed separately by sex, age (adult, fledgling), and length of exposure period (S-EMF, L-EMF exposure). One-way ANOVAs (age) for adult males and fledglings, and two-way ANOVAs (clutch production, age) for adult females, were used to determine potential differences prior to further analyzes for treatment effects. One-way ANOVAs tested for treatment effects on melatonin concentrations in adult females at 70 d. Melatonin results from males were analyzed by a Mann-Whitney U-test (1995 70 d sample), Repeated Measures ANOVA and subsequent one-way ANOVAs to identify EMF effects at specific 1996 sampling periods (see below).

Plasma concentrations of melatonin between sexes were compared using paired t-tests. T-tests were used to identify EMF effects on melatonin concentrations in fledglings within sex. Pearson Product-Moment Correlation with Bonferroni corrections or Spearman Rank Order Correlation (1995 adult males only) were used to analyse associations between melatonin levels and body mass, with statistical outliers (Sokal and Rohlf 1981) removed prior to correlation analyses. Statistical significance was considered to be at the $P<0.05$ level. All values reported are means ± S.E.M.

Results

i) Plasma melatonin concentrations and body mass of male adult American kestrels

Plasma melatonin concentrations in S-EMF and L-EMF adult male American kestrels did not show any age-related differences in 1995, at individual sampling periods in 1996, or overall in 1996 (1-way ANOVA, all $Ps > 0.05$). Consequently, data for melatonin levels of males were grouped by S-EMF or L-EMF exposure for further analyses.

In 1995, melatonin levels of S-EMF male kestrels showed no effect from 70 d of EMF exposure ($U=657.5$ $P>0.05$; see Table 1, Figure 1). In 1996, S-EMF exposure affected overall melatonin concentrations in males ($F_{3,22}=13.44$ $P<0.001$). Time ($F_{2,23}=41.39$ $P<0.001$) and treatment*time interactions ($F_{2,23}=20.25$ $P<0.001$) were significant. Specifically, plasma melatonin concentrations were similar at 14 d ($F_1=0.48$ $P>0.05$), but while melatonin levels in the controls had slightly increased at 42 d, melatonin concentrations in S-EMF males had declined at 42 d and were significantly lower than in controls ($F_1=27.13$ $P<0.001$). Melatonin concentrations in control males then declined at 70 d, but concentrations in S-EMF males at 70 d had increased from 42 d, and were significantly higher than those of controls at 70 d ($F_1=9.25$ $P<0.01$; see Table 1, Figure 1).

L-EMF males showed a similar melatonin pattern as the 1996 S-EMF males (see Table 1, Figure 1). Treatment effects for melatonin levels were significant ($F_{2,24}=25.62$ $P<0.001$), as were time ($F_{2,24}=6.08$ $P<0.001$) and treatment*time interactions ($F_{2,24}=25.62$ $P<0.001$; see Table 1, Figure 1). Again, plasma melatonin concentrations were similar between control and EMF males at 14 d ($F_1=1.39$ $P>0.05$). Melatonin concentrations declined in both control and L-EMF males at 42 d, but were significantly lower in L-EMF males than

controls at 42 d (F_1=42.59 P<0.001). Melatonin concentrations in control males continued declining at 70 d, but melatonin levels in L-EMF males increased at 70 d from 42 d levels, and were significantly higher than in controls at 70 d (F_1=4.89 P<0.05).

In 1996, melatonin levels of S-EMF males at 42 d were similar to those of control males at 70 d (t_{25}=1.89 P>0.05 Figure 1). Melatonin levels of L-EMF males at 42 d were similar to melatonin levels of control males at 70 d (t_{25}=0.253 P>0.05, Figure 1).

There was no correlation between actual plasma concentrations of melatonin and body mass at sampling periods for S-EMF males in 1995 (Spearman) and 1996, or L-EMF males in 1996 (Pearson, all Ps > 0.05). One outlier (1-tail t-test: t_{15}=2.018 P<0.05; 14 d: 107.34 pg/ml vs. 87.15 ± 2.43 pg/ml for S-EMF males) was removed from the 1996 S-EMF males prior to analysis. Changes in melatonin and body mass between periods were correlated but significant for L-EMF males only. In control males, the decline in melatonin from 42 d through 70 d was positively correlated to increasing body mass in 56 d through 70 d (r=0.7245 P<0.01; see Fig. 2). In L-EMF males, declining melatonin from 14 d through 42 d was negatively correlated with declining body mass from 42 d through 56 d (r=-0.6078 P<0.01), and melatonin increases from 42 d through 70 d was positively correlated with increasing body mass from 56 d through 70 d (r=0.5632 P<0.05; see Figure 2).

ii) Plasma melatonin concentrations and body mass of females

Melatonin data for adult female American kestrels were grouped as there were no age differences, or differences between layers (incubation versus abandonment) and non-layers (2-way ANOVAs, all Ps > 0.05). Plasma concentrations of melatonin were similar between adult control and EMF females (see Table 2), showing no effect from 70 d of S-EMF exposure in 1995 (F_1=0.56 P>0.05) and 1996 (F_1 =0.05 P>0.05), or from 70 d of L-EMF exposure (F_1=2.50 P>0.05; see Table 2).

Correlation of plasma concentrations of melatonin and body mass for adult female American kestrels was conducted for all females regardless of clutch production, and then restricted to those females which laid and incubated a clutch for 28 d, when hatching should or did occur. Melatonin and body mass at 70 d were not correlated for any female group regardless of clutch production, or for layers from the 1995 S-EMF females, 1996 S-EMF,

L-EMF and control females (Pearson, all Ps > 0.05; see Table 2 for correlation coefficients). Only 1995 control layers demonstrated a positive but weak correlation between melatonin and body mass at 70 d after pairing ($r=0.5593$ $P<0.01$; see Table 2); one outlier (one-tail t-test, $t_{19}=1.9205$ $P<0.05$, 113.39 pg/ml vs. 71.06 ± 4.93 pg/ml of population) was removed prior to correlation analysis.

iii) Comparison of plasma melatonin levels in adult male to adult female kestrels

As there were no treatment differences for each sex in 1995, we combined 1995 control and EMF birds by sex. Adult male kestrels had higher melatonin levels than females at the end of the breeding season ($t_{77}=-2.99$ $P<0.01$; see Table 3). Differences in melatonin levels between sexes in 1996 were tested within treatment groups due to the significant treatment effects. Regardless of exposure length and treatment group, males had higher melatonin levels than females (see Table 3 for correlation coefficients and P-values).

iv) Fledgling American kestrels: plasma melatonin levels and body mass at 35 d of age

For the 1995 fledgling data, we grouped an external control group with the true control group of the EMF experiment to increase sample size (n = 16 ♀, 9 ♂), as there were no significant differences in melatonin levels or body mass within sex of these two control groups (1-way ANOVAs, all Ps > 0.05).

In 1995, melatonin and body mass were similar between control and S-EMF fledglings within sex (1-way ANOVAs, all Ps > 0.05; see Table 4). Consequently, treatment groups were combined to examine differences by sex. There were no significant differences in plasma melatonin between male and female fledglings ($F_1=2.05$ $P>0.05$), although females were significantly heavier than males ($F_1=11.37$ $P<0.01$).

In 1996, only one control and two S-EMF young hatched and were excluded from the analysis. Comparisons in 1996 were made strictly within the EMF experiment, control versus EMF groups. At 35 d of age, L-EMF exposure had suppressed plasma melatonin levels in fledgling females ($t_7=3.14$ $P<0.05$) and males ($t_6=2.66$ $P<0.05$), but did not affect body mass of females ($t_4=-0.37$ $P>0.05$) or males ($t_6=-1.53$ $P>0.05$; see Table 4). Overall differences by sex in melatonin and body mass of the 1996 young were not examined because of the significant treatment differences and small sample sizes. Plasma melatonin concentrations and

body mass were not correlated for female or male fledglings regardless of treatment group or year (Pearson, all Ps > 0.05; see Table 4 for correlation values).

Discussion

While annual melatonin profiles have been reported for the nocturnal Indian spotted owlet (*Athene brama*, Haldar and Ghosh 1995), our data likely represent the first melatonin profiles for a diurnal raptor species. EMF exposure affected plasma melatonin concentrations in adult males, but only mid-way through the breeding season at 42 d (i.e., suppressed) and at season's end (i.e., increased at 70 d of exposure). However at 70 d, plasma melatonin concentrations in adult females and 1995 males were not affected by EMF exposure. S-EMF exposure of adults had no effect on plasma melatonin concentrations in fledgling kestrels at 35 d of age, but L-EMF exposure of adults significantly suppressed plasma melatonin in male and female fledgling birds.

We make the assumption that the suppression of plasma melatonin concentrations in the kestrels between 0800 and 1000 under light conditions, would also occur during the night, but with greater exaggeration as nocturnal patterns are more pronounced. While the 1995 results for melatonin concentrations in males conflict with 1996 results, the 1996 results provide a more accurate assessment of temporal patterns because multiple sampling periods more accurately identify temporal patterns (Wiens 1981, McIntosh 1991).

It is unlikely that EMFs created a 24 h phase-shift in plasma melatonin levels of male adult and L-EMF fledgling kestrels. Feeding, light, noise, and vibrations can act as zeitgebers, causing a phase-shift in melatonin levels. These factors were controlled during the experiment. Each day, feeding was conducted at the same time daily, and alternately started in the control or EMF rooms. While poultry may perceive 'lights on' versus 'lights off' only when there is a 10x difference in light intensity (Etches 1996), we found no differences in light intensity. In addition, noise conditions were similar in both rooms. There were no differences in noise or vibrations when EMFs were on or off, and both rooms had ventilation fans providing a constant background noise throughout the breeding season. Rather than causing a 24-hr phase-shift, EMFs affected plasma melatonin concentrations in adult male and L-EMF fledgling kestrels.

The timing of blood-sampling in our study further suggests that EMFs did not cause a phase-shift in melatonin concentrations. In both rooms, male adults were blood-sampled four to six hours after 'lights on,' and females and fledglings 10 hours after 'lights on.' Light for 12 or 80 min during the scotophase reduced melatonin levels by 50% and 85%, respectively, in the pigeon (Vakkuri *et al.* 1985). This suppression of melatonin to near daytime levels occurred within a shorter period than our time period between 'lights on' and blood sampling. In addition, chickens (Cockrem and Follet 1985, Liou *et al.* 1987, Doi *et al.* 1995) and Zebra finches (*Poephila guttata*, Van't Hof and Gwinner 1996) have consistent melatonin levels with small fluctuations during daylight. We suspect that kestrels' melatonin levels are also consistent with minimal variations throughout the 'lights on' period, so that it is unlikely we were sampling during periods of major fluctuations in daytime melatonin levels of our kestrels.

The absence of EMF effects on plasma melatonin levels in males at 14 d or at 70 d (1995 only), females at 70 d, and S-EMF fledgling kestrels, is consistent with other EMF studies. EMF exposure had no effect on serum and pineal melatonin levels in adult AMES mice and male Sprague Dawley rats (Lerchl *et al.* 1990), even when mice were exposed to a much stronger magnetic field (Levine *et al.* 1995) than used in our study (i.e., 2.0 T vs. 30 μT). Serum melatonin levels of female Suffolk lambs (*Ovis aries*) were unaffected by exposure to weaker EMFs (Lee *et al.* 1995) than ours (i.e., 6.3 kV/m vs. 10 kV/m, 3.77 μT vs. 30 μT).

The lack of EMF effects on melatonin levels in adult males at 14 d may be associated with the short cumulative EMF exposure time from the start of the experiment, and the delay in melatonin changes observed in other species. Serum melatonin concentrations in rats were reduced only after 15 d exposure to magnetic fields (Soriano *et al.* 1992), while melatonin in laying chickens changed after 10 d when switched to a longer photoperiod (Liou *et al.* 1987).

The suppression of plasma melatonin concentrations in S-EMF and L-EMF males at 42 d and L-EMF fledgling birds is consistent with previous EMF-melatonin research in mammals, particularly following six weeks exposure. Compared to our treatment conditions, stronger EMFs (i.e., 65 kV/m; 5, 50, 250 μT) but similar daily exposure times (e.g., 20 h/d)

for 28 to 42 d, suppressed serum melatonin of young male rats (Grota *et al.* 1994) and pineal melatonin in Wistar-King male rats (Kato *et al.* 1993). Exposure for six weeks to weaker magnetic fields (i.e., 0.02 μT and 1 μT vs. 30 μT) suppressed plasma and pineal melatonin concentrations in Long-Evans rats (Kato *et al.* 1994a, b), while the nocturnal melatonin profile of adult male and female Djungarian hamsters (*Phodopus sungorus*) was suppressed by acute magnetic field exposure (1 gauss, 60 Hz; Yellon 1994). The suppression of melatonin levels in other species exposed for approximately 42 d, and the suppression of melatonin in L-EMF but not S-EMF fledglings, further supports the hypothesis that melatonin changes may occur only after prolonged EMF exposure (e.g., > 14 d).

The differences in plasma melatonin concentrations between S-EMF, L-EMF and control males in 1996 increased with time. From 14 d to 42 d after pairing, melatonin declined in S-EMF, L-EMF and longer-term control males, but not short-term controls. At 42 d, melatonin levels were significantly lower in the S-EMF and L-EMF males than the controls. From 42 d to 70 d, melatonin concentrations in S-EMF and L-EMF males increased and were significantly higher than those of respective control males. We can offer no explanation for these results, but hypothesize that the pattern is a seasonal phase-shift or compression of the melatonin profile for the breeding season. Most avian melatonin is produced by the pineal gland and retinas (e.g., 54% and 33%, respectively, in the Japanese quail, Underwood *et al.* 1984) which are photoreceptive (e.g., Wilson 1991 and references therein, Lamašová *et al.* 1995). The pineal gland transduces EM radiation energy into melatonin (Pang *et al.* 1991), and EMFs are likely perceived as light (Reiter 1992, 1993). Photoperiod and melatonin rhythms are closely correlated in domestic (Liou *et al.* 1987, Doi *et al.* 1995) and wild (Miché *et al.* 1991) avian species. Nocturnal plasma melatonin changes reflected photoperiodic regulation of pineal melatonin synthesis under light:dark cycles (Liou *et al.* 1987). Exposure to artificially long photoperiods initially stimulates gonadal development followed by photorefractoriness in birds (Maitra and Dey 1992, 1996), and may be reflected in plasma melatonin levels. Consequently, we hypothesize that at 42 d, the EMFs, acting as a longer photoperiod, have resulted in the EMF males becoming photorefractory in advance of the controls. This is suggested by the EMF plasma melatonin concentrations at 42 d being

consistent with melatonin levels of control males at 70 d, i.e., the end of the reproductive season. The rise in EMF melatonin concentrations from 42 d through 70 d may represent a rise to possible post-breeding season levels, but this requires further investigation.

Some avian species (e.g., House sparrow *Passer domesticus*, Barfuss and Ellis 1971; Indian jungle bush quail *Perdicula asiatica*, Haldar and Ghosh 1995), including the raptorial Indian spotted owlet (Haldar and Ghosh 1995), showed an inverse relationship between melatonin levels and gonadal development and regression. While the significant effect of time in this study clearly indicates plasma melatonin concentrations in male kestrels declined over the breeding season, male American kestrels do not appear to show such an inverse melatonin-gonadal relationship. While we did not measure testicular size or mass, Bird and Buckland (1976) showed that fertility is greatest prior to and during clutch production, a period when gonadal development is maximal in other seasonally breeding avian species (Wingfield *et al.* 1992, Haldar and Ghosh 1995). Melatonin concentrations in male kestrels taken during clutch production (i.e., at 14 d) are at the highest level during the breeding season, when testes are likely at or near maximal size. Melatonin concentrations in males then decline as testes regress through the season, thereby contrasting with the inverse melatonin-gonadal profile of the Indian spotted owlet and other avian species.

Adult female kestrels showed no EMF-melatonin effects at 70 d exposure. The differential reactions in plasma melatonin concentrations of males and females in 1996 to the same EMF conditions suggests a sexually-dimorphic response of melatonin to EMFs. As we found in American kestrels, melatonin levels of the adult Indian spotted owlet also exhibited a sex-dependent nature, with male owlets having a higher seasonal basal level and amplitude of melatonin than females (Haldar and Ghosh 1995). This sexually-dimorphic response may be linked to males being more sensitive than females to environmental factors (Murton and Westwood 1977, Maitra and Dey 1995), including EMFs as observed with the body mass of these kestrels (Fernie and Bird, submitted) and with gerbils (*Meriones unguiculatus*; Stehle *et al.* 1988).

S-EMF and L-EMF male kestrels were heavier than controls, particularly at 56 d and 70 d of exposure, yet feed-intake was unaffected by EMF exposure in overwintering kestrels

(Fernie, in prep; Fernie and Bird, submitted). Melatonin injections have been associated with increased body mass in hamsters and chickens (Osei *et al.* 1989, Zeman *et al.* 1993, Lamošová *et al.* 1997 and references therein), but melatonin via drinking water had no effect on feed intake or body mass of Japanese quail (Zeman *et al.* 1993). We did not examine the melatonin-feed intake relationship in kestrels, but found the melatonin concentrations which occurred in our adult and fledgling American kestrels were not directly associated with body mass. The lack of correlation between plasma melatonin concentrations and body mass in our fledglings is consistent with results for growing Japanese quail (Zeman *et al.* 1993). However, in the longer-term male groups, declining melatonin was only associated with subsequent increasing body mass in controls; declining and increasing melatonin levels in EMF males were associated with decreasing and increasing body mass, respectively. These results suggest that either L-EMF exposure affects the association of plasma melatonin and body mass in male American kestrels, or indicate no direct association between plasma melatonin and mass for kestrels. Furthermore, these correlation results for adults may point to taxonomic differences in the association of plasma melatonin and body mass, as seen in the association of melatonin and circadian rhythms in sparrows and starlings compared to gallinaceous birds (Zeman *et al.* 1993 and references therein).

In summary, EMFs affected plasma melatonin concentrations in male American kestrels, particularly after 14 d exposure. Melatonin concentrations were suppressed at 42 d of EMF exposure and then increased at 70 d exposure. As plasma melatonin levels in EMF males at 42 d were comparable to melatonin concentrations in control males at 70 d, our results indicate a compression of the seasonal melatonin profile of male kestrels by EMF exposure. Melatonin was suppressed in L-EMF fledgling birds, but not S-EMF fledgling birds or adult females at 70 d exposure. Plasma melatonin concentrations of adult males were significantly higher than adult female kestrels, which may explain the 1996 differential melatonin response of the two sexes at 70 d exposure. Melatonin and body mass are not directly associated in adult or fledgling American kestrels.

Table 1: A comparison of plasma melatonin concentrations and body mass of short-term EMF (S-EMF) or longer-term EMF (L-EMF) to control adult male American kestrels: actual levels, changes [shown in brackets], and correlations of plasma melatonin levels and body mass. EMFs are electric and magnetic fields. Body mass results previously presented (Fernie and Bird, submitted; Fernie, in prep.). Means ± S.E.M. and correlation coefficients. P-values: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

			Melatonin (pg/ml)			Body mass (g)					
		n	14 d	42 d	70 d	14 d	28 d	42 d	56 d	70 d	Correlation
S-EMF, 1 season	Control '95	26	lost	lost	82.53±6.62 Median 76.7	107.85±1.56	113.03±2.20	108.23±2.06	106.63±2.08	109.85±1.91	n.s. [n.s.]
	EMF '95	24	lost	lost	83.04±3.42 Median 78.8	109.95±1.33	113.39±1.54	111.61±1.59	111.36±1.74*	110.86±1.57	n.s. [n.s.]
	Control '96	13	86.09±3.54	89.71±4.68*** [3.62±5.37]	49.65±3.51 [-40.06±7.18]	112.28±3.03	113.00±3.13 [0.73±1.17]	109.83±2.84 [-3.18 ±1.83]	103.88±2.04 [-5.95±1.39]	104.44±2.52 [0.57±1.21]	n.s. [n.s.]
	EMF '96	15	87.15±2.43	58.08±3.94 [-30.88±4.44]	60.87±3.12** [3.27±3.37]	111.93±1.50	112.86±2.49 [0.95±1.47]	112.40±2.32 [-1.93 ±1.01]	112.35±2.31** [-0.05±0.83]	113.53±2.29** [1.17±1.12]	n.s.[1] [n.s.]
L-EMF, 2 seasons	Control	13	93.05±3.21	89.71±2.69*** [-3.34±3.68]	51.40±4.79 [-38.45±4.69]	110.13±3.05	109.96±3.23 [-0.18±1.59]	109.18±3.49 [-0.78±1.03]	105.06±2.79 [-4.13±1.14]	109.20±3.00 [3.46±0.90]	n.s. [2]
	EMF	15	104.86±7.74	53.03±4.20 [-51.83±9.79]	65.50±4.14* [12.47±4.89]	116.07±2.55	115.24±2.22 [-0.83 ±1.15]	112.91±2.22 [-2.32 ±0.97]	111.53±1.69 [-1.38±0.82]	112.88±1.95 [1.34±0.77]	n.s.[1] [3], [4]

[1] No significant correlation after Bonferroni correction; [2] L-EMF control males: declining melatonin, 42 d to 70 d, and increasing body mass, 56 d to 70 d ($r=0.72$ $P<0.01$); [3] L-EMF males: declining melatonin, 14 d to 42 d, and declining body mass, 42 d to 56 d ($r=-0.61$ $P<0.01$); [4] L-EMF males: increasing melatonin, 42 d to 70 d, and increasing body mass, 56 d to 70 d ($r=0.56$ $P<0.05$).

