EFFECTS OF CAPTIVITY ON THE MORPHOLOGY, NESTING SUCCESS, AND GROWTH OF THE AMERICAN KESTREL (*FALCO SPARVERIUS*): IMPLICATIONS FOR CAPTIVE WILDLIFE MODELS AND REINTRODUCTION PROGRAMS

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ABSTRACT

With the increased use of captive breeding for species conservation and for wildlife research there is a rising concern over potential effects of captivity on animal populations. Changes arising within populations as a result of captivity could reduce their ability to survive and reproduce after release into the wild, or make research on captive specimens incomparable to their wild counterparts. Research into the effects of captive breeding is relatively new and is often hampered by the difficulty of separating origin and rearing environment effects on animals. The overall aim of this study was to determine the effects of captivity on the morphology, nesting success and nestling growth of a common wildlife model, the American kestrel (*Falco sparverius*). From 2006 to 2009 data were collected on wild and captive kestrel pairs, including adult size, egg volume, fertility, clutch initiation date, hatching success, and nestling survival. From 2007 to 2009, repeated measures of nestlings were taken to determine growth rates. To separate the effects of origin and rearing environment, whole clutches were cross-fostered between pairs in each environment and compared to control clutches. In 2009, captive-bred and wild kestrels reared in captivity (F1 birds) from the 2006 to 2008 cross-fostered clutches were paired in combinations (captive / captive, captive male / F1 female, F1 male / captive female, F1 / F1) and data were collected on adults, nesting success and nestling growth.

Results of the combined studies suggest that captive birds and eggs are significantly larger than those in the wild, and that F1 results are intermediate between the two. When comparing reproductive variables, wild clutches had significantly higher fertility and nestling survival than the other groups. Fertility and hatchability were similar between captive and F1 clutches, but nestling survival was greater in captive clutches. Overall, nest productivity was highest in wild pairs and lowest in F1 pairs. Reproductive variables were significantly affected by sex in F1 pairings, with F1 males exhibiting greater nest failure and lower productivity than captive males regardless of female mate. Nestling growth rates differed significantly between captive, wild and F1 birds with captive birds generally having faster growth rates, though asymptotic size did not differ between groups. Carotenoid concentrations in plasma did not vary greatly between groups, though external flesh color in kestrels differed between environment and sex; wild kestrels had significantly higher color scores than captive birds, and males had higher scores than females. Overall, rearing environment had a greater impact on adult kestrel size, nestling growth rates and nesting success than origin, though sufficient differences were noted between captive and F1 birds to suggest that some level of selection in captivity had occurred.

RÉSUMÉ

L'utilisation croissante de la reproduction en captivité pour les espèces en danger et pour l'étude de la faune suscite davantage d'intérêt quant aux effets potentiels de la captivité sur les populations animales. Des changements engendrés par la captivité pourraient réduire la capacité des animaux de survivre et se reproduire suite à leur réintroduction dans le milieu naturel, ou rendre les résultats d'études sur les spécimens captifs inapplicables à leurs homologues sauvages. L'étude des effets de la reproduction en captivité est relativement nouvelle et souvent entravée par la difficulté de distinguer les effets environnementaux des effets génétiques sur les animaux. L'objectif principal de la présente étude était de déterminer les effets de la captivité sur la morphologie, le succès reproductif et la croissance des oisillons chez une espèce-modèle commune, la crécerelle d'Amérique (*Falco sparverius*). De 2006 à 2009, des données ont été recueillies sur des couples de crécerelles sauvages et captives, dont la grosseur des adultes, le volume des œufs, la fertilité, la date d'initiation des couvées, le taux d'éclosion et la survie des oisillons. De 2007 à 2009, des mesures à répétition ont été prises pour évaluer les taux de croissance des oisillons. Afin de séparer les effets environnementaux des effets génétiques, des couvées entières ont été échangées entre couples dans chacun des milieux et comparées à des couvées de contrôle. En 2009, des individus nés en captivité ainsi que des individus sauvages élevés en captivité (génération F1) issus des couvées échangées de 2006 à 2008 ont été accouplés selon toutes les combinaisons possibles (mâle captif / femelle captive, mâle captif / femelle F1, mâle F1 / femelle captive, mâle F1 / femelle F1) et des données ont été recueillies sur les adultes, le succès reproductif et la croissance des oisillons.