Table 2: A comparison of short-term EMF (S-EMF), longer-term EMF (L-EMF), and control adult female American kestrels (*Falco sparverius*): plasma melatonin concentrations, body mass and correlation coefficients of the two variables at 70 d after pairing. EMFs are electric and magnetic fields. Body mass results have been presented elsewhere (Fernie and Bird, submitted; Fernie, in prep.). $^{**}P<0.01$, $^{*}P<0.05$; one statistical outlier removed.

		n	Melatonin (pg/ml) mean ± S.E.M.	Body mass (g) mean ± S.E.M.	Pearson Correlation (r)
Overall: S-EMF, L-EMF	Control '95	26	71.84 ± 3.67	124.04 ± 1.88	0.4263
	EMF '95	27	67.25 ± 2.18	127.19 ± 2.13	-0.0488
	Control '96	9	18.37 ± 4.12	123.59 ± 2.45	0.4700
	EMF '96	15	23.22 ± 5.21	125.10 ± 3.15	-0.1970
	Control, L-EMF	10	35.19 ± 3.97	120.36 ± 2.43	0.3990
	EMF, L-EMF	15	25.79 ± 3.46	126.38 ± 2.34	0.05651
Layers: S-EMF, L-EMF	Control '95	19	71.06 ± 4.93	125.39 ± 9.32	0.5593**
	EMF '95	18	67.32 ± 2.55	130.26 ± 10.35	0.1966
	Control '96	9	24.12 ± 3.37	123.12 ± 8.08	0.4801
	EMF '96	6	34.86 ± 5.74	127.35 ± 6.78	-0.2408
	Control, L-EMF	9	35.95 ± 4.36	119.97 ± 8.04	-0.2785
	EMF, L-EMF	13	26.71 ± 3.85	126.93 ± 9.49	0.46561

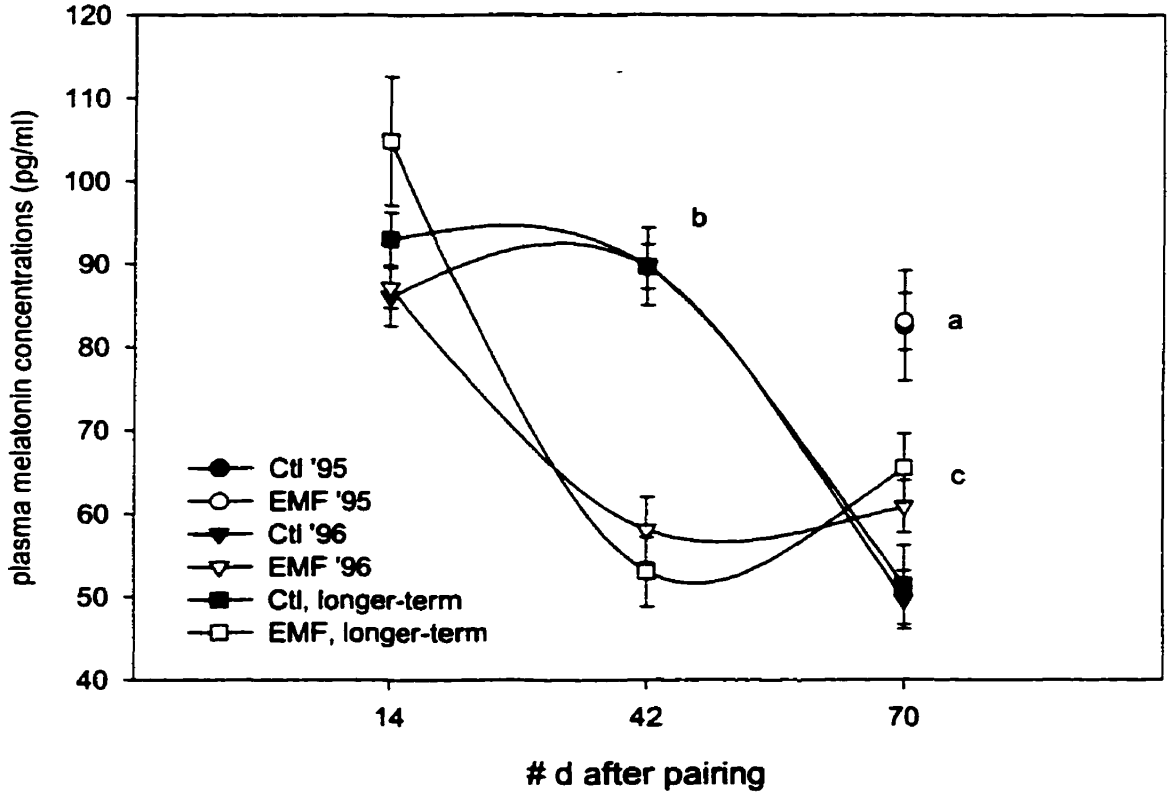
Table 3: A comparison of plasma melatonin concentrations between adult male and female American kestrels, 70 days after pairing. Short-term groups were used for one breeding season, and longer-term groups for two breeding seasons. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$

			Melatonin 70 d after pairing (pg/ml)	
Experimental group	Sex	n	Mean ± S.E.M.	t- value [df]
1995, all adults	Males	50	$82.77 \pm 3.78^{**}$	-2.99 [77]
	Females	54	69.83 ± 2.10	
1996, short-term controls	Males	12	$46.6 \pm 3.51^{**}$	-3.97 [9]
	Females	12	24.1 ± 3.02	
1996, short-term EMF	Males	16	$60.9 \pm 3.12^{***}$	-7.92 [14]
	Females	16	32.2 ± 4.43	
Longer-term controls	Males	11	$51.4 \pm 4.79^{*}$	-2.25 [9]
	Females	11	35.2 ± 3.97	
Longer-term EMF	Males	15	$65.5 \pm 4.14^{***}$	-7.73 [14]
	Females	15	25.8 ± 3.46	

Table 4: A comparison of plasma melatonin concentrations and body mass of EMF and control fledgling male and female American kestrels at 35 d of age. EMFs are electric and magnetic fields. Means ± S.E.M. and correlation coefficients. P-values: $^{*}P<0.05$ $^{**}P<0.01$.

		n	Melatonin (pg/ml)	Body mass (g)	Pearson Correlation
Short-term EMF birds					
1995 females	Control	16	60.20 ± 5.14	122.20 ± 2.41	0.461
	EMF	4	70.86 ± 8.29	125.75 ± 4.51	0.836
1995 males	Control	9	77.90 ± 6.03	113.42 ± 1.67	0.25
	EMF	5	62.73 ± 9.12	115.94 ± 2.25	0.059
Overall, 1995	Male	14	72.48 ± 5.25	114.32 ± 1.33	0
	Female	20	62.33 ± 4.45	122.95 ± 2.09**	0
Longer-term EMF birds					
1996 females	Control	5	65.44 ± 6.15*	117.50 ± 10.60	0.43
	EMF	4	44.75 ± 3.34	114.45 ± 3.42	0.855
1996 males	Control	4	45.24 ± 2.43*	105.05 ± 3.24	0.875
	EMF	4	37.61 ± 1.53	111.33 ± 2.52	-0.2

Fig. 1: A comparison of plasma melatonin concentrations (pg/ml) in reproducing adult male American kestrels (*Falco sparverius*) exposed to electric and magnetic fields (EMFs) to control males at 14, 42 and 70 d after pairing. Means and S.E.M. presented.



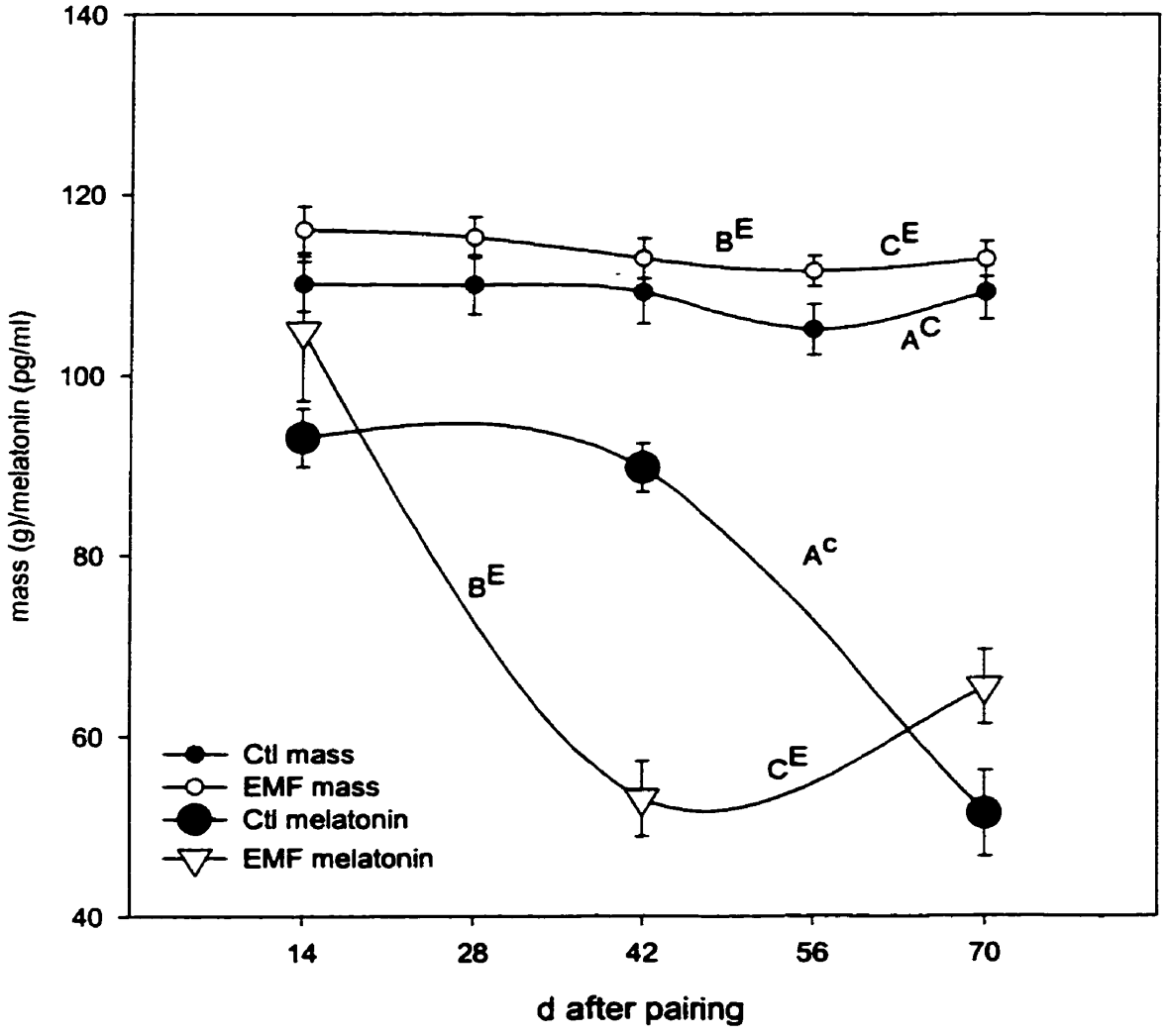
a: 1995 samples at 70 d only, n.s.

Overall in 1996, adult male melatonin levels showed significant treatment, time and treatment*time interactions for short- and longer-term EMF groups.

b: EM significantly lower than controls in short- and longer-term EMF groups at 42d.

c: EM significantly higher than controls in short- and longer-term EMF groups at 70d.

Fig. 2: A comparison of changes in body mass (g) and plasma melatonin concentrations (pg/ml) in reproducing male American kestrels (*Falco sparverius*) exposed to electric and magnetic fields (EMFs) to control males used for two breeding seasons. Means and S.E.M. presented.



A^C control males: declining melatonin (42-70 d) and increasing body mass (56-70 d) correlated ($r=0.7245$ $P<0.01$).

B^E EMF males: declining melatonin (14-42d) and declining body mass (42-56 d) correlated ($r=-0.6078$ $P<0.01$).

C^E EMF males: increasing melatonin (42-70 d) and increasing body mass (56-70 d) correlated ($r=0.5632$ $P<0.05$).

Bonferonni corrections were used for all correlations.

CONNECTING STATEMENT 2.

I have established that electric and magnetic fields from electric transmission lines affect plasma melatonin concentrations in reproducing male kestrels, likely causing a shift in the seasonal melatonin profile. Furthermore, EMF exposure suppressed plasma melatonin concentrations in fledgling kestrels produced by adults which were exposed for two breeding seasons. Given the crucial role of melatonin in avian circannual and circadian rhythms, it is important to determine if the exposure periods used for the captive study are comparable to the length of time free-ranging kestrels are exposed to EMFs during the reproductive period. If EMF exposure affects reproductive success, it is important to understand if these effects are linked to behavioural changes from EMF exposure.

CHAPTER 3. IMPLICATIONS OF ELECTRIC AND MAGNETIC FIELDS FOR BEHAVIOUR OF REPRODUCING FREE-RANGING AND CAPTIVE AMERICAN KESTRELS.

Abstract

We determined the amount of time that wild American kestrels reproducing in nest boxes were exposed to electric and magnetic fields (EMFs) throughout the breeding season, and conducted a controlled laboratory experiment to examine the effects of EMF exposure on behaviour and reproduction of captive kestrels. EMF exposure of wild birds near the nest box progressively declined for females following incubation, although females were more inclined to be exposed than males when nestlings were present. Females sunned more often than males during incubation and nestling phases, and carried prey more often during incubation. These differences are due to spatial and labour partitioning of sex roles. Potential EMF exposure from incubation and perch-hunting from transmission lines, raises maximal EMF exposure of females to approximately 90% and males to 80% of daylight hours. Captive reproducing kestrels were exposed to EMFs that wild kestrels would be exposed to by nesting under 735 kV powerlines for 87.5% of the time. EMF females were more active than controls during courtship, and spent less time grooming and resting during the nestling phase. EMF males were more likely than controls to be active during courtship, alert during incubation, and to perch on the rope during the nestling phase. These behavioural changes would have had no effect on clutch size or fertility, and fledging success was higher in the EMF group as previously reported. We conclude that EMF exposure, rather than the increased alertness of EMF males which was the only behavioural change during incubation, reduced hatching success. Behavioural changes of captive reproducing kestrels exposed to EMFs may be observed in wild kestrels, but these behavioural changes will likely not affect reproductive success.

Introduction

Exposure to electric and magnetic fields (EMFs) has implications for physiology, reproduction and development of insects (e.g., Greenberg *et al.* 1981), birds (e.g., Ossenkopp and Shapiro 1972), mammals (e.g., Persinger 1969, Persinger *et al.* 1972, Burchard *et al.* 1996), primates (e.g., Feldstone *et al.* 1980) and humans (e.g., Anderson and Phillips 1985). Such studies often involve experiments on captive animals, but rarely examine the extent of EMF exposure for free-ranging species which may affect reproduction and species survival.

Many avian species, including American kestrels (*Falco sparverius*), nest in close proximity to electrical transmission structures (e.g., Steenhof *et al.* 1993, Doherty and Grubb 1996). As such, adults are exposed throughout the breeding season, and over repeated breeding seasons, and embryos and chicks are exposed during prenatal and postnatal development.

In this paper we report on a two-part study examining potential behavioural implications of EMF exposure for wild and captive American kestrels. The first part determines the amount of time free-ranging kestrels nesting near transmission lines are exposed to EMFs during the breeding season. The second part of the study examines the effects of EMFs on the behaviour of captive reproducing American kestrels in a controlled laboratory setting.

Methods

Free-ranging Kestrels

Observations of wild kestrels were made during the 1996 reproductive season within St. Clet-Vaudreuil, Quebec (45°20'N, 75° 04'W), a region with typical grassland and agricultural habitat (Bowman and Bird 1985). We used 12 kestrel pairs that nested in wooden boxes. Each nest box was attached to a hydro pole about one metre below the transmission line, and kestrels had a maximum of two alternative perch sites.

A wild control group was not selected as our objective was to identify the amount of time wild kestrels spent within an EMF area. Moreover, an experimental design with wild control and EMF groups was beyond the scope of this project. To minimize potential confounding difficulties of variations in habitat and mate quality, prey availability, and

electrical currents, we elected to study the effects of EMFs on kestrel reproductive behaviour in a controlled laboratory setting.

We observed the time budget of the kestrel pairs only within the EMF area centred around the nest box ($80m^2$ x $80m^2$; henceforth the EMF area) as EMFs can radiate up to 40 m from transmission lines (Hydro Quebec 1989, 1996). The wild kestrels were exposed to an approximate 1.2 μT magnetic field (Hydro Quebec 1996) and 0.05 ± 0.01 kV/m electric field (Fernie, unpubl. data) beneath the wires. While these EMFs are low, we were identifying the EMF exposure time of wild reproducing kestrels rather than possible behavioural effects.

For each nest site, we confirmed that kestrels were nesting through two visitations at the onset of the breeding season and 10 d post-hatching, in order to minimize disturbance. Dependent on the number of pairs successfully nesting in locations fulfilling the habitat criteria, one pair of kestrels was observed during the courtship phase, six pairs during the incubation and nestling phases, and three pairs during the fledgling phase. Different pairs were observed in each phase.

All observations took place between 0900 and 1600 and were made from a stationary vehicle with a spotting scope and binoculars. The duration of each behavioural activity performed by an individual bird was recorded on an actigram (cf. Walter 1983). Each nesting pair was observed for 20 to 45 min/d for a cumulative total of 110 h of observation. Observation periods were rotated daily among the kestrel pairs.

Behavioural activities recorded included: courtship feeding (Willoughby and Cade 1964) and displays (Balgooyen 1976), aggression/attacks toward conspecifics and heterospecifics (Balgooyen 1976), nest box inspection, searching, capturing, and carrying prey (Rudolph 1982), incubation/care of eggs or nestlings, perching (Rudolph 1982), grooming (Saumier *et al.* 1988), and sunning (Saumier 1986, Walter 1983). The total time a kestrel spent within the EMF area was the summation of time an individual was involved in the various activities for each reproductive phase.

Statistical analyses were performed on actual observation times of behaviours, including time observed in the nest box. EMF exposure and behavioural differences for each sex from incubation through fledgling phases were analyzed using Kruskal-Wallis tests as

pairs differed between phases. Differences between sexes within a given reproductive phase were compared using t-tests or Mann-Whitney *U*-tests (Sokal and Rohlf 1981). Differences were considered significant at the $P < 0.05$ level. Means with S.E.M. are presented.

Captive Kestrels

A total of 56 pairs of American kestrels from the captive colony of the Avian Science and Conservation Centre (ASCC) at McGill University was randomly assigned to the control (C: 10 m x 8 m x 3 m; L x W x H) or EMF room (E: 15 m x 10 m x 3 m). Individuals were unrelated for seven generations, had previous breeding experience, and were similar in age (2 - 5 a; all Ps >0.05).

Temperature, humidity, and photoperiod clocks reflected natural conditions of the May through August period for the Montreal region (45°26'N, 73°56'W), with no significant differences in humidity or temperature between the two rooms (t-tests, all Ps > 0.05). Each room had a ventilation fan. There were no significant differences in light intensity between the two rooms (t_{24}=-1.1990 P>0.05), and measurements were identical with the EMFs on or off (lights on). Noise levels, indicative of vibrations, were similar when EMFs were on or off (t_8=0.64 P>0.05; D. Nguyen, pers. comm.).

In the EMF room, a 29.35 ± 0.03 μT magnetic field was generated by copper wires placed at 1 m intervals within the ceiling, floor and walls. Two charged metal plates suspended from the ceiling produced an electric field of 9.67 ± 0.04 kV/m (Nguyen *et al.* 1991). The EMFs were controlled by computer to provide a consistent and uniform field, were equivalent to that which kestrels are exposed to when nesting under a 735-kV transmission line running at peak capacity, and could penetrate all housing materials (D. Nguyen, pers. comm.). The magnetic field of the control room was 2.02 ± 0.27 μT, and the electric field was 0.03 ± 0.01 kV/m.

Each kestrel pair was housed in a visually-isolated pen made of reinforced corrugated cardboard and covered with nylon netting. A standard wooden nest box was attached to the pen wall, and a rope perch placed diagonally across the pen. Wood shavings provided bedding and nesting material. A small one-way mirror permitted discrete behaviour observations. Metal materials were minimized to reduce EMF disturbance and possible shock to the birds

(F. Renaud, pers. comm.)

Kestrels were exposed for 95 d, from pairing until 7 d after the last young fledged. Daily EMF exposure was 21 hours (87.5%). The EMFs were switched off for approximately three hours per day to allow for data collection, feeding and cleaning of pens. Kestrels were provided with day-old cockerels and water *ad libitum* at the same time daily when leftover food from the previous day was removed.

A total of 20 pairs was randomly selected for behavioural observations from 56 pairs of the larger reproductive EMF study (Fernie *et al.*, submitted - b,c, Fernie and Bird, submitted, Fernie, in prep.). For the courtship phase, 11 control pairs and nine EMF pairs were observed. Pairs were changed to include all incubating pairs (C=10, E=6) and pairs with nestlings (C=4, E=4). Courtship began one week after the birds were paired and lasted until the fourth egg was laid, when incubation began (Bird 1988) for 28 to 30 d until the first egg hatched. The nestling phase of 28 to 30 d terminated when the last young fledged. Nest boxes were checked daily for the presence of eggs, nestlings and fledglings, with fertility determined by a portable candler (Weller 1956) 20 d after the last egg was laid.

Observations were made during two morning sessions, 0830 to 1030 and 1030 to 1230. Within each session, each pair was observed for 10 min and behaviours recorded by instantaneous sampling every 20 sec (Martin and Bateson 1986). Initial observation sessions were rotated daily by treatment and observation order of pairs.

The following behaviours were recorded; perch location, perching distance between mates, inside/outside the nest box, sleeping/resting (Saumier 1986), alertness (Saumier 1986), grooming and feeding (Village 1990), and activeness. Aggression and courtship feeding (Village 1990) during courtship, and food transfers between adults and nestlings were recorded (Village 1990). Attempted but not true flight was recorded because there was insufficient pen space to allow for flight. Attempted flight consisted of a kestrel flying to the roof mesh from its rope perch, onto the pen floor, then back onto its rope perch. Flight was the only behaviour impeded by pen size.

Data were changed to proportions for each individual within a particular reproductive phase, then analysed by Mann-Whitney U tests to determine behavioural differences between

the control and EMF groups (Zar 1984). Differences were considered significant at the $P<0.05$ level, and means with S.E.M. are presented.

Results

Free-ranging kestrels

The total EMF exposure time of wild kestrels in the EMF area was similar between the sexes during each reproductive phase (all Ps > 0.05). During courtship, the female and male were exposed to EMFs for 13.07% and 11.64% of their respective daylight time budget within the EMF area (see Table 5), and for 40.24% and 47.85%, respectively, during incubation. Females and males were exposed for 32.11% and 18.76% of their respective nestling daylight budget, and 14.58% and 21.75% of respective daylight budgets in the EMF area during the fledgling period.

EMF exposure from incubation on through the breeding season significantly declined for females ($\chi^2_3 = 7.46$ P<0.05) but not males ($\chi^2_3 = 2.19$ P>0.05; see Table 5). Comparing the exposure time between sexes within each reproductive phase showed that males and females were exposed to EMFs for similar periods during the incubation ($t_{10}=0.94$ P>0.05) and fledgling phases ($t_4=0.41$ P>0.05). A trend ($t_9=2.10$ P=0.06) is evident during the nestling period when females were exposed to EMFs for a longer period than males.

We observed no behavioural differences as the season progressed for either sex (all Ps>0.05). Most behaviours of both sexes were similar within each phase during incubation, nestling, and fledgling phases (all Ps>0.05). However, females sunned more often than males in the EMF area during incubation (♀ 202.5 sec vs. ♂ 1.0 sec; U=52.5 P<0.05) and nestling phases (♀ 80.0 sec vs. ♂ 0.0 sec; U=40.5 P<0.05), and carried prey while flying in the EMF area during the nestling phase (♀ 46.0 sec vs. ♂ 0.0 sec; U=42.0 P<0.05).

Captive Kestrels

During courtship, no significant differences were found between the control (C) and EMF (E) kestrels in perching distance between mates, aggression, or courtship feeding (all Ps>0.05). Compared to control females, EMF females were more active (E 0.4, C 0.0; U=20.5 P<0.05). Several behavioural trends were evident during courtship. EMF females were slightly more alert than control females (E 8.2, C 6.1; U=25.5 P=0.06), and perched on

the roof more frequently (E 0.3, C 0.0; U=26.0 P=0.07). EMF male kestrels were more active than controls (E 1.0, C 0.0; U=27.5 P=0.09).

During incubation, the only behavioural change from EMF exposure observed was a trend of EMF males being more alert than control males (E 95.1, C 77.0; U=14.0 P=0.08). Behaviours of females during incubation, including the amount of time spent in the nest box, was unaffected by EMF exposure.

During the nestling phase, EMF females were observed grooming/preening (E 0.0, C 3.0; U=0.0 $P<0.05$) and resting/sleeping (E 0, C 5.0; U=-2.02 P<0.05) less often than control females. Compared to control males, EMF males perched on the floor less often (E 0, C 7.0; U=1.0 P<0.05) as they tended to favour the rope perch (E 30.0, C 17.0; U=2.0 P=0.08).

Discussion

Wild reproducing female kestrels spent progressively less time exposed to EMFs near the nest box following incubation. EMF exposure times of males, and individual behaviours of both sexes, showed no variation through the season. A trend was evident during the nestling period only, when females were more exposed to EMFs than males. Compared to males, females sunned more often in the EMF area during incubation and nestling phases, and carried prey during the nestling period. These exposure and behavioural differences are likely because kestrel pairs partition their labour and space (Willoughby and Cade 1964, Porter and Wiemeyer 1972, Balgooyen 1976, Rudolph 1982, Bird 1988), with the male providing food for the female and young (Balgooyen 1976) by hunting predominantly away from the nest site (Rudolph 1982).

Reproductive behaviour of captive kestrels changed when exposed to EMFs, especially during courtship and nestling phases. EMF females were more active than controls during courtship, with a similar trend among EMF males. Trends were also evident of EMF females being more alert and perching from the roof during courtship, and of EMF males being more alert during incubation. With nestlings present, EMF females spent less time grooming and resting/sleeping, and EMF males spent more time perching on the rope than controls.

The wild male kestrel spent less time in the EMF area than the female during courtship

(see Table 1), and was the only one to perform courtship displays, food offerings and transfers. This sexual dimorphic response is consistent with kestrel courtship behaviours used to establish and maintain a pair bond (Willoughby and Cade 1964, Balgooyen 1976, Bird 1988). Females increase both their time near the nest box and demands for feeding by the male. Consequently, males spend more time hunting away from the EMF area during courtship (Rudolph 1982).

During courtship, captive EMF females were more active than controls, with a similar trend in EMF males. This increase in activity is likely due to EMF exposure as increased activity was observed in mice (Moos 1964) and rats (Persinger *et al.* 1973) exposed to EMFs. The increased activity of captive EMF males and the pen's small dimensions, rather than EMF exposure, probably explain the tendency of EMF females preferring to perch on the rope and be more alert. However, the increased activity of both sexes exposed to EMFs had no effect on clutch production or size, which were similar between treatment groups, or fertility as it was higher in the EMF group (50.9%) than the control group (46.8%; Fernie, in prep., Fernie *et al.* submitted - b). As the captive exposure period was longer during courtship than the actual observed exposure period for wild kestrels, it is unlikely that courtship behavioural changes in wild kestrels from EMF exposure will affect clutch parameters related to reproductive success.

The increased activity levels of the captive EMF kestrels during courtship is unlikely associated with EMF effects on melatonin levels. Melatonin is correlated with locomotor rhythms and activity patterns in some avian species (Turek *et al.* 1976, Hendel and Turek 1978, Chabot and Menaker 1992). EMF males tended to be more active during courtship, yet melatonin levels were similar between EMF and control males during courtship in 1996, and between 1995 male groups during the nestling phase (Fernie *et al.* submitted - c, Fernie in prep.). This link between melatonin and activity levels of kestrels requires further investigation.

During incubation, activeness of the captive birds was similar between groups regardless of EMF exposure. The female kestrel is the primary incubator (Balgooyen 1976), which decreases her food requirements and suppresses highly active behaviours (Balgooyen

1976). Consequently, male kestrels must provide less food (Balgooyen 1976, Village 1990), and so, are less active. Reduced activity and an increase in exposure time from courtship to incubation was observed with wild kestrels, with both males and females spending more time near the EMF nest area.

Alertness of captive males tended to increase during incubation when exposed to EMFs. We can offer no explanation for this trend. EMF exposure had no effect on behaviours of females, including time spent incubating, and while hatching success was poor in both groups, it was significantly lower in the EMF clutches (11.1%) compared to controls (13.6%; Fernie in prep., Fernie *et al.* submitted - b). The wild kestrels incubated for a comparable period (♀ 70.4 ± 6.8%; ♂ 21.2 ± 11.8% of actual observed time) to that of our captive unexposed kestrels at the ASCC (♀ 70.2 ± 4.4%, ♂ 8.7 ± 2.3% of observed time; K. Fernie unpubl. data). Consequently, while EMF exposure of wild kestrels is unlikely to affect adult behaviour during incubation, it reduces hatching success (Fernie *et al.* submitted - b, Fernie in prep).

As the reproductive season progressed to the nestling and fledgling stages, wild females spent increasingly less time in the EMF area, with females being slightly more exposed to EMFs than males in the nestling period. During this time, only females sunned and carried prey in the vicinity. These exposure and behavioural differences are also a result of the labour division between kestrel mates (Balgooyen 1976). While the male is capable of hunting and providing enough prey during the courtship, incubation, and initial nestling phases (Cavé 1968), the female resumes hunting to meet the demands of growing nestlings beyond seven to 10 d of age (Balgooyen 1976). With females hunting near the nest box more often than males, it is not surprising that females carried prey and sunned in the EMF area when nestlings were present.

During the nestling period, captive EMF females groomed and rested/slept less often than control females. Decreased grooming activity in wild females exposed to EMFs may lead to heavier ectoparasitic loads (Woodroffe 1953) and an associated reduction in health and vigour (Möller 1993). Despite these behavioural changes, fledging success was higher in the EMF room indicating EMF behavioural changes had no effect on fledging success. We

conclude that if wild birds exposed to EMFs experienced similar behavioural changes, fledging success is unlikely to be affected.

Restricting our wild kestrel observations to the EMF area surrounding the nest box likely underestimated the EMF exposure time of kestrels in their home range. Kestrels preferentially perch-hunt (Rudolph 1982) from perches averaging 6.8 m in height (Balgooyen 1976), with females perch-hunting 28% and males 34% of daylight hours during courtship and post-brooding phases (Rudolph 1982). Transmission lines fall within the preferred perch category, especially if the home range has few natural perch sites (e.g., trees) as with our wild kestrels. This would increase EMF exposure for wild females and males during courtship to approximately 40-45% of daylight hours, to 50-60% during the late nestling phase, and 40-55% when fledglings are present. These potential figures exclude our observed perch-hunting behaviour times.

EMF exposure for male and female kestrels is further underestimated in our study since the accurate identity of the presence and sex of the incubating kestrel was not always possible. Estimated EMF exposure through incubation alone may be 75% to 80% of the female's time budget, and 20% of the male's time budget (Balgooyen 1976). Consequently, males are potentially exposed to EMFs for 70-80% of daylight hours during incubation and early nestling phases, when estimated times for males perch-hunting (i.e., 34.5%, Rudolph 1982) and incubating (i.e., 20%, Balgooyen 1976), are added to our modified observed EMF exposure times. Similarly, females are potentially exposed for more than 90% during the incubation and early nestling phases, when we include incubation estimates of 80% (Balgooyen 1976) and perch-hunting estimates of 5.0% (Rudolph 1982) to our modified observed times. Moreover, the total EMF exposure for a 24 hour period depends on the kestrel's roosting location. Gessaman and Findell (1979) indicated that wild incubating American kestrels remain in their nest boxes at night for 10.5 h, with females incubating more often than males (Balgooyen 1976).

In summary, the extent of EMF exposure of free-ranging kestrels depends on the sex of the bird and the reproductive phase, because of partitioning of space and duties by a reproducing pair. Daily EMF exposure of captive kestrels (87.5%) was greater than potential

daytime EMF exposure of wild kestrels during courtship (40 - 50%) and late nestling (50 - 60%) phases, but comparable during incubation and early nestling phases (♀ 90%, ♂ 70 - 80%) which constitute the majority of the breeding season. If roosting locations involve EMF exposure, then wild kestrels are exposed to EMFs for a similar period as experienced by our captive kestrels. Consequently, behavioural changes in captive kestrels from EMF exposure may be experienced by wild kestrels, with captive changes occurring predominantly during courtship and nestling phases. The increased activity of captive EMF birds had no impact on clutch production, size or fertility. Behavioural changes during incubation were restricted to a trend of EMF males being more alert, a behavioural change unlikely to affect hatching success which was significantly lower in the EMF room. While wild females were more likely to be exposed to EMFs than males when nestlings were present, and captive EMF females rested and preened less often than control females, captive fledging success was higher in the EMF room indicating no effect from the behavioural changes and likely not from the greater EMF exposure of female kestrels. We conclude that given roosting locations involving EMF exposure, wild kestrels can be exposed to EMFs for a similar period to our captive birds, and consequently may experience similar behavioural changes. These behavioural changes are unlikely to affect reproductive success of wild birds nesting under EMF conditions.

Table 5: Observed times of exposure to electric and magnetic fields (EMFs) near nest boxes for wild American kestrels. Based on behavioural activities performed within the surrounding EMF area centred about the nest box (80 x 80 m^2). Observation times and percentages include observed perch-hunting and incubation times when the sex of the incubating kestrel could be accurately identified.

Reproductive	Sample Sizes		Females		Males	
Phase	Female	Male	minutes	%	minutes	%
Courtship	1	1	41.5	13.1	21.53	11.64
Incubation	6	6	127.76 ± 29.25	40.2	88.54 ± 29.77	47.85
Nestling	6	6	101.93 ± 28.05[§]	32.1	34.71 ± 17.59	18.76
Fledging	3	3	46.28 ± 11.39	14.6	40.26 ± 9.27	21.75

EMF exposure of females progressively declined following incubation (χ^2_3=7.46 P<0.05), while exposure time did not vary significantly for males as the season developed.

[§]Females were slightly (t_9=2.10 P=0.06) more exposed to EMFs than males with nestlings.

CONNECTING STATEMENT 3.

The second paper clearly indicates that free-ranging American kestrels may be exposed to EMFs during the reproductive season for periods comparable to that used in the captive reproductive study. Currently, it is unknown if EMFs affect reproductive success of raptorial species which commonly nest on transmission structures under EMF conditions (Steenhof et a. 1993). The second paper showed EMF exposure affected the reproductive behaviour of the captive kestrels, and briefly indicated that EMFs affect reproductive success of kestrels. However, the extent of EMF effects on eggs, embryos, and hatchlings requires further investigation.

CHAPTER 4. EFFECTS OF ELECTRIC AND MAGNETIC FIELDS ON THE REPRODUCTIVE SUCCESS OF AMERICAN KESTRELS.

Abstract

Reduced reproductive success of birds nesting near transmission lines has been documented, but never directly attributed to electric and magnetic fields (EMFs) from the power lines. Laboratory studies using domestic species have identified EMF effects on embryological development, but reproductive success of wild species is dependent on many other additional factors. We used captive American kestrels under controlled or EMF conditions for one (S-EMF) or two (L-EMF) breeding seasons to test the hypothesis that EMFs that kestrels are exposed to when nesting under 735 kV power lines affect reproductive success. Clutch size, initiation date, and the length of the laying sequence were unaffected by EMF exposure. Fertility was higher in S-EMF clutches than control clutches, but hatching success was lower in S-EMF clutches. L-EMF exposure had no effect on fertility, but was associated with increased hatching success. Mean egg volume and mass per clutch were greater in the S-EMF clutches in 1995 only. Correcting for the larger egg volume of EMF eggs, S-EMF eggs of 1995 had thinner eggshells and slightly more dried albumen. S-EMF embryos were larger in overall size than control embryos, but hatchlings were similar in size and body mass to controls. L-EMF hatchlings were heavier than control hatchlings. Fledging success was higher under S-EMF conditions in 1995, but lower under L-EMF conditions. Clearly, EMF exposure affects reproductive success of kestrels, with increased mortality of embryos or young dependent on the length of exposure of adults.

Introduction

Electrical transmission lines and towers have proved beneficial to birds, providing alternative sites for perching, roosting, hunting, and nesting (Olendorff *et al.* 1981, Steenhof *et al.* 1993); however, these birds are exposed to electric and magnetic fields (EMFs) generated by electric powerlines. Overall nest success of raptors and ravens (*Corvus corax*) nesting on a 500 kV transmission line in southern Idaho-Oregon, was similar to or higher than pairs nesting on surrounding substrates (Steenhof *et al.* 1993). However, reproductive success of Tree swallows (*Tachycineta bicolor*), but not Eastern bluebirds (*Sialia sialis*) and House wrens (*Troglodytes aedon*), was reduced under power lines, although results were inconclusive in directly attributing this to EMFs (Doherty and Grubb 1996).

While effects of EMF exposure on avian fertility and hatching success are ambiguous, it has affected embryonic development, delaying development of Sea urchins (*Stronglyocentrotus purpuratus*, Cameron *et al.* 1993) and chickens (*Gallus domesticus*, Delgado *et al.* 1982, Ubeda *et al.* 1983, 1994, Juutilainen *et al.* 1987, Martin 1988, but see Maffeo *et al.* 1988, Martin 1992, Coulton and Barker 1991, Veicsteinas *et al.* 1996), and increasing body mass and/or bone length of mice (Kowalczuk *et al.* 1994) and chickens (Rooze and Hisenkamp 1985). EMF laboratory experiments on birds have utilized artificial incubators which may be affected by EMFs, particularly temperature and humidity, thereby having implications for successful embryonic development and hatching (Martin 1992).

Birds nesting on or near transmission lines may be exposed to EMFs throughout long and repeated breeding seasons (Steenhof *et al.* 1993). Most previous EMF studies have used domestic species and artificial incubators, but produced inconsistent findings. Consequently, it is important to determine if reproductive success is directly affected by EMF exposure by using adult birds, eggs and young of a wild species. Avian reproductive success is a function of fertility, hatching and fledging success, as well as egg size with possible implications for embryo development, hatching success, hatchling size, sex ratios, and fledging success (see Williams 1994). We tested the hypothesis that EMFs affect reproductive success of avian species using pairs of American kestrels to lay and incubate eggs and rear young under EMF and control conditions. Kestrels were exposed for one breeding season to determine short-

term EMF (S-EMF) effects, and for two breeding seasons to determine longer-term EMF (L-EMF) effects.

Methods and Materials

We used 56 pairs of captive American kestrels from the Avian Science and Conservation Centre of McGill University for each year of study. In 1995, 28 pairs were randomly assigned to a control room, and 28 pairs to an EMF room. In 1996, new pairs were randomly assigned to each room (13 control, 15 S-EMF pairs) for determining S-EMF effects. In 1996, 13 control pairs and 15 EMF pairs from 1995 were used for a second breeding season to identify possible L-EMF effects. Not all pairs laid clutches.

A control room (10 m long, 8 m wide, 3 m high) and an EMF room (15 m long, 10 m wide, 3 m high) were used to house the kestrels. In the control and EMF rooms, humidity, temperature and photoperiod clocks simulated natural conditions (lat. 45°30', long. 73°26'), with no significant differences between the two rooms (t-tests, all Ps > 0.05). Each room had a ventilation fan. Average light intensity at the birds' head level was not significantly different with EMFs on or off (lights on; t_{24}=-1.1990 P>0.05). Noise levels, indicative of vibrations (D. Nguyen, pers. comm.), were similar when EMFs were on or off (t_8=0.64 P>0.05).

A magnetic field of 29.35 ± 0.03 μT in the EMF room was created by a current running through coils in the walls, floor, and ceiling of the room. The electric field of 9.67 ± 0.04 kV/m was generated by two plates suspended from the ceiling (Nguyen *et al.* 1991, Burchard *et al.* 1996). The EMFs, controlled by a computer to provide consistent and uniform fields, were equivalent to that which kestrels are exposed to when nesting under a 735-kV transmission line running at peak capacity. The magnetic field of the control room was 2.02 ± 0.27 μT, and the electric field was 0.03 ± 0.01 kV/m.

Each pair was housed in a visually-isolated breeding pen made of reinforced corrugated cardboard and covered with nylon netting. A standard wooden nest box and rope perch were provided, as were wood shavings for bedding and nesting material. Metal materials were minimized to reduce disturbance of the electric field and possible shocks to the birds (F. Renaud, pers. comm.), while magnetic fields penetrated all housing materials (D. Nguyen, pers. comm.).

Kestrel pairs were genetically unrelated within the past seven generations. Each bird had previous breeding experience. Within each sex, there were no significant differences in age (2 - 5 y), size (wing chord), body mass, or condition (body mass: wing chord index) at pairing (t-tests, all Ps > 0.05). Kestrels were provided *ad libitum* frozen-thawed day-old whole cockerels and water each day at the same time. All leftovers were removed daily.

Kestrels were paired on 11 May 1995 and 13 May 1996, with exposure of EMF pairs beginning immediately and lasting for 95 d in 1995, and 91 d in 1996, until young were 35 d, one week after fledging. The birds were exposed to EMFs for approximately 21 hr, or 87.5% of a 24 hr period, in 1995, and to approximately 23.5 hr or 98% of 24 hr in 1996. These exposure periods are comparable to that which free-ranging kestrels are potentially exposed to during the reproductive period (Fernie, in prep., Fernie *et al.*, submitted - a).

Nest boxes were checked every morning at feeding (approximately 0800 h). New eggs were deemed as laid that morning, when fresh egg mass was weighed to the nearest 0.0001 g (Sartorius analytical balance; Mettler AJ100). Each egg was labelled with a non-toxic marker indicating its egg sequence position within the clutch. Twenty days after incubation began as calculated by the lay date of the penultimate egg (Bird 1988), eggs were candled (Weller 1956) for fertility, and categorized as living or dead embryo, addled or infertile. Egg length and width were measured to the nearest 0.01 mm using digital calipers (Mitutoyo Digital Caliper 0.01-150 mm, model 500-115). Volume was calculated using Hoyt's (1979) equation: volume (mm^3) = $width^2$ * length * 0.51. All measurements were performed by the same individual to reduce observer effects.

In 1995, we collected the third eggs immediately following laying for composition analysis. As it appeared that using the third egg may have adversely affected hatching success in 1995 (I. Ritchie, pers. comm.), infertile eggs restricted to the middle position of the laying sequence were collected at the end of the incubation period for composition analysis in 1996. Initial and fifth eggs of American kestrel clutches can be smaller than other eggs within the clutch (Wiebe and Bortolotti 1996). Immediately upon collection, eggs were boiled until hard for 10-13 minutes (Ricklefs 1982) and frozen (-20°C) until composition analysis. Frozen eggs were thawed at room temperature for one hour, weighed to the nearest 0.0001 g (Sartorius

analytical balance; Mettler AJ100), and then separated into shell with associated membranes, albumen, and yolk components. Eggshell width, measured to the nearest 0.001 mm using a micrometer (Mitutoyo digit outside micrometer, 0-25 mm, series 193), was determined using five measurements around the egg circumference. Egg components were air-dried at 65°C (VWR 1645D oven, VWR Scientific Inc.) to constant mass (<24 h). Lipids were extracted from yolks for 24 h in two baths of a 5:1 mixture of petroleum ether (30-60°C b.p.) and chloroform, and air-dried to constant mass. Lipid content of yolks was calculated by dry mass minus nonlipid dry mass; water content by wet minus dry albumen mass.

Fertile eggs which failed to hatch seven days after the estimated hatch date were opened for morphometric measurements of embryos. Date of death was calculated using the aging criteria established for American kestrel embryos (Bird *et al.* 1984). Wet mass was recorded to the nearest 0.1 g (Sartorius scale PT600). Morphometric measurements were taken to the nearest 0.01 mm on the right side of embryos, aged > 12 d, using a digital caliper (Mitutoyo Digital Caliper, 0.01-150 mm Model 500-115). Measurements were taken of total length of the uncurled embryo, tarsus, mid-toe, antebrachium, and eyeball at its widest point (Bird and Laguë 1982, Bird *et al.* 1984).

Prior to morning feeding, day-old kestrels were weighed to the nearest 0.1 g (Sartorius scale, PT600), and length of tarsus and antebrachium were measured to the nearest 0.01 mm using a digital caliper (Mitutoyo Digital Caliper, 0.01 - 150 mm model 500-115). The length of the tarsus and antebrachium were used as indicators of the structural size of the hatchlings.

Statistical Analyses

Ratio estimators (Cochran 1977) with t-tests were used to compare differences in fertility, hatching, and fledging success between control and EMF groups within years. Fertility was measured as the number of fertile eggs to the total number of eggs within a clutch, and hatching success measured as the number of eggs hatched to the total number of fertile eggs within a clutch. Fledging success was measured as the number of young fledging to the number of eggs hatched within a clutch.

Intra-clutch egg size variation was analysed using the mean egg volume and mass for

the clutch. We used a two-way ANOVA to determine S-EMF and L-EMF treatment effects, iteratively removing insignificant interaction and year terms from the model to increase power (Alasaskas and Ankney 1994). Effects of EMFs on clutch size were analyzed by one-way ANOVA. Egg component data for lipid content were log-transformed because of the high coefficient of variation (Sokal and Rohlf 1981), with all component data analyzed using one-way ANOVAs.

Embryonic growth analyses were restricted to 1995 data (due to sample sizes in 1996) from 23-27 d old embryos as age effects on embryo growth were linear (see below) and most embryos died at this age. Within this sample, a significant amount of variation could be explained by age for all measurements except mass ($P > 0.45$, $n = 42$). We controlled for age effects by regressing each variable, except mass, against age, and using the residual values. EMF effects on embryonic development were tested in two ways. First, overall growth effects were tested in a general way using a composite measure of size; the six linear measurements were used as input variables in a principle components analysis (PCA; Rising and Somers 1989), and the first component (PC1) of the PCA was used as an index of overall embryo size.

Body mass and structural size (tarsus, antebrachium length) of day-old kestrels were analyzed using nested one-way ANOVAs as more than one young hatched in some clutches. Means with S.E.M. are recorded.

Results

The mean number of eggs laid per clutch was similar between S-EMF, L-EMF and control clutches (one-way ANOVA, all Ps>0.05; see Table 6). The number of days from pairing to clutch initiation, and the length of the laying sequence, was similar between S-EMF, L-EMF and respective control pairs in 1995 and 1996 (t-test, all $Ps > 0.05$).