L’ensemble des résultats suggère que les oiseaux captifs ainsi que leurs œufs sont significativement plus gros que leurs homologues sauvages, la génération F1 étant intermédiaire. Quant au succès reproductif, la fertilité des couples sauvages et la survie de leurs oisillons étaient supérieures aux autres groupes. La fertilité et le taux d’éclosion étaient semblables entre les couvées F1 et captives, bien que la survie des oisillons ait été supérieure chez ces dernières. La productivité globale était la plus élevée chez les couples sauvages et la plus faible chez les couples F1. Les mâles F1 avaient un taux d’échec de nidification plus élevé et une productivité globale moins élevée que les mâles captifs peu importe l’origine de la femelle. Des différences significatives dans les taux de croissance ont été observées entre oisillons captifs, sauvages et F1 : la croissance était généralement plus rapide chez les oisillons captifs malgré que la grosseur asymptotique ne variât pas entre les groupes. Les concentrations de caroténoïdes dans le plasma ne variaient pas de façon significative entre les groupes; cependant des différences dans le teint de la chair ont été observées selon le milieu et le sexe: les crécerelles sauvages avaient le teint plus foncé que les crécerelles captives; et les mâles plus foncé que les femelles. Dans l’ensemble, le milieu d’élevage a eu un impact plus prononcé sur la grosseur adulte des crécerelles, les taux de croissance des oisillons et le succès reproductif que l’origine génétique, bien que suffisamment de différences aient été constatées entre individus captifs et F1 pour suggérer qu’un certain niveau de sélection en captivité a eu lieu.

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This thesis is written in manuscript style in the formats of the journals Biological Conservation (Ch 1 – 3, 6), Zoo Biology (Ch 4), and Physiological and Biochemical Zoology (Ch 5). With the exception of the first (*General introduction*) and sixth chapters (*Summary and conclusions*) which were written by the primary author for this thesis, D.M. Bird and I.J. Ritchie served as co-authors for the manuscript chapters which will be submitted for publication. In addition, Marianne Gagnon was a co-author for chapter 5. The second (*Effects of captivity on adult morphology and nesting success in American kestrels* (Falco sparverius)) and third chapters (*Effects of captivity on nestling growth in American kestrels* (Falco sparverius)*: Using a cross-fostering design to separate the effects of origin and rearing environment in captive and wild nests*) will be submitted to Biological Conservation. The fourth chapter (*Comparisons of adult morphology, nest success and nestling growth between captive-bred, first-generation captive and wild American kestrels* (Falco sparverius)) will be submitted to Zoo Biology. The fifth chapter (*Plasma carotenoid concentrations and external color in captive-bred, first-generation captive and wild adult and nestling American kestrels*) is intended for the journal Physiological and Biochemical Zoology.

Each chapter to be submitted for publication was the result of a collaborative effort by the co-authors, and each made the following contributions to the completion of this research. D.M. Bird, thesis supervisor, served as advisor and provided direction for the project. He also contributed to the editing of the entire thesis. I.J. Ritchie provided expertise on the study species and on captive breeding methods, and provided advice on data collection and protocols for field and captive research. Marianne Gagnon worked with the primary author on all aspects of the laboratory analyses of plasma carotenoid samples.