Mean egg volume per clutch was significantly larger ($F_1=4.44$ $P<0.05$) for EMF clutches than control clutches in 1995 only (see Table 6), but S-EMF and L-EMF exposure in 1996 had no effect on mean egg volume (all Ps>0.05; see Table 6). In 1995 only, mean egg mass per clutch was significantly heavier in S-EMF clutches ($F_1=7.19$ $P<0.01$) than controls, with no S-EMF or L-EMF effects observed (all $Ps > 0.05$) in 1996.

Components of 1995 eggs were corrected for egg volume as EMF eggs were larger than control eggs. S-EMF eggs had significantly thinner eggshells (F_1=8.80 P<0.01) and slightly more dried albumen (F_1=3.42 P=0.07) than control eggs, but were similar to controls in dried albumen and yolk mass, lipid and water content (see Table 7; one-way ANOVAs, all Ps>0.05). Small sample sizes in each group (n < 6) prevented analysis of eggs collected in 1996.

Based on the total number of eggs within a clutch, fertility was significantly higher in S-EMF clutches than control clutches in 1995 (t_{47}=-2.10 P<0.05) and 1996 (t =-7.18 P<0.001; see Table 6). There was no significant difference in fertility related to L-EMF exposure (t_{22}=0.89 P>0.05).

In 1995, S-EMF embryos were structurally larger overall than control embryos as measured by PC1 ($F_{1,21}$=5.93 P<0.05). Of the five individual morphological size measurements, only uncurled embryo length showed significant differences between the two groups (see Table 8). EMF embryos were longer than controls (F_1=11.67 P<0.01).

Hatching success in 1995 (13.6 ± 4.2 %) proved to be significantly lower (t_{35}=-3.36 P<0.01) than in 1996 (33.3 ± 28.7 %). Hatching success of fertile eggs within a clutch was significantly lower among S-EMF clutches than control clutches in 1995 (t_{47}=2.00 P<0.05) and 1996 (t_{18}=-2.11 P<0.05; see Table 6), but significantly higher in the L-EMF clutches than the control clutches (t_{22}=-2.24 P<0.05; see Table 6). Of the 1996 S-EMF exposure and control groups, only three S-EMF and one respective control kestrel hatched. Further analyses of hatchling body mass and structural size exclude these hatchlings.

Individual eggs successfully hatching, not the clutch mean, were significantly heavier when initially laid in 1995 S-EMF clutches (F_1=15.41 P<0.01) and 1996 L-EMF clutches (F_1=4.03 P<0.05) compared to respective controls. However, the 1995 S-EMF hatchlings were similar to control hatchlings in body mass and structural size (all Ps>0.05; see Table 8). L-EMF hatchlings were significantly heavier than control hatchlings (F_1=4.19 P<0.01) but similar in structural size (all Ps>0.05; see Table 8).

Fledging success was significantly higher (t_{47}=-9.17 P<0.001) in the 1995 S-EMF group (71.4 ± 6.2 %) than control group (57.1 ± 4.7 %), but significantly lower (t_{22}=-10.89

$P<0.001$) in the 1996 L-EMF group (60.0 ± 7.6 %) than the control group (88.9 ± 4.7 %).

Discussion

EMF exposure affected reproductive success of American kestrels. Fertility and fledging success (1995) were higher in S-EMF clutches than control clutches, but hatching success of fertile eggs was reduced in S-EMF clutches. Fertility was similar in the longer-term groups, with hatching success higher and fledging success lower in the L-EMF clutches. Mean egg volume and mass per clutch were greater in the S-EMF clutches in 1995 only; no effects were observed in 1996. S-EMF eggs had thinner eggshells and slightly more albumen than control eggs in 1995. In 1995, embryonic growth was greater in the S-EMF group than the control group, but hatchling size and body mass was similar between groups. L-EMF hatchlings were heavier than controls, but similar in structural size.

EMFs had no effect on clutch size, sequence length, or initiation date. Although the kestrels likely perceived the EMFs as light as indicated by previous melatonin results (see Fernie *et al.*, submitted - b), possible effects from photoperiod changes did not occur within the initial 11 to 14 d of exposure when clutch initiation began (Fernie *et al.*, submitted - b).

EMF effects on kestrel fertility were restricted to the initial breeding season of exposure, being higher in the S-EMF groups than controls. In contrast to the S-EMF kestrels, fertility was unaffected in rats exposed for 30 days (20 h daily) to a stronger electric field (100 kV/m) than in our study (10 kV/m; Sikov *et al.* 1984). EMFs may be perceived as light (Reiter 1992, 1993), and fertility in domestic avian species has been affected by light intensity (Davis *et al.* 1993 but see Siopes 1984, 1991) and elevated ambient temperatures (McDaniel *et al.* 1995). However, light intensity during the photophase, as well as temperatures, were similar between the EMF and control rooms.

The larger egg volume and mass per S-EMF clutch in 1995 is the result of EMF exposure rather than other environmental factors, parental or clutch characteristics (Williams 1994). Egg size can increase with laying date and rising temperatures (Perrins 1996), whereas cold temperatures during follicular and egg development reduced (Magrath 1992) or increased egg size (Williams and Cooch 1996). Yet, clutch initiation and completion dates were similar for control and EMF pairs, as were temperature and humidity between the two

experimental rooms. Prey or food availability influenced egg size of American kestrels (Wiebe and Bortolotti 1995) and Tengmalm's owls (*Aegolius funereus*; Hakkarainen and Korpimäki 1994), but not Blackbirds (*Turdus merula*; Macgrath 1992). However, kestrels in this study were fed *ad libitum* prior to and throughout the reproductive season, and there were no EMF effects on courtship feeding rates (Fernie *et al.*, submitted - a) or feed-intake of wintering kestrels (Fernie and Bird, submitted).

The larger egg size per S-EMF clutch in 1995 is unlikely related to parental qualities of body condition, age and reproductive experience. Mean egg volume was positively correlated with pre-clutch body condition of female American kestrels, and females in good condition laid larger eggs (Wiebe and Bortolotti 1995). However, EMF and control females were similar in body size, mass, and condition when paired with males, approximately 10 to 14 d prior to clutch initiation, and at mid-clutch (Fernie and Bird, submitted). While younger females and females with minimal breeding experience produce smaller eggs in some species (Croxall *et al.* 1992, Williams *et al.* 1993, Hipfner *et al.* 1997, but see Hakkarainen and Korpimäki 1994), age and breeding experience were controlled for and similar between EMF and control groups.

The larger S-EMF egg size per clutch in 1995 is also unlikely a function of clutch size and laying sequence as in some species (Slagsvold *et al.* 1984, Williams 1994). Clutch sizes were similar between control and EMF pairs, and the effect of laying sequence has been eliminated by using the mean mass or volume per clutch. Eggs laid after incubation was initiated were heavier than eggs laid when incubation began after clutch completion (Nilsson and Svensson 1993), but American kestrels start incubation with the penultimate egg (Porter and Wiemeyer 1972), and no S-EMF effects on incubation behaviours were observed (Fernie *et al.*, submitted - a). Consequently, increased egg size in mass and volume per clutch were likely a function of S-EMF exposure rather than environmental factors, parental or clutch characteristics.

Egg composition analysis in 1996 was not completed as the sample sizes of mid-sequence eggs was too small ($n < 6$ per group). In 1995, S-EMF eggs had thinner eggshells and slightly more dried albumen. Thinner eggshells in raptorial species have been observed

in relation to DDT/DDE exposure (Lundholm 1997 and references therein). The change in albumen is consistent with other results indicating egg components vary with egg size (Mills 1979, Ankney 1980, Finkler *et al.* 1998), particularly that albumen content increases disproportionately with egg size. The slightly larger amount of albumen in the EMF eggs may partly account for the larger-sized embryos in these eggs, as albumen and water content are the primary determining factors of near-term embryo size and hatchling size of chickens (Finkler *et al.* 1998). However, the EMF eggs were similar to control eggs in terms of water content.

EMF exposure increased embryonic growth of American kestrels in 1995, likely as a function of the larger mass or volume of eggs per EMF clutch, and increased albumen of these eggs. S-EMF embryos had longer bodies and their tarsi were slightly longer than controls just prior to hatching. Early stages of embryonic development of Sea urchins, rats, mice and chicks, were affected by 50 to 100 Hz EMFs (see Cameron *et al.* 1993). Initial development of embryonic chickens was disturbed by EMF exposure (Leal *et al.* 1988-89, Delgado *et al.* 1982, Ubeda *et al.* 1983, 1994, Juutilainen *et al.* 1987, Martin 1988), while EMF mice fetuses were longer and heavier at term (Kowalczuk *et al.* 1994) and EMF 8 day-old chicks were heavier with longer tarsi (Rooze and Hisenkamp 1985). The larger EMF kestrel embryos in our study contrast with others in which no embryonic effects were found in mice (Kowalczuk *et al.* 1994) or white leghorn chickens (Maffeo *et al.* 1988, Martin 1992, Veicsteinas *et al.* 1996). Temperature levels, critical to the successful development of avian embryos (Martin 1992), were probably similar between treatments as no differences were observed in the proportion of time females incubated (Fernie *et al.*, submitted - a).

Unhatched kestrel embryos and eggs showed no visible deformities, and most died within five days prior to hatching. Death of Ostrich (*Struthio camelus*) embryos in the last 10 to 14 d of artificial incubation was related to malpositioning and oedema, the latter correlated to water loss and in turn to egg size (Brown *et al.* 1996). Heavier eggs of Japanese quail lose proportionally less water during incubation (Martin and Arnold 1991), with water loss and oxygen regulation partly a function of eggshell thickness (Vleck and Vleck 1996). The S-EMF eggs per clutch were heavier and eggshells thinner than control eggs, but control and

EMF eggs were similar in water loss after 20 days of incubation (K. Fernie, unpubl. data). When opening unhatched eggs for embryo analysis, malpositioning was unfortunately not recorded. Inadequate turning by S-EMF parent kestrels may partly account for the reduced hatching success, but is unlikely as EMF males were slightly more alert and females showed no behavioural differences from controls during incubation (Fernie *et al.*, submitted - a). In 1996, seven EMF (four) and control (three) females died when laying their clutch as a result of egg peritonitis due to *E. coli* (determined by necropsy). *E. coli* reduces hatching success (Edens *et al.* 1997), but both S- (three) and L-EMF (four) females were equally affected, and hatching success was higher in the L-EMF group.

Hatching success of kestrels was affected by EMF exposure, being lower in S-EMF clutches but higher in L-EMF clutches. We can offer no explanation for why the pattern of hatching success was inconsistent between S-EMF and L-EMF groups. Reproductive success of Tree swallows, but not Eastern bluebirds or House wrens, was also lower under EMF conditions from powerlines compared to control areas (Doherty and Grubb 1996). In contrast, prenatal mortality and litter size was unaffected in rats exposed during pregnancy to stronger electric fields (80 and 100 kV/m), but for shorter periods (22 and 30 d; Burack *et al.* 1984 and Sikov *et al.* 1984, respectively), compared to this study (30 kV/m; adults 50 d, day-old hatchlings 28 d exposure). The reduced hatching success of the larger S-EMF eggs was surprising, as larger eggs have better hatching success in American kestrels (Wiebe and Bortolotti 1995) and other species (Schifferli 1973, Croxall *et al.* 1992, Perrins 1996, Amundsen *et al.* 1996). Poorer hatching success was observed with smaller female kestrels (Bortolotti and Wiebe 1993), but EMF and control females were similar in size and body mass throughout the study (Fernie and Bird, submitted).

Sex ratios *within* each clutch are impossible to assess as in all but one clutch, only one or two young hatched, and we did not identify the sex of unhatched embryos. However, based on feather development at approximately 14 d, the sex ratio of young was similar (approximately 50:50) within each room in 1995 and 1996. Sex ratios within rat litters were unaffected by EMF exposure (Sikov *et al.* 1984). It is possible that our small sample size based on 14 d old young may obscure sex-ratio effects from EMF exposure, as 1995 S-EMF

eggs were larger, and larger volume eggs produced more males than females within the same colony of American kestrels (Anderson *et al.* 1997). Further investigations of possible EMF effects on sex ratios of kestrels is required.

The S-EMF day-old kestrels were similar in body mass and structural size to controls, yet hatched from individual eggs which were heavier when initially laid. Compared to control hatchlings, L-EMF hatchlings were heavier and hatched from heavier individual eggs. Our sample sizes are small which likely accounts for the contradiction of S-EMF egg size-hatchling results, but the L-EMF results are consistent with previous research in which large egg size was positively correlated with larger hatchlings in American kestrels (Anderson *et al.* 1997) and other species (Schifferli 1973, Ricklefs 1984, Reid and Boersma 1990, Nilsson and Svensson 1993, Smith *et al.* 1995, Amundsen *et al.* 1996, Dawson and Clark 1996).

Fledging success was higher in the S-EMF group despite lowered hatching success. Fledging success in the L-EMF group was lower, but with higher hatching success and larger hatchlings. We can offer no explanation for this pattern of hatching-fledging success and EMF exposure, but suggest that EMFs increase the mortality of young either prior to hatching or fledging. The L-EMF results, larger hatchlings but reduced survival, contradict other research in which larger hatchlings had better survival (Dawson and Clark 1996 but see Perrins 1996).

In conclusion, EMF exposure of adult American kestrels affected reproductive success, particularly fertility, egg size and components, embryo and hatchling size, hatching and fledging success. S-EMF clutches had better fertility but poorer hatching success, and among 1995 S-EMF clutches, mean egg volume and mass per clutch, dried albumen mass, and embryonic growth were larger, but eggshells were thinner than controls. L-EMF clutches were similar in mean egg size (mass, volume) and fertility, had better hatching success and larger hatchlings, but poorer fledging success compared to controls. The pattern of EMF exposure and mortality of young kestrels may indicate that mortality at the pre-hatching or pre-fledging stage is increased by EMF exposure. EMF effects on reproductive success has important implications for wild species nesting within EMFs generated by powerlines.

Table 6. Reproductive traits of American kestrels exposed to short-term or longer-term electric and magnetic fields (EMFs) within years. Means ± S.E.M. are presented. (***P<0.001; **P<0.01; *P<0.05).

Variable	Short-term Exposure				Longer-term Exposure	
	Ctl '95	EMF '95	Ctl '96	EMF '96	Ctl	EMF
Sample size (clutches)	27	22	10	10	11	13
Pairs laying one clutch (%)	96.4	78.6	76.9	66.7	84.6	86.7
Clutch size (# eggs)	4.7 ± 0.1	4.8 ± 0.2	4.9 ± 0.3	4.5 ± 0.2	4.5 ± 0.4	4.9 ± 0.2
Pairing to first egg (d)	11.2 ± 1.1	11.9 ± 1.0	14.2 ± 2.9	12.2 ± 1.3	11.7 ± 1.1	12.0 ± 0.9
Laying sequence length (d)	8.2 ± 0.3	8.7 ± 0.4	8.0 ± 0.7	8.0 ± 0.6	7.2 ± 0.5	7.8 ± 0.4
Mean egg size/clutch						
- volume (cm^3)	13.6	14.3*	13.2 ± 0.2	13.4 ± 0.5	13.7 ± 0.3	14.1 ± 0.2
- mass (g)	14.6 ± 0.2	15.4 ± 0.2**	14.0 ± 0.2	14.3 ± 0.5	14.6 ± 0.3	15.0 ± 0.2
Fertility/ total eggs (%)	46.8 ± 7.1	50.9 ± 6.5*	6.1 ± 3.1	35.6 ± 12.6***	61.2 ± 11.0	57.1 ± 11.2
Hatch/fertile eggs (%)	13.6 ± 4.2*	11.1 ± 4.3	33.3 ± 28.7*	12.5 ± 12.1	26.7 ± 9.7	36.1 ± 10.7***
Fledging/ hatched (%)	57.1 ± 4.7	71.4 ± 6.2***	100[1]	100[1]	88.9 ± 4.7***	60.0 ± 7.6

[1]Only one control kestrel and two S-EMF kestrels hatched and fledged in 1996.

Table 7.Egg components of control and short-term electric and magnetic field (EMFs) exposure groups. Only the third egg of each clutch was used. Data were corrected for egg volume, but means ± S.E.M. of uncorrected data are presented. (**P<0.01)

Variable	Short-term Exposure			
	Ctl '95	EMF '95	F value	P value
sample size (# clutches)	25	19		
Mass with shell[1]	13.6 ± 0.2	14.3 ± 0.20	0.07	0.79
Shell width	0.145 ± 0.002	0.138 ± 0.004	8.8	0.01
Water content of albumen	8.1 ± 0.2	8.6 ± 0.2	0.29	0.59
Dried albumen mass	0.4 ± 0.02	0.5 ± 0.02	3.42	0.07
Dried yolk mass	1.5 ± 0.02	1.7 ± 0.04	1.34	0.25
Lipid content	0.06 ± 0.07	0.09 ± 0.02	1.56	0.22

[1]All measurements are in g except shell width (mm) and sample size.

Table 8. Morphological characters of 23-28 d old embryo or day-old hatchling American kestrels exposed to electric and magnetic fields (EMFs) and control conditions. Hatchlings from the 1996 short-term EMF or control groups were excluded from analysis due to small sample sizes (n < 3). Means ± S.E.M. are presented.

Character	Control	EMF	F	P
1995 Embryo Size, sample size	11	12		
Body mass[1]	11.1 ± 0.6	11.4 ± 0.8	0.11	0.74
Body length	41.3 ± 0.9	45.5 ± 0.8	11.67	0.003
Tarsus length	8.3 ± 0.3	9.1 ± 0.3	4.02	0.06[2]
Middle toe	4.1 ± 0.1	4.5 ± 0.1	4.25	0.05[2]
Antebrachium	6.8 ± 0.2	7.1 ± 0.2	0.85	0.37
Eye	9.4 ± 0.2	9.8 ± 0.2	2.61	0.12
1995 Short-term Hatchlings, sample size	7	7		
Body mass	10.3 ± 0.5	11.6 ± 0.5	2.64	0.31
Tarsus length	12.9 ± 0.3	13.1 ± 0.8	2.97	0.28
Antebrachium length	11.3 ± 0.7	12.6 ± 0.4	3.44	0.25
1996 Longer-term Hatchlings, sample size	9	15		
Body mass	10.4 ± 0.2	10.5 ± 0.3	4.19	0.01
Tarsus length	13.5 ± 0.3	12.6 ± 0.2	0.84	0.61
Antebrachium length	13.8 ± 0.2	12.9 ± 0.3	0.67	0.74

[1]Mass measured in g, all other measurements in mm.

[2]not significant using sequential Bonferroni corrected alpha.

CONNECTING STATEMENT 4.

I have determined that EMF exposure affects plasma melatonin concentrations in reproducing kestrels, and that the exposure times used in the laboratory study were comparable to that potentially experienced by free-ranging kestrels. According to the melatonin study, the kestrels perceived the EMFs as light, thereby altering the photoperiod in the EMF room. Changes in photoperiod are known to advance the onset of molt in other species (Dawson 1998), and melatonin is involved in plumage colour changes (Gupta *et al.* 1987) which would occur during molting. At the onset of molt, body mass of American kestrels increases. The next step is to determine whether EMFs affect body mass and molting in American kestrels in light of EMF effects on melatonin levels in reproducing male kestrels.

CHAPTER 5. EFFECTS OF ELECTRIC AND MAGNETIC FIELDS ON BODY MASS AND FEED-INTAKE OF AMERICAN KESTRELS.

Abstract

Raptors commonly nest and roost on transmission towers and hydro poles which exposes them to electric and magnetic fields (EMFs) from power lines. Captive and wild American Kestrels (*Falco sparverius*) were used to determine implications of EMFs, while captive kestrels only were used to examine EMF effects during reproduction and development. In this study, we hypothesized that EMF exposure affects the body mass of reproducing adult male and female kestrels, and determined whether increased body mass is a function of increased feed intake related to EMF exposure. Captive kestrels were paired for one (S-EMF) or two (L-EMF) breeding seasons to determine EMF effects. S-EMF and L-EMF exposure of males affected overall mean body mass during the reproductive season, and S-EMF males were significantly heavier than controls when molting began at approximately 56 d of exposure. In contrast, the body mass and pectoral muscle scores of females were unaffected by EMF exposure during laying, 20 days post-laying, and after 70 d of EMF exposure. There were no significant effects on body mass or feed-intake of wintering kestrels related to 10 d of EMF exposure. Our results can be explained by EMFs manipulating photoperiods indicated by altered melatonin levels, and that EMF effects are likely restricted to high resting metabolic rate periods in kestrels.

Introduction

Transmission towers have proved beneficial to birds, providing alternative sites for perching, nesting, roosting and hunting (Steenhof *et al.* 1993). Nest platforms and boxes have been erected on transmission towers and hydro poles for use by raptors (Olendorff *et al.* 1981, Steenhof *et al.* 1993). A 10-year study revealed that 133 pairs of raptors and ravens (*Corvus corax*) established new nests along a 500-kV transmission line in southern Idaho-Oregon, had overall nest success rates similar to or higher than pairs nesting on surrounding substrates, and 82.4% of pairs repeatedly nested on tower nest sites (Steenhof *et al.* 1993).

Power lines and structures have also electrocuted many individuals of species, particularly birds of prey (Olendorff *et al.* 1981, Ferrer *et al.* 1991, Bevanger 1994, Negro and Ferrer 1995). However, it remains unknown whether birds spending considerable time in the vicinity of power lines are affected by electric and magnetic fields (EMFs). EMF exposure did affect reproductive success of American kestrels (*Falco sparverius*, Fernie *et al.*, submitted - c), and while inconclusive in attributing reduced reproductive success directly to EMF exposure, reproductive success of Tree swallows (*Tachycineta bicolor*) was lower under power lines than in control areas (Doherty and Grubb 1996).

EMF exposure can affect hormone levels, feed intake, and embryonic development. Melatonin concentrations in male kestrels were affected by EMF exposure, suggesting kestrels perceived EMFs as light. Periodic manipulations can alter the onset and rate of molt in avian species (Dawson 1998). Prolactin and testosterone of adult male rats were affected by EMF exposure (Free *et al.* 1981, Oroza *et al.* 1987), and Holstein cows had elevated plasma progesterone levels and increased dry matter intake when exposed to EMFs (Burchard *et al.* 1996). Embryonic development of American kestrels (Fernie, in prep., Fernie *et al.* submitted - b), among other species, has been affected by EMF exposure (Delgado *et al.* 1982, Juutilainen *et al.* 1987, Cameron *et al.* 1993, Kowalczuk *et al.* 1994).

We determined whether EMF exposure for one and two breeding seasons affected behavior, reproduction, and development of captive American Kestrels, and determined the extent of EMF exposure experienced by wild kestrels (Fernie *et al.*, submitted - a, Fernie, in prep.). The melatonin results indicate a compression of the reproductive season for the EMF

males through a lengthening of the photoperiod (Fernie *et al.*, submitted - c). Longer photoperiods are known to advance the onset of molt in several avian species (Dolnik and Gavrilov 1980, Dawson and Goldsmith 1985, Meijer 1989), and molt is associated with changes in body mass in American kestrels (Fernie, unpubl. data) and the onset of gonadal regression in European kestrels (Meijer 1989). Consequently, we hypothesize that EMF exposure affects body mass of reproducing adult male and female kestrels, and that increased body mass may also be a function of increased feed intake related to EMF exposure. The body mass data for reproducing kestrels has also been presented in a paper concerning EMF effects on plasma melatonin concentrations in American kestrels (Fernie *et al.*, submitted - c).