STATEMENT OF ORIGINALITY

This thesis contains four manuscript chapters of which I am the first author, having collected and analysed all of the data and served as the primary writer and editor. This study fulfills the requirement of originality for the Doctor of Philosophy degree in the following respects:

1) This is the first study conducted for the express purpose of determining the effects of captivity on the morphology, nesting success, and nestling development of the American kestrel, or of any other raptorial species.
2) This is the first study to determine whether captive-bred American kestrels, a common wildlife model maintained in captivity for many generations, can adequately serve as representatives of wild-type specimens for research purposes.
3) This is the first study where a clutch cross-fostering design was used for research on the effects of origin and rearing environment on a raptorial species, and one of only a handful of studies to use this method to study the effects of captivity in birds.
4) This is the first study to compare coloration and plasma carotenoid concentrations between multiple-generation captive, first-generation captive, and wild adult and nestling birds.
5) This is the only comparison of captive and wild birds to have access to: 1) a captive population that descends from the same ancestral regional population as the wild birds examined in this study, removing any potential effects of regional differences between the birds, 2) a captive population of over 10 generations of documented captive ancestry without any intentional selection for particular

traits, and 3) first-generation captive birds reared in captivity from the egg-stage under identical conditions as captive-bred birds, allowing for easier separation of the effects of origin and rearing environment on results.

CHAPTER 1

GENERAL INTRODUCTION

1 Historical and current uses of captive breeding

Captive breeding can be defined as breeding and rearing an animal in a captive setting for some portion of the animal's life (Araki et al., 2008). Producing animals in captivity was largely adopted to satisfy human needs for transportation, labour, companionship, and food (Price, 1984). Within the last century, and particularly within the last few decades, captive breeding has also been used to 1) enhance knowledge in wildlife research by using captive populations of wild-type animals as models (Honess et al., 2010), and 2) as a means of conserving and restoring declining species to the wild (Araki et al., 2007).

Historically, captive breeding was aimed at producing animals whose qualities would better serve human needs with little concern for how they differed from their wild counterparts (Price, 1984). However, animals that serve as wildlife models and those bred for eventual reintroduction into the wild must retain as much as possible their original wild state if they are to serve their intended purposes (Price, 1984; Künzl et al., 2003). The maintenance of wild phenotypes after generations of captive breeding can be difficult, and as a result, the study of the impact of artificial environments on animals has become more prominent in recent decades, though little research has been conducted on captive-bred animals not intensively selected for laboratory research or commercial use (Wisely et al., 2005).

Most studies on the effects of captive breeding to date have centered on animal behaviour and genetic diversity (e.g. McPhee, 2003; Mathews et al., 2005; Ivy et al., 2009; Jones et al., 2010; Lesobre et al., 2010; Montgomery et al., 2010). Studies of possible changes in morphology or physiology are less common, in part because it is often assumed that any differences noted between captive animals and their wild counterparts are the result of abnormal development in a captive setting that would not occur otherwise (O'Regan and Kitchener, 2005). They remain important factors to animal welfare and survival however, as seen in a recent comparison of the genetic diversity between wild and captive populations of the threatened lesser kestrel (*Falco naumanni*). The study revealed that while captive and wild populations shared a comparable amount of genetic diversity, successful breeding in captivity was unevenly distributed among pairs (Alcaide et al., 2010). With young for reintroduction being produced by a limited number of the total captive pairs, this could lead to an eventual decrease in genetic diversity in the wild upon their release and integration into the natural population.

A recent study using beach mice (*Peromyscus polionotus subgriseus*) to test a model estimating release numbers for reintroduction programs included as variables not only genetic diversity and population sizes but also behaviour and morphology (McPhee and Silverman, 2004). Based on that study and reviews of reintroduction programs, a recommendation was made that additional data, including nesting success, behavioural and foraging skills, life span, and causes of mortality, should be gathered in addition to traditional survival data when planning reintroduction programs, as survival alone does not guarantee a successful restoration of a wild population (McPhee and Silverman, 2004; Jule et al., 2008).