Methods and Materials

Reproductive Study

We used 56 pairs of captive American Kestrels from the Avian Science and Conservation Centre (ASCC) of McGill University. In 1995, 28 pairs were randomly assigned to the control room, and 28 to the EMF room. In 1996, new birds were randomly assigned to each room © n=13 pairs, E n=15 pairs), and another 13 control and 15 EMF pairs from 1995 were assigned for a second season to the control and EMF room, respectively. Kestrels used in the experiment for one breeding season identified effects of 'short-term' EMF exposure (S-EMF), while those used for two seasons identified 'longer-term' EMF effects (L-EMF).

Pairs were genetically unrelated within the past seven generations. Each bird had previous breeding experience. Within each sex, adults were similar in age (2 - 5 y), size (wing chord), body mass, and condition (body mass:wing chord index) at pairing (1-way ANOVAs, all Ps > 0.05).

In the control and EMF rooms, humidity, temperature and photoperiod clocks followed natural conditions (lat. 45°30', long. 73°26') and were similar (t-tests, all Ps > 0.05). The control room measured 10 m x 8 m x 3 m, and the EMF room was 15 m x 10 m x 3 m high. Each room had a ventilation fan. Average light intensity at birds' head level was similar with EMFs on or off (lights on; t_{24}=-1.1990 P>0.05). Noise levels, indicative of vibrations (D.

Nguyen, pers. comm.), were similar when EMFs were on or off (t_s=0.64 P>0.05).

A magnetic field of 29.35 ± 0.03 μT in the EMF room was created by a current running through coils in the walls, floor, and ceiling of the room. The electric field of 9.67 ± 0.04 kV/m was generated by two plates suspended from the ceiling (Nguyen *et al.* 1991, Burchard *et al.* 1996). The EMFs, controlled by a computer to provide consistent and uniform fields, were equivalent to that which kestrels are exposed to when nesting under a 735-kV transmission line running at peak capacity. The magnetic field of the control room was 2.02 ± 0.27 μT, and the electric field was 0.03 ± 0.01 kV/m.

Kestrels were paired on 11 May 1995 and 13 May 1996, with exposure of EMF pairs beginning immediately and lasting for 95 d in 1995, and 91 d in 1996, until young were 35 d old, one week after fledging. Kestrels were exposed to EMFs for approximately 21 hr, or 87.5% of a 24 hr period, in 1995, and to approximately 23.5 hr or 98% of 24 hr in 1996. These exposure periods are comparable to those potentially experienced by free-ranging kestrels during the reproductive season (Fernie *et al.*, submitted - a).

Each pair was housed in a visually-isolated breeding pen made of reinforced corrugated cardboard and covered with nylon netting. A standard wooden nest box and rope perch were provided, as were wood shavings for bedding and nesting material. Metal materials were minimized to reduce disturbance of the electric field and possible shocks to the birds (F. Renaud, pers. comm.), while magnetic fields penetrated all housing materials (D. Nguyen, pers. comm.).

Kestrels were weighed to the nearest 0.1 g (Sartorius scale, model PT600). Male kestrels were weighed every 14 d, between 08:00 and 10:00 prior to morning feeding. Female kestrels were measured three times, between laying of the third and fourth egg (mid-clutch), 20 d post-laying, and at 70 d after pairing. Females were weighed at 15:00 to avoid disturbing egg laying which generally occurs in the early morning (Liou *et al.* 1987, I. Ritchie, pers. comm.). Females' pectoral muscles were scored from one (poor) to four (excellent; Gosler 1991) to monitor protein reserves throughout the experiment. Kestrel pairs were provided daily with *ad libitum* day-old cockerels and water at the same time when leftover food was removed.

For the reproductive study, Repeated Measures ANOVA were used to analyze EMF effects (Sokal and Rohlf 1995) using S.A.S. (version 6.11, 1985) software. Analyses were conducted separately within sex and length of exposure period. When necessary, data were transformed using the Box-Cox transformation (Sokal and Rolf 1995). Friedman's Repeated Measures ANOVA on Ranks test was used to analyze female pectoral muscle scores (Sokal and Rolf 1995). Statistical significance was considered at the $P<0.05$ level, and means ± S.E.M. are reported.

Feed-Intake Study

A cross-over experimental design using 32 captive kestrels was used for the feed-intake study, conducted between 4 December 1996 and 11 January 1997. Sixteen kestrels were housed in each of the control and EMF rooms for trial one, then switched to the opposite room for trial two. In each trial and room, eight male kestrels and eight females were housed individually in the breeding pens without nest boxes. Before the initial EMF trial began, the kestrels went through a 6 d acclimation period in the heated rooms (EMF 20.4°C ± 0.5°C, control 20.6°C ± 1.3°C) as they had been housed at ambient temperatures (0° C ± 5°C). Once feed intake was stabilized, EMF exposure began for 10 d, followed by a 4 d 'wash-out' period without EMFs to eliminate any residual EMF effects. Kestrels then switched rooms, followed by another 4 d acclimation period without measurements taken. Another 10 d trial and 4 d 'wash-out' period followed.

The feed-intake kestrels were exposed to EMFs for 23 h daily, comparable to the reproductive study and potentially experienced by reproducing free-ranging kestrels (Fernie, in prep., Fernie *et al.*, submitted - a). In both rooms, photoperiod was natural, and humidity temperature, noise and vibration conditions were similar (1-way ANOVA, all $Ps > 0.05$).

Kestrels in the feed-intake study were similar in age (3 - 4 a), and had been exposed (short-term) to EMFs in the reproductive study. As in the reproductive study, kestrels were provided with *ad libitum* water and day-old whole cockerel between 08:00 and 10:00 each day. Kestrels were weighed daily as described earlier. To facilitate leftover feed collection, pens were lined with wax paper which was changed daily. Leftover food from each bird was immediately frozen at -20°C until thawing 24 h prior to drying. Food samples were dried to

a constant weight in a Despatch convection oven at 177°C for 24 hr, and weighed to the nearest 0.01 g (Mettler PE3600 Deltarange scale).

Statistical analyses for the feed-intake study was based on the approximate dried feed-intake. Analysis involved Repeated Measures ANOVA. The model controlled for residual effects from the initial acclimation period or the two EMF trials. Data were standardized for analysis due to the large variation within the raw data (Sokal and Rohlf 1995).

Results

Reproductive Study

S-EMF exposure of male kestrels affected body mass in 1995 ($F_{7,42}$=2.65 P<0.05) and 1996 ($F_{6,20}$=3.92 P<0.01; see Figure 3). Time (1995 $F_{5,44}$ =8.21 P<0.001, 1996 $F_{5,21}$ =3.92 P<0.01) and treatment*time interactions (1995 $F_{5,44}$=3.64 P<0.01; 1996 $F_{5,21}$=4.94 P<0.01) were significant in both years. S-EMF males were heavier than controls at 56 d in 1995 (4.4%, χ^2_1=5.46 P<0.05) and 1996 (8.2%, F_1 =7.65 P<0.01), and at 70 d in 1996 (8.7%, F_1=7.10 P<0.01).

L-EMF male kestrels were also heavier than control males ($F_{6,20}$=5.41 P<0.01; see Figure 4), with a trend evident at 56 d of L-EMF males being heavier than controls (6.2%, F_1=3.77 P=0.06). Time ($F_{5,21}$=140.33 P<0.001) and treatment*time interactions ($F_{5,21}$=4.29 P<0.01) were significant.

S-EMF exposure of female kestrels had no effect on body mass in 1995 ($F_{3,31}$=0.05 P>0.05) or 1996 ($F_{4,14}$=1.25 P>0.05), and nor did L-EMF exposure ($F_{4,17}$=0.79 P>0.05; see Table 9). Time effects were significant for S-EMF females in 1996 only ($F_{3,15}$=70.01 P<0.001), and for L-EMF females ($F_{3,18}$=56.99 P<0.001). There were no significant treatment*time interactions (all Ps>0.05). Pectoral protein reserves of females were unaffected by EMF exposure, and there were no significant time or treatment*time interactions (Friedman's R.M. ANOVA, all Ps>0.05; see Table 9).

Feed Intake Results

Initially, control and EMF kestrels within each sex were similar in body mass (1-way ANOVAs, all Ps > 0.05), with males weighing 129.9 ± 4.5 g, and females weighing 142.8 ± 5.5 g. No significant differences were observed in body mass of males or females through any

trial periods (R.M. ANOVAs, all Ps>0.05), except the initial wash-out period when EMF males were heavier than control males (2% or 2.7 g; $F_{4,11}$=3.51 P<0.05). Results for body mass of males showed significant time effects during the initial acclimation period ($F_{5,10}$=13.26 P<0.001), and significant treatment*time interactions during the initial wash-out period ($F_{3,12}$=5.08 P<0.05). During the initial EMF trial, body mass of females varied over time ($F_{9,6}$=5.47 P<0.05), and the heavier group alternated daily (treatment*time $F_{9,6}$=5.18 P<0.05).

There were no differences in feed intake for either sex during the acclimation period, or during the two EMF trials and wash-out periods (R.M. ANOVAs, all Ps>0.05; see Table 10). Males ate progressively less over time during the initial EMF trial ($F_{5,10}$=80.81 P<0.001). Females ate progressively more during the initial acclimation period (time: $F_{5,10}$=136.5 P<0.001), but the group eating more varied daily (time*treatment interaction) during the initial trial ($F_{9,5}$=5.26 P<0.05) and wash-out ($F_{3,12}$=3.54 P<0.05) periods.

Discussion

S-EMF and L-EMF exposure affected the body mass of reproducing male kestrels, particularly when molting at 56 d and 70 d after pairing, and differences in body mass between EMF and control males increased over time. S-EMF and L-EMF exposure of female kestrels had no effect on body mass or pectoral muscle scores. EMF exposure for 10 d had no affect on body mass or feed intake of wintering male or female kestrels. Average daily dried feed intake of wintering adult males was 11.8 g ± 1.0 g, and feed intake of wintering adult females was 13.3 g ± 1.1 g, equivalent to 43.0 - 48.6 g (wet mass) daily.

Body mass of reproducing female kestrels was unaffected by S-EMF or L-EMF exposure, yet declined following clutch production which is typical of this species (Balgooyen 1976, Bird 1988). Pectoral protein reserves of females were unaffected by EMF exposure, except the 1996 S-EMF females at 70 d had higher pectoral scores than controls. We can offer no explanation for the larger pectoral reserves of these S-EMF females.

Compared to control males, S-EMF and L-EMF male kestrels were more stable in body mass, with body mass differences increasing between the two groups as the season progressed (treatment*time interaction; see Figs. 3, 4). In contrast to the reproducing kestrels, the body mass of wintering kestrels showed no effect from 10 d of EMF exposure.

The lack of EMF effects on winter mass is consistent with body mass results for male Pigtailed macaques (*Macaca nemestrina*) exposed to EMFs for three 21 d trials (Wolpaw *et al.* 1989), and for male Sprague-Dawley rats after 30 d exposure (Free *et al.* 1981).

The lack of EMF effects on body mass of wintering kestrels is likely partly related to their winter body mass being 20 g to 30 g above their post-clutch reproductive body mass. Wintering kestrels experienced consistent warm temperatures, approximately 20°C above ambient temperatures, which were unlikely to induce excess eating or fattening for overnight winter survival (Dawson and Marsh 1986). European kestrels, and likely American kestrels (I. Ritchie, pers. comm.), experience their lowest periods of daily energy expenditure (DEE) during winter (Masman *et al.* 1988). Body mass or feed intake was unlikely to increase regardless of EMF exposure as wintering kestrels had likely reached their maximum seasonal weight gain and were experiencing a relatively low DEE period.

The lack of EMF effects on feed intake of kestrels contrasts with the increased dried matter intake of Holstein cows exposed to the same EMF and room conditions (Burchard *et al.* 1996). However, the cows were exposed for a longer time period (28 d) compared to the kestrels (10 d) which suggests that EMF effects on feed intake may only appear after continuous, extended exposure ($\geq$ 28 d). EMF effects on mass of reproducing males only appeared at 56 d, and effects on male melatonin levels at 42 d in our study (Fernie, in prep., Fernie *et al.*, submitted - b). A lag period for EMF effects to occur would explain the lack of EMF effects on body mass of wintering kestrels.

Feed intake of kestrels is unlikely to be affected by an extended photoperiod if EMFs are perceived as light (Reiter 1993), as appears likely with the reproducing kestrels (Fernie *et al.*, submitted - b). Total daily food intake of molting White-crowned sparrows (*Zonotrichia leucophrys gambelii*) was unaffected by photoperiod length, although feed intake was less in the last 4 h photophase period of a 20L:4D photoperiod (Murphy and King 1990). Consequently, if the kestrels perceived EMFs as light during the scotophase (Fernie *et al.*, submitted -b), the longer photophase would not necessarily increase feed intake regardless of reproductive, molting, or overwintering demands.

The extended exposure of reproducing males to EMFs (e.g., $\leq$ 42 d) likely

manipulated the photoperiod compared to controls (Fernie *et al.*, submitted - b), which would have induced the onset of molt or increased the rate of molt (Dawson 1998). EMFs may be perceived as light (Reiter 1992, 1993), and both light and EMFs suppress melatonin (Stehle *et al.* 1988, Kato *et al.* 1993, 1994, Grota *et al.* 1994, Yellon 1994, Rogers *et al.* 1995). Plasma melatonin levels in the reproducing S-EMF and L-EMF male kestrels were suppressed at 42 d exposure (Fernie *et al.*, submitted - b, Fernie, in prep.), strongly suggesting EMF kestrels were exposed to a longer photoperiod than controls. Photoperiod manipulations have induced molt in several species (Gwinner 1996), including American kestrels (Willoughby 1966, Bird *et al.* 1980), and long photoperiods have advanced the onset of molt in species (Blackmore 1969, Chilgren 1978, Dolnik and Gavrilov 1980, Dawson 1991, 1998), including male European kestrels (Meijer 1989). Under long photoperiods (18L:6D, 16L:8D), a decrease in daylength prior to gonadal regression advanced molt onset in House sparrows (*Passer domesticus*), with 18L males beginning to molt two weeks in advance of 16L males (Dawson 1998). In the reproductive study, the longest photoperiod was set at 16L:8D and the photophase was decreasing at 42 d, which was prior to the onset of molt in male kestrels. The melatonin results strongly suggest that EMF kestrels were exposed to a longer photoperiod than controls, and the timing of the decrease in daylength would have advanced molt onset in EMF males.

Molting is accompanied by an increase in body mass in some avian species (Newton 1966, Orell and Ojanen 1980, Dijkstra *et al.* 1988, Masman *et al.* 1988, Dietz *et al.* 1992), and an increase in lipid or fat scores (Newton 1968, Morton and Welton 1973, Chilgren 1977, Lindström *et al.* 1993). Male kestrels at the ASCC initially lost weight (7.7 g, paired t-test: t=-5.87 P<0.001) at molt onset, then 7 d later, gained 5 g with concomitant increases in fat scores (K. Fernie, unpubl. data). These changes in body mass are similar to the differences in body mass between the EMF and control males at 56 d or approximately when molting began (S-EMF 1995 4.73 g, 1996 8.47 g, L-EMF 6.48g). Consequently, an advance of molt onset in EMF males over controls would account for body mass differences at 56 d.

The mass of growing new feathers may explain our results of S-EMF males being heavier at 70 d in 1996. Growing feathers initially weigh more (Newton 1968), and can

represent 10-12% of body mass (Chilgren 1977). In the ASCC kestrels, feather loss occurred at molt onset with a two-week delay until initial feather growth, coinciding with the 56 - 70 d period when EMF males were heavier. Feather growth would account for the S-EMF males being heavier than controls at 70 d (1996). We calculated the potential feather mass of S-EMF males at 70 d using Turček's (1966) formula, $Fw = 0.09*W^{0.95}$ where W is mass in grams. Plumages of S-EMF males (8.06 ± 0.17 g) were heavier (F_1=7.12 P<0.05) than those of controls (7.45 g ± 0.15g), partly explaining body mass differences between S-EMF groups at this time.

EMFs did not affect body mass of females when molting. The plasma melatonin results for the reproductive study (Fernie *et al.*, submitted - b), also suggested that male kestrels were more sensitive to EMFs than females, which has also been observed in gerbils (*Meriones unguiculatus*; Stehle *et al.* 1988). We offer several explanations for the body mass results of females, which are not necessarily mutually exclusive. First, female kestrels begin to molt earlier than males during mid-incubation (Bird 1988) which marked approximately 28 d of EMF exposure. This exposure period is shorter than when EMF effects appeared in males (i.e., 42 and 56 d), and again indicates that EMF effects only occur after continuous, extended exposure. Second, EMF females reabsorb the oviduct when molting, which occurred prior to daylength decline in our study. If females are similar to males, the decrease in daylength after the onset of gonadal regression increases the rate of molt, not its onset (Dawson 1998), so we would not expect to see an advance in molt onset with associated mass differences in EMF females. Third and as discussed below, the molting period of female kestrels is not a peak resting metabolic rate period (RMR; as defined by Bennett and Harvey 1987), and EMFs appear to affect kestrels only when RMR peaks. Finally, if female body mass was indeed affected by EMF exposure during molt, any effects may have been masked by the significant weight loss (approximately 40 g) experienced during incubation.

The sexually dimorphic response in body mass of kestrels to EMFs is likely linked to differential peak RMR periods. Our results indicate EMF effects only occur during the annual RMR peak of American kestrels. RMR increases with body mass gains, molting, and associated feather-synthesis costs (Winjandts 1984, Daan *et al.* 1989, Dietz *et al.* 1992,

Lindström *et al.* 1993). Male European Kestrels have a higher RMR (12% to 20%, Masman *et al.* 1988, Daan *et al.* 1989) than females because males are the predominant food provisioner of young (Masman *et al.* 1988). The higher RMR of males may be responsible for more pronounced differences in this sex compared to females when molting under long photoperiods (Meijer 1989). American kestrel males are also the main providers of food to young, and molt at this time (Balgooyen 1976, Rudolph 1982). However, female kestrels molt during incubation (Bird 1988), when RMR is reduced in many species (Williams 1996) including American kestrels (Gessaman and Findell 1979). Yet, S-EMF females produced significantly heavier eggs (Fernie, in prep., Fernie *et al.*, submitted - c), which is a high RMR period for female birds (Walsberg 1983, Masman *et al.* 1988). Our results indicate that EMFs affect kestrels either after an extended exposure period, and/or only during peak RMR periods.

In summary, S-EMF and L-EMF exposure affected body mass of male reproducing American Kestrels, particularly during molt at 56 d and 70 d of EMF exposure. The body mass of female kestrels was not affected by S-EMF or L-EMF exposure. Wintering male and female kestrels exposed to EMFs for 10 d were similar in body mass and feed-intake compared to respective control kestrels. These results suggest EMF effects may only occur after continuous and extended EMF exposure of more than 14 d as seen in male kestrels. EMFs were likely perceived as light by the reproducing kestrels, which would affect the photoperiod and advance the onset or rate of molt depending on gonadal regression. These hypotheses are supported by the results for plasma melatonin levels and body mass of male kestrels. Finally, EMFs likely affect kestrels when the RMR peaks, as seen with the results for body mass of male kestrels during molt and because larger eggs per clutch were produced by S-EMF females. The link between EMFs and peak RMR bears further investigation.

Table 9: A comparison of body mass and pectoral muscle scores (Gosler 1991) of adult female American kestrels (*Falco sparverius*) exposed to electric and magnetic fields (EMFs) or control conditions for the short-term (one season) or longer-term (two seasons) during the reproductive season. Means ± S.E.M. are presented. No significant differences occurred.

Exposure		Body mass (g)/ Pectoral muscle score (scale 1 - 4)		
group	n	mid-clutch	20 d post laying	70 d post pairing
Short-term				
Control '95	28,26, 26	167.22 ± 2.53	127.47 ± 2.27	124.04 ± 1.88
		3.18 ± 0.17	2.98 ± 0.11	3.09 ± 0.16
EMF '95	29, 28,27	168.52 ± 2.84	125.99 ± 2.07	127.19 ± 2.13
		3.09 ± 0.16	3.00 ± 0.10	2.93 ± 0.14
Control '96	11, 10, 10	157.15 ± 4.55	129.55 ± 2.73	123.59 ± 2.45
		3.25 ± 0.21	3.00 ± 0.24	2.88 ± 0.12
EMF '96	11, 10, 10	158.42 ± 4.42	128.44 ± 3.15	125.10 ± 2.58
		3.35 ± 0.15	2.78 ± 0.15	3.57 ± 0.21
Longer-term				
Control	9, 10, 10	160.56 ± 4.23	124.35 ± 2.94	120.36 ± 2.43
		2.78 ± 0.26	2.81 ± 0.17	3.04 ± 0.19
EMF	13, 13, 13	165.58 ± 5.21	132.45 ± 3.61	126.93 ± 2.63
		3.15 ± 0.20	2.41 ± 0.13	2.92 ± 0.15

Table 10: Dried feed (day-old cockerel) intake of wintering, captive adult male and female American kestrels exposed to electric and magnetic fields (EMFs). Cross-over experimental design used with two 10d EMF trial periods. Uncorrected means ± S.E.M. presented. No significant differences in dried feed intake occurred.

		Males		Females	
		Control (n = 8)	EMF (n = 8)	Control (n = 8)	EMF (n = 8)
Run-in pd.	day 1	8.96 ± 1.46	12.88 ± 1.15	10.95 ± 1.36	8.40 ± 1.35
	day 2	12.44 ± 0.93	11.97 ± 0.69	9.76 ± 1.32	10.60 ± 1.16
	day 3	14.03 ± 0.79	14.78 ± 0.80	13.48 ± 1.13	15.07 ± 0.65
	day 4	14.51 ± 0.78	13.77 ± 0.76	13.89 ± 0.87	14.42 ± 0.73
	day 5	12.70 ± 0.77	11.42 ± 0.74	13.31 ± 1.37	12.69 ± 0.74
	day 6	13.15 ± 0.75	13.91 ± 0.60	13.69 ± 0.86	13.82 ± 1.03
EMF Trial 1	day 1	13.88 ± 1.08	14.32 ± 0.78	14.27 ± 1.70	16.13 ± 1.12
	day 2	13.20 ± 1.03	13.51 ± 1.27	12.60 ± 1.27	13.69 ± 1.01
	day 3	11.42 ± 0.64	14.86 ± 2.71	11.35 ± 1.54	14.80 ± 1.03
	day 4	12.69 ± 1.05	13.59 ± 0.51	10.65 ± 1.02	15.21 ± 0.47
	day 5	11.73 ± 1.25	9.87 ± 0.89	11.05 ± 1.20	12.20 ± 1.36
	day 6	12.39 ± 0.78	11.31 ± 0.33	13.01 ± 1.24	14.06 ± 0.77
	day 7	10.26 ± 0.87	9.16 ± 0.77	10.02 ± 1.03	10.04 ± 1.37
	day 8	9.63 ± 0.54	10.83 ± 0.87	12.05 ± 1.20	16.33 ± 2.03
	day 9	10.06 ± 0.78	10.38 ± 1.01	11.29 ± 1.00	10.75 ± 0.80
	day 10	10.73 ± 0.67	10.72 ± 0.68	12.68 ± 1.07	9.78 ± 0.63
Wash-out pd.	day 1	10.17 ± 0.77	12.64 ± 1.16	11.87 ± 0.84	13.16 ± 1.36
	day 2	10.87 ± 0.44	10.31 ± 0.77	12.70 ± 1.12	12.63 ± 1.11
	day 3	10.71 ± 0.89	9.72 ± 0.34	10.77 ± 1.02	9.22 ± 0.78
	day 4	10.57 ± 0.40	9.57 ± 0.73	11.37 ± 1.25	10.58 ± 0.53
EMF Trial 2	day 1	8.39 ± 1.28	10.27 ± 0.62	10.84 ± 0.69	10.64 ± 0.64
	day 2	8.23 ± 1.25	10.13 ± 0.23	9.91 ± 0.97	11.65 ± 0.67
	day 3	7.85 ± 1.51	9.70 ± 0.54	10.37 ± 1.05	10.96 ± 1.19
	day 4	7.12 ± 1.19	9.22 ± 0.77	10.67 ± 0.31	10.51 ± 0.43
	day 5	8.29 ± 1.18	10.17 ± 0.78	9.97 ± 1.05	10.98 ± 0.81
	day 6	9.68 ± 0.39	9.06 ± 0.75	9.64 ± 0.55	10.64 ± 0.97
	day 7	9.94 ± 0.89	9.78 ± 0.63	10.20 ± 0.79	10.59 ± 0.60
	day 8	10.79 ± 0.78	8.46 ± 0.77	9.80 ± 0.68	10.11 ± 0.49
	day 9	10.82 ± 0.87	10.01 ± 0.46	9.49 ± 0.60	10.25 ± 0.70
	day 10	9.46 ± 1.81	8.28 ± 0.81	9.93 ± 0.94	8.56 ± 1.38
Wash-out pd.	day 1	8.13 ± 1.19	9.51 ± 0.74	9.61 ± 0.54	9.47 ± 0.60
	day 2	9.96 ± 0.83	8.68 ± 0.41	9.92 ± 0.71	9.31 ± 0.52
	day 3	9.86 ± 0.91	9.22 ± 0.96	9.57 ± 0.92	11.12 ± 0.61
	day 4	10.41 ± 0.67	9.76 ± 0.54	11.67 ± 1.06	11.01 ± 1.08

Fig. 3: Effects of short-term electric and magnetic field (EMF) exposure on body mass of reproducing male American kestrels: A comparison of EMF males to control (Ctl) males within years, using birds for one breeding season. Means with SE presented. EMF males were heavier than controls at 56 d in 1995 (X^2_1 =5.46 P<0.05) and 1996 (F_1=7.65 P<0.01), and 70 d (1996 F_1=7.10 P<0.01).

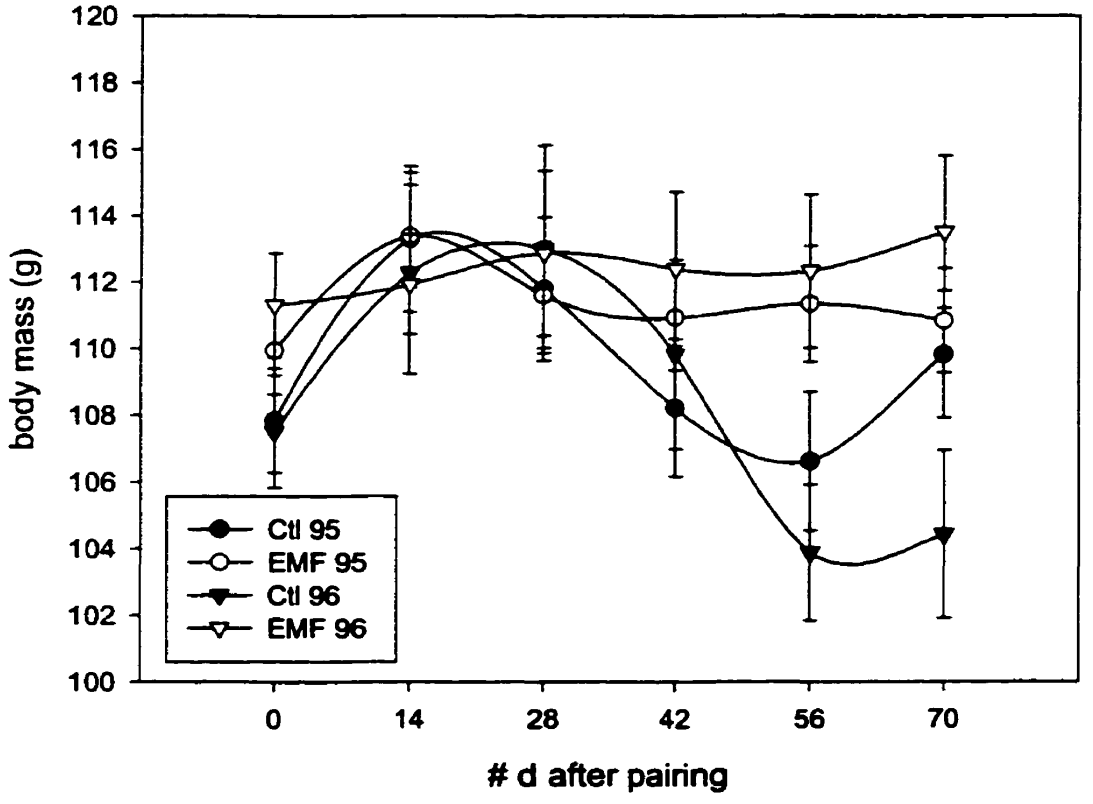
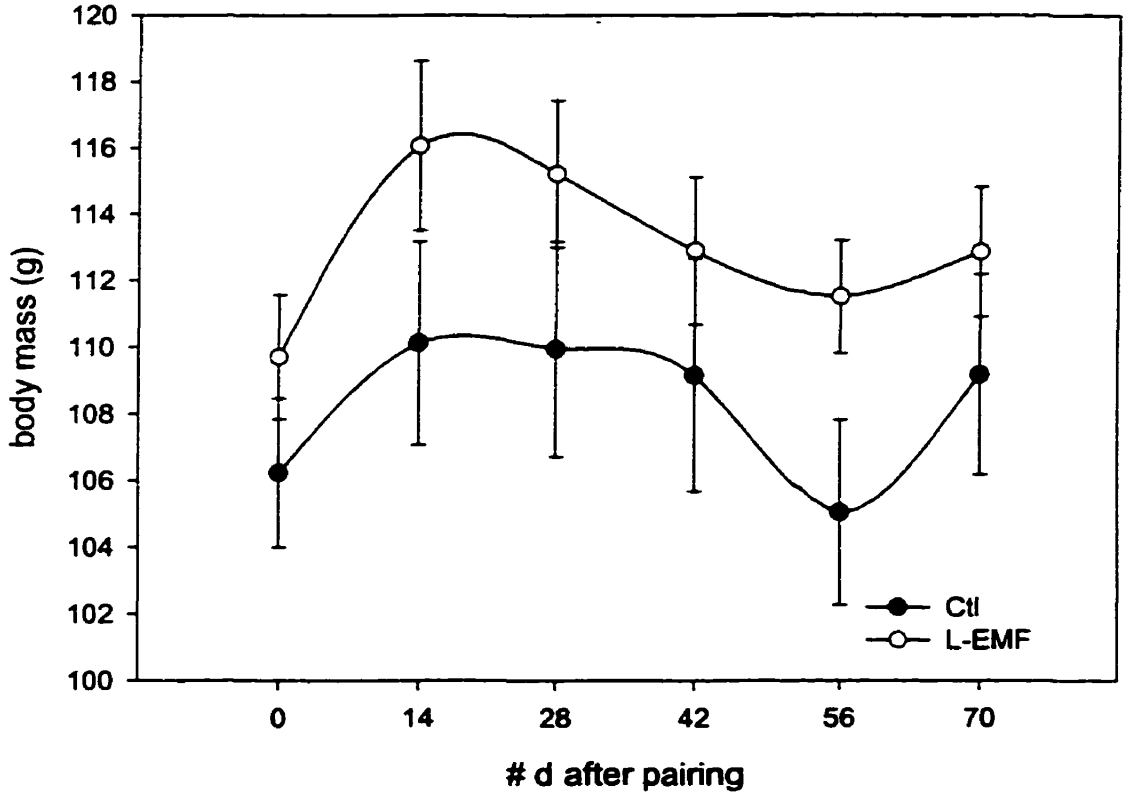


Fig. 4: Effects of longer-term exposure to electric and magnetic fields (L-EMF) on body mass of reproducing male American kestrels: A comparison of L-EMF vs. control males used for two breeding seasons. Means and SE presented. L-EMF males were slightly heavier than controls at 56 d, near the onset of molt (F_1=3.77 P=0.06).



Photoperiod variations govern avian circannual and circadian rhythms, and affect nocturnal pineal melatonin synthesis and release. Melatonin has been equivocally suppressed in mammalian species by light, changes in the geomagnetic field (Olcese *et al.* 1985, 1988, Reiter 1992), pulsed magnetic fields (e.g., Kato *et al.* 1993, 1994a,b, Yellon 1994), and alternating EMFs (Reiter 1985). Changes in an artificial magnetic field suppressed melatonin in migrating Pied flycatchers (Schneider *et al.* 1994a), but whether EMFs from electrical transmission lines suppress plasma melatonin concentrations in reproducing birds was not known. Birds readily nest under EMF conditions, but the reduced reproductive success of at least one species nesting under EMF conditions could not be directly attributed to EMFs (Doherty and Grubb 1996).

Given the importance of melatonin in circannual and circadian rhythms, and the importance of reproductive success to species survival, I conducted a study to test the hypotheses that EMF exposure affects plasma melatonin concentrations in reproducing American kestrels and that EMF exposure affects reproductive success of kestrels. Captive kestrels were paired in either a control or EMF room for one season in 1995 (28 pairs per room) and 1996 (Ctl 13 pairs, EMF 15 pairs) to examine short-term effects of EMF exposure (S-EMF). Kestrel pairs from 1995 (Ctl 13 pairs, EMF 15 pairs) were exposed for a second breeding season to examine longer-term EMF (L-EMF) effects.

This study demonstrated that exposure of reproducing American kestrels to electric and magnetic fields (EMFs) affects plasma melatonin concentrations and had implications for the circannual events of reproduction and molting. Four main conclusions are evident from this research:

1. Plasma melatonin in adult males was affected by EMF exposure, being suppressed at 42 d exposure and elevated at 70 d of EMF exposure. The similarity in melatonin levels between EMF males at 42 d and controls at 70 d likely indicates a seasonal phase-shift of the melatonin profile of male kestrels by EMF exposure. Plasma melatonin was suppressed in L-EMF fledgling birds only, not in adult females or 1995 adult males at 70 d of EMF exposure.

Results from the 1996 males are more powerful than 1995 as multiple sampling accounts for natural heterogeneity. Plasma melatonin of adult males was significantly higher than that of adult female kestrels, possibly explaining the differential melatonin response of the two sexes at 70 d exposure. Melatonin and body mass are not directly associated in adult or fledgling American kestrels.

2. The results of the captive reproductive study concerning EMF effects on plasma melatonin levels and reproductive success (to be discussed) are relevant to wild American kestrels nesting under EMF conditions, as exposure of wild birds is potentially equivalent to the exposure times (88%, 98%) experienced by the captive reproducing birds. Potential and maximal EMF exposure of wild kestrels during the reproductive season is approximately 90% for females and 80% for males during daylight hours. Differences in EMF exposure within a reproducing pair are likely due to spatial and labour partitioning.

The behaviour of captive reproducing kestrels was affected by S-EMF exposure; behavioural effects of L-EMF exposure were not investigated. S-EMF females were more active than controls during courtship, and spent less time grooming and resting during the nestling phase. EMF males were more likely than controls to be active during courtship, alert during incubation, and to perch during the nestling phase. These behavioural changes had no effect on clutch size and fertility, and fledging success was higher in the S-EMF group. Behavioural changes were minimal during incubation, and likely did not contribute to the reduced hatching success of the S-EMF group.

3. EMF exposure affected reproductive success of American kestrels, but not clutch size, initiation date, and the length of the laying sequence. Fertility was higher in S-EMF clutches than controls, but hatching success was lower in S-EMF clutches. L-EMF exposure had no effect on fertility, but was associated with increased hatching success. Mean egg volume and mass per clutch was greater in the S-EMF clutches of 1995. Correcting for the larger egg volume of these EMF eggs, S-EMF eggs had thinner eggshells and slightly more dried albumen. S-EMF embryos were larger in overall size than control embryos, but S-EMF hatchlings were similar in size and body mass to controls. L-EMF hatchlings were heavier than control hatchlings. Fledging success was higher under S-EMF conditions in 1995, but

lower under L-EMF conditions. Clearly, EMF exposure affected reproductive success of kestrels, increasing mortality of young at pre- and post-hatching stages dependent on the length of exposure of parents.

4. The melatonin results of the reproducing male kestrels suggest that the birds perceived EMFs as light, thereby affecting the photoperiod in the EMF room. Photoperiodic manipulations advance the onset of molt, which in the male kestrel, is associated with increases in body mass. S-EMF males were heavier than controls at 56 d of exposure, when the onset of molt would have occurred. It is unlikely that this increased body mass was the result of increased feed intake as there were no effects on feed-intake of wintering kestrels during 10 d of EMF exposure. The body mass and pectoral muscle scores of reproducing females showed no EMF effects during laying, incubation (20 d post-laying), or after 70 d of EMF exposure. Female kestrels start to molt earlier than males, i.e., during incubation. The differential response of the two sexes in terms of body mass and melatonin concentrations suggests that male kestrels may be more sensitive to EMF exposure than females which has been observed in gerbils (*Meriones unguiculatus*; Stehle *et al.* 1988). This differential response of the sexes may also be the result of male kestrels having a higher (12%) resting metabolic rate than females (Daan *et al.* 1989), which was at an annual peak during the molting period for male but not female kestrels (Masman *et al.* 1988, Daan *et al.* 1989).

Possible Mechanisms

EMF effects in reproducing kestrels occurred during peak resting metabolic rate periods in the annual cycle. In comparison to controls, EMF females laid significantly larger eggs in volume and mass, EMF embryos were structurally larger, and males were significantly heavier at the onset of molt. The thyroid hormones, thyroxiine (T_4) and thyriiodothyronine (T_3), are the primary determinants in regulating the metabolic rate; as the level of circulating thyroid hormones increase, metabolic rate increases correspondingly. T_4 is associated with reproduction and molt, while T_3 is associated with calorigenesis and lipogenesis especially during migration (Wentworth and Ringer 1986). Thyroid hormones, especially the ratio of T_4:T_3, play an important role in embryonic muscle growth, particularly of the heart and liver as well as body mass, and carbohydrate metabolism (Christensen *et al.* 1996). The metabolism

of chicken embryos increases greatly and then plateaus during the last 7 d of incubation (Finkler *et al.* 1998 and references therein). Thyroid activity during the breeding season appears to be controlled or associated with the pineal gland, and hence melatonin; pinealectomized pigeons had higher serum thyroid hormones during the breeding season (Ramachandran *et al.* 1996 and references therein). Melatonin is also known to affect growth, body mass and metabolism in young quail (Zeman *et al.* 1993) and embryonic chickens (Lamošová *et al.* 1997). Given the connection between the pineal gland and thyroid hormones, the effects of EMFs on melatonin, and the occurrence of EMF effects on reproducing kestrels during high metabolic periods of the annual cycle of each sex, I suggest that a possible mechanism explaining these EMF results involves the interaction of EMFs, the thyroid gland, and melatonin.

Future Research

Future research concerning effects of EMF exposure on birds should investigate whether EMF effects are restricted to high resting metabolic rate (RMR) periods. Resting metabolic rates are high for males during molting when young are present, and for females during egg production. In my study, EMF males, compared to controls, were significantly heavier at the onset of molt. While not directly affecting the body mass of EMF females, females produced heavier and larger eggs per clutch when exposed to EMFs. However, EMF exposure of 10 d had no effect on overwintering kestrels. This suggests EMF effects only occur during high RMR periods, and/or only occur after extended exposure (e.g., > 14 d).

Another worthwhile study would be to determine if the reduction of melatonin during the reproductive season has implications for proper migration, particularly orientation, of kestrels raised and/or breeding under EMF conditions. Melatonin concentrations in male kestrels were affected from the middle of the breeding season onwards, and melatonin in L-EMF fledglings was lower at 35 d of age. Following reproduction and molting, kestrels and other temperate species migrate. Migratory distance and proper direction, particularly during the initial migration, are determined by melatonin.

Further research should also address a possible circannual mechanism, as yet unidentified, involving the annual variations in the *amplitude* of the circadian melatonin

rhythm. The amplitude of melatonin during the scotophase is higher under long-day photoperiods than short-day photoperiods; melatonin peaks were higher under 20L:4D compared to 16L:8D in laying chickens (Liou *et al.* 1987). The amplitude of melatonin is also suppressed at the summer and winter solstice (Miché *et al.* 1991), and during zugunruhe activity (Gwinner 1996). Furthermore, seasonal variations in the duration of nocturnal melatonin do not appear to act as a reproductive signal for avian species as opposed to mammalian species (Juss *et al.* 1993). Total melatonin production in Japanese quail was similar under short (8L:16D) and long photoperiods (16L:8D), with the greatest secretion from the pineal gland occurring under intermediate photoperiods (12L:12D; Cockrem and Follett 1985). Such short photoperiods occur during the winter, long photoperiods during the summer, and intermediate photoperiods during the vernal and autumnal equinoxes. Furthermore, in the EMF males, melatonin concentrations were altered and the reproductive season and molt advanced. The geomagnetic field fluctuates on a circadian and circannual basis. Together, these results suggest that changes in the *amplitude* of melatonin occurring on a circadian and circannual basis in avian species may be a possible mechanism involved in circannual rhythms of avian species.

Able, K.P. 1995. Orientation and navigation: A perspective on fifty years of research. Condor 97:592-604.

Able, K.P. and M.A. Able. 1990. Calibration of the magnetic compass of a migratory bird by celestial rotation. Nature 347:378-380.

Able, K.P. and M.A. Able. 1993. Daytime calibration of magnetic compass of a migratory bird by celestial rotation. Nature 347:523-525.

Able, K.P. and M.A. Able. 1995. Interactions in the flexible orientation system of a migratory bird. Nature 375:230-232.

Able, K.P. and M.A. Able. 1997. Development of sunset orientation in a migratory bird: no calibration by the magnetic field. Anim. Behav. 53:363-368.

Adachi, A., Hasegawa, M. and S. Ebihara. 1995. Measurement of circadian rhythms of ocular melatonin in the pigeon by *in vivo* microdialysis. NeuroReport 7:286-288.

Adachi, A., Hasegawa, M. and S. Ebihara. 1995. Measurement of circadian rhythms of ocular melatonin in the pigeon by *in vivo* microdialysis. NeuroReport 7:286-288.

Alasaskas, R.T. and C.D. Ankney. 1994. Nutrition of breeding female ruddy ducks: the role of nutrient reserves. Condor 96:878-897.

Amundsen, T., Lorentsen, S.-H. and T. Tveraa. 1996. Effects of egg size and parental quality on early nestling growth: an experiment with the Antarctic petrel. J. Animal Ecol. 65:545-555.

Anderson, L.E. and R.D. Phillips. 1985. Biological effects of electric fields: an overview. In: M. Grandolfo, S.M. Michaelson, and A. Rindi (eds.). Biological effects and dosimetry of static and ELF electromagnetic fields. pp. 345-377. New York: Plenum Press.

Anderson, D.J., Reeve, J. and D.M. Bird. 1997. Sexually dimorphic eggs, nestling growth and sibling competition in American kestrels *Falco sparverius*. Funct. Ecol. 11:331-335.

Ankney, C.D. 1980. Egg weight, survival, and growth of lesser snow goose goslings. J. Wildl. Manage. 44:174-182.

Balgooyen, T.G. 1976. Behaviour and ecology of the American kestrel (*Falco sparverius* L.) in the Sierra Nevada of California. Univ. Calif. Publ. Zool. 103:1-83.

Barfuss, D.W. and L.C. Ellis. 1971. Seasonal cycles in melatonin synthesis by the pineal gland as related to testicular function in the house sparrow (*Passer domesticus*). Gen. Comp. Endocrinol. 17:183-193.

Basco, P.S., Rashotte, M.E. and F.K. Stephan. 1996. Photoperiod duration and energy balance in the pigeon. Phsyiol. Behav. 60:151-159.

Beason, R.C. 1987. Interaction of visual and non-visual cues during migratory orientation by the Bobolink (*Dolichonyx oryzivorus*). J. Ornithol. 128:317-324.

Bennett, P.M. and P.H. Harvey . 1987. Active and resting metabolism in birds: allometry, phylogeny, and ecology. J. Zool. 213:327-363.

Bennett, A.T.D., Cuthill, I.C., Partridge, J.C. and E.J. Maier. 1996. Ultraviolet vision and mate choice in Zebra finches. Nature 380:433-435.

Berthold, P. 1975. Migration: control and metabolic physiology. Avian Biol. 5:77-127.

Bevanger, K. 1994. Bird interactions with utility structures: collision and electrocution, cases and mitigating measures. Ibis 136:412-425.

Bingman, V.P. 1981. Savannah sparrows have a magnetic compass. Anim. Behav. 29:962-963.

Binkley, S. and E. Geller. 1975. Pineal *N*-acetyltransferase in chickens: rhythm persists in constant darkness. J. Comp. Physiol. 99:67-70.

Binkley, S., Stephens, J.L., Riebman, J.B. and K.B. Reilly. 1977. Regulation of pineal rhythms in chickens: photoperiod and dark-time sensitivity. Gen. Comp. Endocrinol. 32:411-416.

Bird, D.M. 1982. The American kestrels as a laboratory research animal. Nature 299:300-301.

Bird, D.M. 1985. Evaluation of the American kestrel (*Falco sparverius*) as a laboratory research animal. In. J. Archibald, J. Ditchfield, H.C. Rowsell (eds.). The Contribution of Laboratory Animal Science to the Welfare of Man and Animals: Past, Present and Future. pp. 3-9. Stuttgart: Gustav Fischer.

Bird, D.M. 1988. The American Kestrel. In: R.S. Palmer (ed.). The Handbook of North American birds. Vol. 5. pp. 253-290. New York: Vail-Ballou Press.

Bird, D.M. and R.B. Buckland. 1976. The onset and duration of fertility in the American kestrel. Can. J. Zool. 54:1595-1597.

Bird, D.M. and P.C. Laguë. 1982. Influence of forced renesting and hand-rearing on growth of young captive American kestrels. Can. J. Zool. 60:71-79.

Bird, D.M., Weil, P.G. and P.C. Laguë. 1980. Photoperiodic induction of multiple breeding seasons in captive American kestrels. Can. J. Zool. 58:1022-1026.

Bird, D.M., Gauthier, J. and V. Montpetit. 1984. Embryonic growth of American kestrels. Auk 101:392-396.

Blackmore, F.H. 1969. The effect of temperature, photoperiod and molt on the energy requirements of the House Sparrow, *Passer domesticus*. Comp. Biochem. Physiol. 30:433-444.

Bluhm, C.K., Schwabl, H., Schwabl, I., Perera, A., Follett, B.K., Goldsmith, A.R. and E. Gwinner. 1991. Variation in hypothalamic gonadotrophin-releasing hormone content, plasma and pituitary LH, and in-vitro testosterone release in a long-distance migratory bird, the Garden warbler (*Sylvia borin*), under constant photoperiods. J. Endocrinol. 128:339-345.

Bortolotti, G.R. and K. Wiebe. 1993. Incubation behaviour and hatching patterns in the American kestrel *Falco sparverius*. Ornis Scand. 24:41-47.

Boswell, T., Sharp, P.J., Hall, M.R. and A.R. Goldsmith. 1995. Migratory fat deposition in European quail: a role for prolactin? J. Endocrinol. 146:71-79.

Bowman, R. and D.M. Bird. 1985. Reproductive performance of American Kestrels laying replacement clutches. Can. J. Zool. 63:2590-2593.

Brainard, G.C., Vaughan, M.K. and R.J. Reiter. 1986. Effect of light irradiance and wavelength on the Syrian hamster reproductive system. Endocrinology 119:648-654.

Brown, C.R., Peinke, D. and A. Loveridge. 1996. Mortality in near-term ostrich embryos during artificial incubation. Br. Poult. Sci. 37:73-85.

Burack, G.D., Seto, Y.J., Hsieh, S.T. and J.L. Dunlap. 1984. The effects of prenatal exposure

to a 60-Hz high-intensity electric field on postnatal development and sexual differentiation. J. Bioelectr. 3:451-467.

Burchard, J.F., Nguyen, D.H., Richard, L. and E. Block. 1996. Biological effects of electric and magnetic fields on productivity of dairy cows. J. Dairy Sci. 79:1549-1554.

Cameron, I.L., Hardman, W.E., Winters, W.D., Zimmerman, S. and A.M. Zimmerman. 1993. Environmental magnetic fields: influences on early embryogenesis. J. Cell. Biochem. 51:417-425.

Cassone, V.M. 1990. Effects of melatonin on vertebrate circadian systems. Trends Neurosci. 13:457-464.

Cavé, A.J. 1968. The breeding of the Kestrel, *Falco tinnunculus* L., in the reclaimed area Oostelijk Flevoland. Neth. J. Zool. 18:313-407.

Chabot, C.C. and M. Menaker. 1992. Circadian feeding and locomotor rhythms in pigeons and house sparrows. J. Biol. Rhythms 7:287-299.

Charles, R.G., Robinson, F.E., Hardin, R.T., Yu, M.W., Feddes, J. and H.L. Classen. 1992. Growth, body composition, and plasma androgen concentration of male broiler chickens subjected to different regimens of photoperiod and light intensity. Poult. Sci. 71:1595-1605.

Chilgren, J.D. 1977. Body composition of captive White-crowned Sparrows during postnuptial molt. Auk 94:677-688.

Chilgren, J.D. 1978. Effects of photoperiod and temperature on postnuptial molt in captive White-crowned Sparrows. Condor 80:222-229.

Christensen, V.L., Donaldson, W.E. and J.P. McMurtry. 1996. Physiological differences in late embryos from Turkey breeders at different ages. Poult. Sci. 75:172-178.

Cochran, W.G. 1977. Sampling techniques, 3rd edition. New York: John Wiley & Sons.

Cockrem, J.F. 1995. Timing of seasonal breeding in birds, with particular reference to New Zealand birds. Reprod. Fertil. Dev. 7:1-19.

Cockrem, J.F. and B.K. Follett. 1985. Circadian rhythm of melatonin in the pineal gland of the Japanese quail (*Coturnix coturnix japonica*). J. Endocrinol. 107:317-324.

Coulton, L.A. and A.T. Barker. 1991. The effect of low-frequency pulsed magnetic fields on

chick embryonic growth. Phys. Med. Biol. 36:369-381.

Croxall, J.P., Rothery, P. and A. Crisp. 1992.The effect of maternal age and experience on egg-size and hatching success in Wandering albatrosses *Diomedea exulans*. Ibis 134:219-228.

Daan, S., Masman, D., Strijkstra, A. and S. Verhulst. 1989. Intraspecific allometry of basal metabolic rate: relations with body size, temperature, composition, and circadian phase in the Kestrel, *Falco tinnunculus*. J. Biol. Rhythms 4:267-283.

Davis, G.S., Parkhust, C.R. and J. Brake. 1993. Light intensity and sex ratio effects on egg production, egg quality characteristics, and fertility in breeder Pekin ducks. Poult. Sci. 72:23-29.

Dawson, A. 1991. Photoperiodic control of testicular regression and moult in male House sparrows *Passer domesticus*. Ibis 133:51-55.

Dawson, A. 1994. The effects of daylength and testosterone on the initiation and progress of moult in Starlings *Sturnus vulgaris*. Ibis 136:335-340.

Dawson, A. 1997. Circannual rhythms in starlings. In: S. Harvey and R.J. Etches (eds.). Perspectives in Avian Endocrinology. pp. 149-158.Bristol: Society for Endocrinology.

Dawson, A. 1998. Photoperiodic control of the termination of breeding and the induction of moult in House Sparrows *Passer domesticus*. Ibis 140:35-40.

Dawson, A. and A.R. Goldsmith. 1985.Modulation of gonadotrophin and prolactin secretion by daylength and breeding behaviour in free-living starlings, *Sturnus vulgaris*. J. Zool. 206:241-252.

Dawson, A. and A.R. Goldsmith. 1997. Changes in gonadotropin-releasing hormone (GnRH-I) in the pre-optic and median eminence of Starlings (*Sturnus vulgaris*) during the recovery of photosensitivity and during photostimulation. J. Reprod. Fertil. 111:1-6.

Dawson, A. and P.J. Sharp. 1998. The role of prolactin in the development of reproductive photorefractoriness and postnuptial molt in the European starling (*Sturnus vulgaris*). Endocrinology 139:485-490.

Dawson, A., Follett, B.K., Goldsmith, A.R. and T.J. Nicholls. 1985. Hypothalamic gonadotrophin-releasing hormone and pituitary and plasma FSH and prolactin during

photostimulation and photorefractoriness in intact and thyroidectomized Starlings (*Sturnus vulgaris*). J. Endocrinol. 105:71-77.

Dawson, R.D. and R.G. Clark. 1996. Effects of variation in egg size and hatchling date on survival of Lesser scaup *Aythya affinis* ducklings. Ibis 138:693-699.

Dawson, W.R. and R.L. Marsh. 1986. Winter fattening in the American Goldfinch and the possible role of temperature in its regulation. Physiol. Zool. 59:357-368.

Delgado, J.M.R., Leal, J., Monteagudo, J.L. and M.G. Gracia. 1982. Embryological changes induced by weak, extremely low frequency electromagnetic fields. J. Anat. 134:533-551.

Denbow, D.M., Leighton, A.T. and R.M. Hulet. 1990. Effect of light sources and light intensity on growth performance and behaviour of female turkeys. Br. Poult. Sci. 31:439-445.

Dey, M. and S.K. Maitra. 1995. Exogenous melatonin does not influence photorefractoriness in the testes of roseringed parakeet, *Psittacula krameri* (neumann). Pavo 33:5-9.

Dietz, M.W., Daan, S. and D. Masman. 1992. Energy requirements for molt in the Kestrel, *Falco tinnunculus*. Phys. Zool. 65:1217-1235.

Dijkstra, C., Daan, S., Meijer, T., Cavé, A.J. and R.P.B. Foppen. 1988. Daily and seasonal variations in body mass of the Kestrel in relation to food availability and reproduction. Ardea 76:127-140.

Doherty, P.F., Jr. and T.C. Grubb. Jr. 1996. Effects of high-voltage power lines on birds breeding within the powerlines' electromagnetic fields. Sialia 18:129-134.

Doi, O., Iwasawa, A., Nakamura, T. and Y. Tanabe. 1995. Effects of different photoperiods on plasma melatonin rhythm of the chicken. Anim. Sci. Technol. (Jpn.) 66:16-26.

Dolnik, V.R. and V.M. Gavrilov. 1980. Photoperiodic control of the molt cycle in the Chaffinch (*Fringilla coelebs*). Auk 97:50-62.

Edens, F.W., Parkhust, C.R., Casas, I.A. and W.J. Dobrogosz. 1997. Principles of ex ovo competitive exclusion and in ovo administration of Lactobacillus reuteri. Poult. Sci. 76:179-196.

Emlen, S.T. 1967a. Migratory orientation in the Indigo bunting, *Passerina cyanea*. Part I.

Evidence for use of celestial cues. Auk 84:309-342.

Emlen, S.T. 1967b. Migratory orientation in the Indigo bunting, *Passerina cyanea*. Part II. Mechanism of celestial orientation. Auk 84:463-489.

Emlen, S.T. 1970. Celestial rotation: its importance in the development of migratory orientation. Science 165:716-718.

Etches, R.J. 1996. Reproduction in Poultry. Oxon: CAB International.

Farner, D.S. and B.K. Follett. 1979. Reproductive periodicity in birds. In: E.J.W. Barrington (ed.). Hormones and Evolution. pp. 829-872. New York: Academic Press.

Feldstone, C.S., Polonis, J.J., Gibson, E.G., Smith, H.D. and E.E. Dean. 1980. A preliminary study of possible effects of high intensity 60-Hz electric fields on non-human primate behaviour. The Bioelectromagnetics Society: Second Annual Meeting, September 14 - 18, 1980, San Antonio, Texas.

Fernie, K.J. In prep. Effects of electric and magnetic fields on the reproductive biology of American kestrels *Falco sparverius*. McGill University, Ph.D. Thesis.

Fernie, K.J. and D.M. Bird. submitted. Effects of electric and magnetic fields on body mass and feed-intake of American kestrels.

Fernie, K.J., Leonard, N.J. and D.M. Bird. submitted - a. Effects of electric and magnetic fields on behaviour of free-ranging and captive American kestrels.

Fernie, K.J., Petitclerc, D., Nguyen, D.H., Bird, D.M. and E. Block. submitted - b. Effects of electric and magnetic fields on melatonin levels of reproducing American kestrels.

Fernie, K.J., Dawson, R.D., Bird, D.M. and P. Laguë. submitted - c. Effects of electric and magnetic fields reproductive parameters of American kestrels.

Ferrer, M. 1990. Hematological studies in birds. Condor 92:1085-1086.

Ferrer, M., de la Riva, M. and J. Castroviejo. 1991. Electrocution of raptors on power lines in southwestern Spain. J. Field Ornithol. 62:181-190.

Finkler, M.S., Van Orman, J.B. and P.R. Sotherland. 1998. Experimental manipulation of egg quality in chickens: influence of albumen and yolk on the size and composition of near-term embryos in a precocial bird. J. Comp. Physiol. B 168:17-24.

Follett, B.K. and A. Pearce-Kelly. 1990. Photoperiodic control of the termination of

reproduction in Japanese quail (*Coturnix coturnix japonica*). Proc. R. Soc. Ser. B 242:225-230.

Follett, B.K., Kumar, V. and T.S. Juss. 1992. Circadian nature of the photoperiodic clock in Japanese quail. J. Comp. Physiol. A 171:533-540.

Free, M.J., Kaune, W.T., Phillips, R.D. and H-C. Cheng. 1981. Endocrinological effects of strong 60-Hz electric fields on rats. Bioelectromagnetics 2:105-121.

García-Rodríguez, T., Ferrer, M., Recio, F. and J. Castroviejo. 1987. Circadian rhythms of determined blood chemistry values in buzzards and eagle owls. Comp. Biochem. Physiol. 88A:663-669.

Gessaman, J.A. and P.R. Findell. 1979. Energy cost of incubation in the American Kestrel. Comp. Biochem. Physiol. 63A:57-62.

Gosler, A.G. 1991. On the use of greater covert moult and pectoral muscle as measures of condition in passerines with data for the Great Tit *Parus major*. Bird Study 38:1-9.

Greenberg, B., Bindokas, V.P. and J.R. Gaujer. 1981. Biological effects of a 760 kV transmission line: exposures and thresholds in honeybee colonies. Bioelectromagnetics 2:315.

Grota, L.J., Reiter, R.J., Keng, P. and S. Michaelson. 1994. Electric field exposure alters serum melatonin but not pineal melatonin synthesis in male rats. Bioelectromagnetics 15:427-437.

Gupta, B.B.P., Haldar-Misra, C., Ghosh, M. and J.P. Thapliyal. 1987. Effect of melatonin on gonads, body weight, and luteinizing hormone (LH) dependent coloration of the Indian finch, Lal munia (*Estrilda amandava*). Gen. Comp. Endocrinol. 65:451-456.

Gwinner, E. 1975. Circadian and circannual rhythms in birds. In: D.S. Farner and J.R. King (eds.). Avian Biology. 5:221-285. New York: Academic Press.

Gwinner, E. 1977. Circannual rhythms in bird migration. Annu. Rev. Ecol. Syst. 8:381-405.

Gwinner, E. 1986. Circannual rhythms in the control of avian migrations. Adv. Stud. Behav. 16:191-228.

Gwinner, E. 1989. Photoperiod as a modifying and limiting factor in the expression of avian circannual rhythms. J. Biol. Rhythms 4:237-250.

Gwinner, E. 1996. Circadian and circannual programmes in avian migration. J. Exp. Biol. 199:39-48.

Gwinner, E. and W. Wiltschko. 1978. Endogenously controlled changes in migratory direction of the Garden warbler, *Sylvia borin*. J. Comp. Physiol. 125:267-273.

Gwinner, E. and W. Wiltschko. 1980. Circannual changes in migratory orientation of the Garden warbler, *Sylvia borin*. Behav. Ecol. Sociobiol. 7:73-78.

Gwinner, E., Schwabl-Benzinger, I., Schwabl, H. and J. Dittami. 1993. Twenty-four hour melatonin profiles in a nocturnally migrating bird during and between migratory seasons. Gen. Comp. Endocrinol. 90:119-124.

Gwinner, E., Hau, M. and S. Heigl. 1994. Phasic and tonic effects of melatonin on avian circadian systems. In: T. Hiroshige and K. Honma (eds.) Evolution of Circadian Clock. pp. 127-137. Sapporo: Hokkaido University Press.

Gwinner, E., Konig, S. and M. Zeman. 1995. Endogenous gonadal, LH and molt rhythms in tropical Stonechats: effect of pair bond on period, amplitude, and pattern of circannual cycles. J. Comp. Physiol. A 177:73-79.

Hakkarainen, H. and E. Korpimäki. 1994. Environmental, parental and adaptive variation in egg size of Tengmalm's owls under fluctuating food conditions. Oecologia 98:362-368.

Haldar, C. and M. Ghosh. 1995. Pineal gonadal relationship of the Indian spotted owlet, *Athene brama*. J. Reprod. Biol. Comp. Endocrinol. 7:9-18.

Hau, M. and E. Gwinner. 1992. Circadian entrainment by feeding cycles in House sparrows, *Passer domesticus*. J. Comp. Physiol. A 170:403-409.

Hau, M. and E. Gwinner. 1996. Food as a circadian zeitgeber for House sparrows: the effect of different food access durations. J. Biol. Rhythms 11:196-207.

Hau, M. and E. Gwinner. 1997. Adjustment of House sparrow circadian rhythms to a simultaneously applied light and food zeitgeber. Physiol. Behav. 62:973-981.

Heigl, S. and E. Gwinner. 1995. Synchronization of circadian rhythms of House sparrows by oral melatonin: Effects of changing period. J. Biol. Rhythms 10:225-233.

Helbig, A.J. 1991. Inheritance of migratory direction in a bird species: a cross-breeding

experiment with SE- and SW-migrating Blackcaps (*Sylvia atricapilla*). Behav. Ecol. Sociobiol. 28:9-12.

Helbig, A.J. 1996. Genetic basis, mode of inheritance and evolutionary changes of migratory directions in palearctic warblers (Aves: *Sylviidae*). J. Exp. Biol. 199:49-55.

Helbig, A.J., Berthold, P. and W. Wiltschko. 1989. Migratory orientation of Blackcaps (*Sylvia atricapilla*): population-specific shifts of direction during the autumn. Ethology 82:307-315.

Hendel, R.C. and F.W. Turek. 1978. Suppression of locomotor activity in sparrows by treatment with melatonin. Physiol. Behav. 21:275-278.

Hill, G.E. 1990. Female house finches prefer colourful males: sexual selection for a condition-dependent trait. Anim. Behav. 40:563-572.

Hipfner, J.M., Gaston, A.J. and L.N. de Forest. 1997. The role of female age in determining egg size and laying date of Thick-billed Murres. J. Avian Biol. 28:271-278.

Holberton, R.L. 1993. An endogenous basis for differential migration in the Dark-eyed junco. Condor 95:580-587.

Howe, H.F. 1976. Egg size, hatching asynchrony, sex, and brood reduction in the Common grackle. Ecology 57:1195-1207.

Hoysak, D.J. and P.J. Weatherhead. 1991. Sampling blood from birds: A technique and an assessment of its effect. Condor 93:746-752.

Hoyt, D.F. 1979. Practical methods of estimating volume and fresh weight of bird eggs. Auk 96:73-77.

Hydro Québec. 1989. Electricity in the air. Quebec:Hydro-Québec.

Hydro Québec. 1996. Electric and magnetic fields and human health. 2nd edition. Quebec: Communications for Direction, Services de Santé, Québec.

Jander, R. 1963. Insect orientation. A. Rev. Ent. 8:95-114.

Janik, D., Dittami, J. and E. Gwinner. 1992. The effect of pinealectomy on circadian plasma melatonin levels in House sparrows and European starlings. J. Biol. Rhythms 7:277-286.

Juutilainen, J., Läära, E. and K. Saali. 1987. Relationship between field strength and abnormal

development in chick embryos exposed to 50 Hz magnetic fields. Int. J. Radiat. Biol. 52:787-793.

Kato, M., Honma, K., Shigemitsu, T. and Y. Shiga. 1993. Effects of exposure to a circularly polarized 50-Hz magnetic field on plasma and pineal melatonin levels in rats. Bioelectromagnetics 14:97-106.

Kato, M., Honma, K., Shigemitsu, T. and Y. Shiga. 1994a. Circularly polarized 50-Hz magnetic field exposure reduces pineal gland and blood melatonin concentrations of Long-Evans rats. Neurosci. Lett. 166:59-62.

Kato, M., Honma, K., Shigemitsu, T. and Y. Shiga. 1994b. Recovery of nocturnal melatonin concentration takes place within one week following cessation of 50 Hz circularly polarized magnetic field exposure for six weeks. Bioelectromagnetics 15:489-492.

Kowalczuk, C.I., Robbins, L., Thomas, J.M., Butland, B.K. and R.D. Saunders. 1994. Effects of prenatal exposure to 50 Hz magnetic fields on development in mice: 1. Implantation rate and fetal development. Bioelectromagnetics 15:349-361.

Krause, D.N. and M.L. Dubocovich. 1997. Plasticity of melatonin receptors in the avian body. In: S. Harvey and R.J. Etches (eds.). Perspectives in Avian Endocrinology. pp. 387-403. Bristol: Society for Endocrinology.

Kumar, B.S. and V. Kumar. 1991. Seasonal reproduction in subtropical Brahminy myna, *Sturnus pagodarum*: role of photoperiod. Gen. Comp. Endocrinol. 83:354-365.

Kumar, B.S. and V. Kumar. 1992. Termination of photorefractoriness in the Brahminy myna, *Sturnus pagodarum*: role of photoperiods and gonadal hormones. Indian J. Exp. Biol. 30:48-53.

Kumar, V. and B.S. Kumar. 1991. The development of photorefractoriness in termination of the breeding season in the tropical Brahminy myna: role of photoperiod. Reprod. Nutr. Dev. 31:27-36.

Lack, D. 1968. Ecological adaptations for breeding in birds. London: Methuen.

Lamašová, D., Zeman, M., Macková, M. and E. Gwinner. 1995. Development of rhythmic melatonin synthesis in cultured pineal glands and pineal cells isolated from chick embryo. Experientia 51:970-975.

Lamašová, D., Zeman, M. and M. Juráni. 1997. Influence of melatonin on chick skeletal muscle cell growth. Comp. Biochem. Physiol. C 188:375-379.

Leal, J., Shamsaifar, K., Trillo, M.A., Ubeda, A., Abraira, V. and L. Chacon. 1988-89. Embryonic development and weak changes of the geomagnetic field. J. Bioelectr. 7:141-153.

Lee, Jr., J.M., Stormshak, F., Thompson, J.M., Hess, D.L. and Foster, D.L. 1995. Melatonin and puberty in female lambs exposed to EMF: a replicate study. Bioelectromagnetics 16:119-123.

Lerchl, A., Nonaka, K.O., Stokkan, K.A. and R.J. Reiter. 1990. Marked rapid alterations in nocturnal pineal serotonin metabolism in mice and rats exposed to weak intermittent magnetic fields. Biochem. Biophys. Res. Commun. 169:102-108.

Levine, R.L., Dooley, J.K. and T.D. Bluni. 1995. Magnetic field effects on spatial discrimination and melatonin levels in mice. Physiol. Behav. 58:535-537.

Lindström, A., Visser, G.H. and S. Daan. 1993. The energetic cost of feather synthesis is proportional to basal metabolic rate. Phys. Zool. 66:490-510.

Liou, S.S., Cogburn, L.A. and H.V. Biellier. 1987. Photoperiodic regulation of plasma melatonin levels in the laying chicken (*Gallus domesticus*). Gen. Comp. Endocrinol. 67:221-226.

Lundholm, C.D. 1997. DDE-induced eggshell thinning in birds: effects of p,p'-DDE on the calcium and prostaglandin metabolism of the eggshell gland. Comp. Biochem. Physiol. C 118:113-128.

Maffeo, S., Brayman, A.A., Miller, M.W., Carstensen, E.L., Ciaravino, V. and C. Cox. 1988. Weak low frequency electromagnetic fields and chick embryogenesis: failure to reproduce positive findings. J. Anat. 157:101-104.

Magrath, R.D. 1992. Seasonal changes in egg-mass within and among clutches of birds: general explanations and a field study of the Blackbird *Turdus merula*. Ibis 134:171-179.

Maitra, S.K. and M. Dey. 1992. Importance of photoperiods in determining temporal pattern of annual testicular events in Roseringed parakeet (*Psittacula krameri*). J. Biol.

Rhythms 7:13-25.

Maitra, S.K. and M. Dey. 1995. Pinealocyte-response in Roseringed parakeets (*Psittacula krameri*) to melatonin administration varies in relation to the reproductive phase of the annual testicular cycle. Biol. Rhythm Res. 26:88-99.

Maitra, S.K. and M. Dey. 1996. Melatonin does not modulate testicular responsiveness to altered photoperiods. - a study during different phases of the annual gonadal cycle in Roseringed parakeets (*Psittacula krameri*). Biol. Rhythm Res. 27:72-86.

Martin, A.H. 1988. Magnetic fields and the time dependent effects on development. Bioelectromagnetics 9:393-396.

Martin, A.H. 1992. Development of chicken embryos following exposure to 60-Hz magnetic fields with differing waveforms. Bioelectromagnetics 13:223-230.

Martin, P. and P. Bateson. 1986. Measuring behaviour: an introductory guide. New York: Cambridge University Press.

Martin, P.A. and T.W. Arnold. 1991. Relationships among fresh mass, incubation time, and water loss in Japanese Quail eggs. Condor 93:28-37.

Masman, D., Daan, S. and H.J.A. Beldhuis. 1988. Ecological energetics of the Kestrel: daily energy expenditure throughout the year based on time-energy budget, food intake and doubly labelled water methods. Ardea 76:64-81.

McDaniel, C.D., Bramwell, R.K. and J.L. Wilson. 1995. Fertility of male and female broiler breeds following exposure to elevated ambient temperatures. Poult. Sci. 74:1029-1038.

McIntosh, R.P. 1991. Concept and terminology of homogeneity and heterogeneity in ecology. In: J. Kolasa and S.T.A. Pickett (eds.). Ecological heterogeneity. pp. 24-46. New York: Springer-Verlag.

Meijer, T. 1989. Photoperiodic control of reproduction and molt in the Kestrel, *Falco tinnunculus*. J. Biol. Rhythms 4:351-364.

Merila, J. and E. Svensson. 1997. Are fat reserves in migratory birds affected by condition in early life? J. Avian Biol. 28:279-286.

Meyer, W.E. and J.R. Millam. 1991. Plasma melatonin levels in Japanese quail exposed to

dim light are determined by subjective interpretation of day and night, not light intensity. Gen. Comp. Endocrinol. 82:377-385.

Miché, F., Vivien-Roels, B., Pévet, P., Spehner, C., Robin, J.P. and Y. Le Maho. 1991. Daily pattern of melatonin secretion in an Antarctic bird, the Emperor Penguin, *Aptenodytes forsteri*: seasonal variations, effect of constant illumination and of administration of Isoproterenol or Propranolol. Gen. Comp. Endocrinol. 84:249-263.

Mills, J.A. 1979. Factors affecting the egg size of Red-billed gulls *Larus novaehollandiae scoppulimus*. Ibis 121:53-67.

Möller, A.P. 1993. Ectoparasites increase the cost of reproduction in their hosts. J. Animal Ecol. 62:309-322.

Moore, M.C., Schwabl, H. and D.S. Farner 1983. Biochronometry of testicular regression in White-crowned sparrows (*Zonotrichia leucophrys gambelli*). J. Comp. Physiol. 153:489-494.

Moos, W.S. 1964. A preliminary report on the effects of electric fields on mice. Aerosp. Med. 35:374-377.

Morton, M.L. and D.E. Welton. 1973. Postnuptial molt and its relation to reproductive cycle and body weight in Mountain White-crowned Sparrows (*Zonotrichia leucophrys oriantha*). Condor 75:184-189.

Morton, M.L. and D.E. Welton. 1973. Postnuptial molt and its relation to reproductive cycle and body weight in Mountain White-crowned Sparrows (*Zonotrichia leucophrys oriantha*). Condor 75:184-189.

Murphy, M.E. and J.R. King. 1990. Diurnal changes in tissue glutathione and protein pools of molting White-crowned Sparrows: the influence of photoperiod and feeding schedule. Phys. Zool. 63:1118-1140.

Murton, D.J. and N.J. Westwood. 1977. Avian breeding cycles. Oxford: Clarendon press.

Negro, J.J. and M. Ferrer. 1995. Mitigating measures to reduce electrocution of birds on power lines: a comment on Bevanger's review. Ibis 137:136-137.

Newton, I. 1966. The moult in the Bullfinch *Pyrrhula pyrrhula*. Ibis 108:41-67.

Newton, I. 1968. The temperatures, weights, and body composition of molting Bullfinches.

Condor 70:323-332.

Nguyen, D.H., Richard, L. and G. Turmel. 1991. Salle d'exposition aux champs electrique et magnetique en courant alternatif pour le detail: description et specifications. Institute de recherche d'Hydro-Quebec, Varennes: Quebec.

Nicholls, T.J., Goldsmith, A.R. and A. Dawson. 1988. Photorefractoriness in birds and comparison with mammals. Physiol. Rev. 68:133-176.

Nilsson, J.-Å. and E. Svensson. 1993. Causes and consequences of egg mass variation between and within blue tit clutches. J. Zool., Lond. 230:469-481.

Olcese, J., Reuss, S. and L. Vollrath. 1985. Evidence of the involvement of the visual system in mediating magnetic field effects on pineal melatonin synthesis in the rat. Brain Res. 333:382-384.

Olendorff, R.R., Miller, A.D. and R.N. Lehman. 1981. Suggested practices for raptor protection on power lines: the state of the art in 1981. Raptor Res. Found., Inc. Rep. No. 4.

Orell, M. and M. Ojanen. 1980. Overlap between breeding and moulting in the Great Tit *Parus major* and Willow Tit *P. montanus* in northern Finland. Ornis Scand. 11:43-49.

Oroza, M.A., Calcicedo, L., Sanchez-Franco, F. and L. Rivas. 1987. Hormonal, hematological and serum chemistry effects of weak pulsed electromagnetic fields on rats. J. Bioelectr. 6:139-151.

Osei, P., Robbins, K.R. and H.V. Shirley. 1989. Effects of exogenous melatonin on growth and energy metabolism in chickens. Nutr. Res. 9:69-81.

Ossenkopp, K.P. and L.J. Shapiro. 1972. Effects of prenatal exposure to a 0.5Hz low-intensity rotating magnetic field on White Peking ducklings. Am. Soc. Zool. 12:650.

Pang, S., Lee, P.P.N. and P. Tang. 1991. Sensory receptors as a special class of hormonal cells. Neuroendocrinology 53(suppl.):2-11.

Papi, F. 1991. Olfactory navigation. In: P. Berthold (ed.) Orientation in birds. pp. 52-85. Basel, Switzerland: Birkhauser.

Parry, D.M., Goldsmith, A.R., Millar, R.P. and L.M. Glennie. 1997. Immunocytochemical

localization of GnRH precursor in the hypothalamus of European starlings during sexual maturation and photorefractoriness. J. Neuroendocrinol. 9:235-243.

Payne, R.B. 1972. Mechanisms and control of molt. In: J.R. King and D.S. Farner (eds.), Avian Biology. 2:104-155. New York: Academic Press.

Perrins, C.M. 1970. The timing of bird breeding seasons. Ibis 112:242-255.

Perrins, C.M. 1996. Eggs, egg formation and the timing of breeding. Ibis 138:2-15.

Persinger, M.A. 1969. Open-field behavior in Rats exposed prenatally to a low intensity-low frequency, rotating magnetic field. Devel. Psychobiol. 2:168-171.

Persinger, M.A., Ossenkopp, K.P. and G.B. Glavin. 1972. Behavioral changes in adult rats exposed to ELF magnetic fields. Inter. J. Biomet. 16:155-162.

Persinger, M.A., Ludwig, H.W. and K.P. Ossenkopp. 1973. Psycho-physiological effects of extremely low frequency electromagnetic fields: a review. Percept. Mot. Skills 36:1131-1159.

Pohl, H. 1994. Entrainment properties of the circadian system changing with reproductive state and molt in the canary. Physiol. Behav. 55:803-810.

Porter, R.D. and S.N. Wiemeyer. 1972. Reproductive patterns in captive American Kestrels (Sparrow Hawks). Condor 74:46-53.

Prayitno, D.S., Phillips, C.J. and D.K. Stokes. 1997. The effects of color and intensity of light on behavior and leg disorders in broiler chickens. Poult. Sci. 76:1674-1681.

Ramachandran, A.V., Patel, M.M. and C.S. Patel. 1996. Effects of pineal indoles and parachlorophenylalanine on seasonal reproduction in the pigeon. J. Exp. Biol. 199:793-800.

Ravikumar, G. and P.D. Tewary. 1990. Photorefractoriness and its termination in the subtropical House sparrow, *Passer domesticus*: Involvement of circadian rhythm. Chronobiol. Int. 7:187-191.

Ravikumar, G. and P.D. Tewary. 1991. Endogenous circadian rhythm in the photoperiodic ovarian response of the subtropical sparrow, *Passer domesticus*. Physiol. Behav. 50:637-639.

Reid, W.V. and P.D. Boersma. 1990. Parental quality and selection on egg size in the

Magellanic penguin. Evolution 44:1780-1786.

Reiter, R.J. 1985. Action spectra, dose-response relationships, and temporal aspects of light's effects on the pineal gland. Ann NY Acad Sci 453:215-299.

Reiter, R.J. 1987. The melatonin message: Duration versus coincidence hypotheses. Life Sci. 40:2119-2131.

Reiter, R.J. 1992. Alterations of the circadian melatonin rhythm by the electromagnetic spectrum: a study in environmental toxicology. Reg. Toxicol. Pharmacol. 15:226-244.

Reiter, R.J. 1993. Static and extremely low frequency electromagnetic field exposure: reported effects on the circadian production of melatonin. J. Cell. Biochem. 51:394-403.

Ricklefs, R.E. 1982. Observations on handling procedures and composition of European starling eggs. Condor 84:338-339.

Ricklefs, R.E. 1984. Egg dimensions and neonatal mass of shorebirds. Condor 86:7-11.

Rising, J.D. and K.M. Somers. 1989. The measurement of overall body size in birds. Auk 106:666-674.

Rogers, W.R., Reiter, R.J., Smith, H.D. and L. Barlow-Walden. 1995. Rapid-onset/offset, variably scheduled 60 Hz electric and magnetic field exposure reduces nocturnal serum melatonin concentration in nonhuman primates. Bioelectromagnetics 3 (suppl.):119-122.

Rooze, M. and M. Hinsenkamp. 1985. *In vivo* modifications induced by electromagnetic stimulation of chicken embryos. Reconst. Sug. Traumatol. 19:87-92.

Rudolph, S.G. 1982. Foraging strategies of American Kestrels during breeding. Ecology 63:1268-1276.

SAS Institute Inc. 1985. SAS user's guide: statistics, 5th edition. SAS Inst., Cary, NC, U.S.A.

Saumier, M.D. 1986. Reproduction and behaviour in Trichinella-infected kestrels. M.Sc. Thesis, McGill University.

Saumier, M.D., Rau, M.E. and D.M. Bird. 1988. The influence of *Trichinella pseudospiralis* infection on the behaviour of captive, nonbreeding American kestrels (*Falco*

sparverius). Can. J. Zool. 66:1685-1692.

Sætre, G.P., Dale, S. and T. Slagsvold. 1994. Female pied flycatchers prefer brightly coloured males. Anim. Behav. 48:1407-1416.

Schifferli, L. 1973. The effect of egg weight on the subsequent growth of nestling great tits *Parus major*. Ibis 115:549-558.

Schneider, T. 1995. Distribution of 2-[^{125}I]Iodomelatonin binding sites in the brain of the Pied flycatcher (*Ficedula hypoleuca*) and the Zebra finch (*Taeniopygia guttata*). J. Exp. Biol. 198:1943-1949.

Schneider, T., Thalau, H. and P. Semm. 1994a. Effects of light or different earth-strength magnetic fields on the nocturnal melatonin concentration in a migratory bird. Neurosci. Lett. 168:73-75.

Schneider, T., Thalau, H., Semm, P. and W. Wiltschko. 1994b. Melatonin is crucial for the migratory orientation of Pied flycatchers (*Ficedula hypoleuca* pallas). J. Exp. Biol. 194:255-262.

Sikov, M.R., Montgomery, L.D., Smith L.G. and R.D. Phillips. 1984. Studies on prenatal and postnatal development in rats exposed to 60 Hz electric fields. Bioelectromagnetics 5:101-112.

Silverin, B., Massa, R. and K.A. Stokkan. 1993. Photoperiodic adaptation to breeding at different latitudes in Great tits. Gen. Comp. Endocrinol. 90:14-22.

Siopes, T.D. 1984. The effect of high and low intensity cool-white fluorescent lighting on the reproductive performance of Turkey breeder hens. Poult. Sci. 63:920-926.

Siopes, T.D. 1991. Light intensity effects on reproductive performance of Turkey breeder hens. Poult. Sci. 70:2049-2054.

Siopes, T.D. and M.E. El Halawani. 1989. Pineal and ocular influences on male and female turkeys: plasma luteinizing hormone and prolactin levels during gonadal development. Gen. Comp. Endocrinol. 73:284-292.

Skiles, D.D. 1985. The geomagnetic field: its nature, history, and biological relevance. In: J.L. Kirschvink, D.S. Jones and B.J. McFadden (eds.). Magnetite Biomineralization and Magnetoreception in Organisms. pp. 43-102. New York: Plenum.

Slagsvold, T., Sandvik, J., Rofstad, G., Lorentsen, Ö. and M. Husby. 1984. On the adaptive value of intraclutch egg-size variation in birds. Auk 101:685-697.

Smith, H.G., Ohlsson, T. and K.-J. Wettermark. 1995. Adaptive significance of egg size in the European starling: experimental tests. Ecology 76:1-7.

Sokal, R.R. and F.J. Rohlf. 1981. Biometry, 2nd edition. San Fransisco: W.H. Freeman & Company.

Sokal, R.R., and F.J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research, 3rd ed. San Francisco: W.H. Freeman and Company.

Soriano, F.M., Gonzalez, M.G., Armanazas, E. and A.R. Torner. 1992. Pineal 'synaptic ribbons' and serum melatonin levels in the rat following the pulse action of 52-Gs (50-Hz) magnetic fields: an evolutive analysis over 21 days. Acta Anat. 143:289-293.

Steenhof, K., Kochert, M.N. and J.A. Roppe. 1993. Nesting by raptors and common ravens on electrical transmission line towers. J. Wildl. Manage. 57:271-281.

Stehle, J., Reuss, S., Schroder, H., Henschel, M. and L. Vollrath. 1988. Magnetic field effects on pineal N-acetyltransferase activity and melatonin content in the gerbil: role of pigmentation and sex. Physiol. Behav. 44:91-94.

Sundberg, J. 1995. Female yellowhammers *Emberiza citrinella* prefer yellower males: a laboratory experiment. Behav. Ecol. Sociobiol. 37:275-282.

Turček, F.J. 1966. On plumage quantity in birds. Ekol. Polska, Ser. A. 14:617-633.

Turek, F.W., McMillan, J.P. and M. Menaker. 1976. Melatonin: effects on the circadian locomotor rhythm of sparrows. Science 194:1441-1443.

Ubeda, A., Leal, J., Trillo, M.A., Jimenez, M.A. and J.M.R. Delgado. 1983. Pulse shape of magnetic fields influences chick embryogenesis. J. Anat. 137:513-536.

Ubeda, A., Trillo, M.A., Chacon, L., Blanco, M.J. and J. Leal. 1994. Chick embryo development can be irreversibly altered by early exposure to weak extremely-low - frequency magnetic fields. Bioelectromagnetics 15:385-398.

Underwood, H., Binkley, S., Siopes, T. and K. Mosher. 1984. Melatonin rhythms in the eyes, pineal bodies, and blood of Japanese quail (*Coturnix coturnix japonica*). Gen. Comp. Endocrinol. 56:70-81.

Vakkuri, O., Rintamäki, H. and J. Leppäluoto. 1985. Plasma and tissue concentrations of melatonin after midnight light exposure and pinealectomy in the pigeon. J. Endocrinol. 105:263-268.

Van't Hof, T.J. and E. Gwinner. 1996. Development of post-hatching melatonin rhythm in zebra finches (*Poephila guttata*). Experientia 52:249-252.

Veicsteinas, A., Belleri, M., Cinquetti, A., Parolini, S., Barbato, G. and M.P. Molinari Tosatti. 1996. Development of chicken embryos exposed to an intermittent horizontal sinusoidal 50 Hz magnetic field. Bioelectromagnetics 17:411-424.

Village, A. 1990. The Kestrel. London: T&A D Poyser Ltd.

Villarroel, M.R. 1996. Copulatory behavior and paternity in solitary- and colonial-nesting kestrels. Ph.D. Dissertation, McGill University.

Viltala, J., Korpimäki, E. Palokangas, P. and M. Koivula. 1995. Attraction of kestrels to vole scent marks visible in ultraviolet light. Nature 373:425-427.

Vleck, C.M. and D. Vleck. 1996. Embryonic energetics. In: Cynthia Carey (ed.). Avian energetics and nutritional ecology. pp. 417-460. New York: Chapman & Hall, International Thomson Publishing.

Wada, M., Hatanaka, F., Tsuyoshi, H. and Y. Sonoda. 1990. Temperature modulation of photoperiodically induced LH secretion and its termination in Japanese quail (*Coturnix coturnix japonica*). Gen. Comp. Endocrinol. 80:465-472.

Walsberg, G.E. 1983. Ecological energetics: what are the questions? In: A.H. Brush and G.A. Clark, Jr. (eds.). Perspectives in ornithology. pp. 135-158. Cambridge: Cambridge University Press.

Walter, H. 1983. The raptor actigram: a general alphanumeric notation for raptor field data. J. Raptor Res. 17:1-8.

Webley, G.E., Mehl, H. and K.P. Willey. 1985. Validation of a sensitive direct assay for melatonin for investigation of circadian rhythms in different species. J. Endocrinol. 106:387-394.

Weller, M.L. 1956. A simple field candler for waterfowl eggs. J. Wildl. Manage. 20:111-113.

Wentworth, B.C. and R.K. Ringer. 1986. Thyroids. In: P.D. Sturkie (ed.). Avian Physiology,

4th edition. pp. 452-465. New York: Springer Verlag New York Inc.

Wiebe, K. L. and G.R. Bortolotti. 1995. Egg size and clutch size in the reproductive investment of American kestrels. J. Zool., Lond. 237:285-301.

Wiebe, K. L. and G.R. Bortolotti. 1996. The proximate effects of food supply on intraclutch egg-size variation in American kestrels. Can. J. Zool. 74:118-124.

Wiens, J. 1981. Single-sample surveys of communities: are the revealed patterns real? Am. Nat. 117:90-98.

Williams, J.B. 1996. Energetics of avian incubation. In: C. Carey (ed.) Avian energetics and nutritional ecology. pp. 375-416. New York: Chapman & Hall.

Williams, T.D. 1994. Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. Biol. Rev. 68:35-59.

Williams, T.D. and E.G. Cooch. 1996. Egg size, temperature and laying sequence: why do Snow Geese lay big eggs when it's cold? Funct. Ecol. 10:112-118.

Williams, T.D., Lank, D.B., Cooke, F. and R.F. Rockwell. 1993. Fitness consequences of egg-size variation in the lesser snow goose. Oecologia 96:331-338.

Willoughby, E.J. 1966. Wing and tail molt of the Sparrow Hawk. Auk 83:201-206.

Willoughby, E.J. and T.J. Cade. 1964. Breeding behaviour of the American Kestrel (Sparrow Hawk). Living Bird 3:75-96.

Wilson, F.E. 1990. On the recovery of photosensitivity in two passerine species, American Tree sparrows (*Spizella arborea*) and Harris' sparrows (*Zonotrichia querula*). Gen. Comp. Endocrinol. 79:283-290.

Wilson, F.E. 1991. Neither retinal nor pineal photoreceptors mediate photoperiodic control of seasonal reproduction in American Tree sparrows (*Spizella arborea*). J. Exp. Zool. 259:117-127.

Wilson, F.E. 1997. Photoperiodism in American tree sparrows: role of the thyroid gland. In: S. Harvey and R.J. Etches (eds.). pp. 159-169. Perspectives in Avian Endocrinology. Bristol: Society for Endocrinology.

Wilson, F.E. and B.D. Reinert. 1995. The photoperiodic control circuit in euthyroid American Tree sparrows (*Spizella arborea*) is already programmed for

photorefractoriness by week 4 under long days. J. Reprod. Fertil. 103:279-284.

Wiltschko, W. and R. Wiltschko. 1988. Magnetic orientation in birds. Curr. Ornithol. 5:67-121.

Wiltschko, W. and R. Wiltschko. 1991. Magnetic orientation and celestial cues in migratory orientation. EXS 60:16-37.

Wiltschko, W., Daum, P., Fergenbauer-Kimmel, A. and R. Wiltschko. 1987. The development of the star compass in Garden warblers, *Sylvia borin*. Ethology 74:285-292.

Wingfield, J.C., Hahn, T.P., Levin, R. and P. Honey. 1992. Environmental predictability and control of gonadal cycles in birds. J. Exp. Zool. 261:214-231.

Winjandts, H. 1984. Ecological energetics of the Long-eared Owl (*Asio otus*). Ardea 72:1-92.

Wolfson, A. 1942. Regulation of spring migration in Juncos. Condor 44:237-263.

Wolpaw, J.R., Segal, R.F. and R. Dowman. 1989. Chronic exposure of primates to 60-Hz electric and magnetic fields: 1. Exposure system and measurements of general health and performance. Bioelectromagnetics 10:277-288.

Woodroffe, G.E. 1953. An ecological study of the insects and mites in the nests of certain birds in Br. Bull. Entomol. Res. 44:739-772.

Yellon, S.M. 1994. Acute 60 Hz magnetic field exposure effects on the melatonin rhythm in the pineal gland and circulation of the adult Djungarian hamster. J. Pineal Res. 16:136-144.

Zar, J.H. 1984. Biostatistical analysis. New Jersey: Prentice-Hall.

Zeman, M., Výboh, P., Juráni, M., Lamošová, D., Koštal, L., Bilčík, B., Blažíček, P. and E. Jurániová. 1993. Effects of exogenous melatonin on some endocrine, behavioural and metabolic parameters in Japanese quail *Coturnix coturnix japonica*. Comp. Biochem. Physiol. A 105:323-328.