As captive breeding of wild-type animals becomes more common, increasing demand is being made for research on the effects of captivity on morphology, physiology, behaviour, and genetic diversity in order to create successful captive management programs (Armstrong and Seddon, 2007; Blanchet et al., 2008). Much of the existing documentation on captivity is published in retrospect as part of existing breeding programs (Armstrong and Seddon, 2007). While these results are useful, objective-driven research into the effects of captivity is necessary, as it can identify factors in captive regimes that need to be changed to create more successful breeding programs (Armstrong and Seddon, 2007).

2 Difficulties in captive research

2.1 Comparisons between captive and wild populations

Research into the effects of captive breeding is difficult for a number of reasons, one of which is that captive housing and management techniques vary from one facility to another, even within the same species (McPhee, 2003; Araki et al., 2008). A comparison of laboratory zebra finches (*Taeniopygia guttata*) from three continents and wild Australian populations revealed significant differences in both allelic richness and body size between all four finch populations (Forstmeier et al., 2007).

Another difficulty in studying the effects of captivity is that comparisons between captive populations and their wild counterparts is not always possible; wild source populations can be extinct, or significant regional variations between populations in the wild can make direct comparisons difficult, particularly if captive animals descend from an amalgamation of these populations (McPhee, 2003). Subpopulations of the same species have unique adaptations to the particular environments from which they descend, and in a captive setting this can result in variations in body size and behaviours between individuals, as well as in differences in breeding times. The latter was seen in a captive population of blue tits (*Parus caeruleus*), where clutch initiation times for captive pairs under natural lighting conditions corresponded to breeding times for each pairs' subpopulation of origin (Lambrechts et al., 1999). Difficulty with captive colonies composed of individuals from different subpopulations can also arise from attempts to interbreed individuals; crossed pairs within the blue tit colony suffered almost complete reproductive failure compared to control birds paired with individuals from their own subpopulation (Caro et al., 2007). Individual subpopulations can respond differently to captivity, even when housed under the same conditions (e.g. Lambrechts et al., 1999; Caro et al., 2007).

Finally, it can be difficult to distinguish between environmental effects of captivity that arise within an individual's lifetime, and genetic changes that occur as a result of selection for particular phenotypes over successive generations of captive breeding (Price, 1999; Künzl et al., 2003).

2.2 Environmental effects on captive populations

In a captive environment, most of an animal's experiences, reproductive variables, social interactions and living conditions are dictated by humans (Künzl and Sachser, 1999; Price, 1999). This can affect an individual's nesting success, morphology, health, behaviour and ability to learn. Black-footed ferrets (*Mustela nigripes*) bred in captivity for 6 to 7 generations as part of a species recovery program were found to have significantly smaller cranial and body measurements, and reduced sexual size dimorphism than historical museum specimens of wild ferrets (Wisely et al., 2002; 2005). Once introduced into the wild, offspring of the reintroduced ferrets grew to previous ancestral sizes, suggesting that variations noted in captive animals were the result of captive diets or living space that did not allow captive ferrets to develop their dental and bone structures as they would have normally in the wild (Wisely et al., 2005).

2.3 Genetic changes that arise in captivity

Three factors are necessary for a trait to evolve: genetic variance with phenotypic expression, the inheritance of those phenotypic traits, and the effect those traits have on fitness (McDougall et al., 2006). Strong selective pressures, such as removing animals from their natural environment and introducing them to a captive setting, can result in rapid change within a population (McDougall et al., 2006). Three major types of selection occur in a captive environment: natural selection, where phenotypic expressions change as a result of selection pressures in an environment; relaxed natural selection, as a result of reduced selection pressures (e.g. lack of predation) in captivity; and artificial selection, unique to captivity, whereby selection is influenced by humans through intentional or unintentional selection for particular traits (Künzl and Sachser, 1999; Price, 1999; McPhee, 2003; McPhee and Silverman, 2004). Unintentional selection can result simply from the refusal to breed the most aggressive members of a captive population due to handling difficulties, or from selecting a particular cage design over another, and thus can be difficult to prevent (McDougall et al., 2006).

In captivity, particularly in conservation programs where maintaining as many wild genes as possible is a goal, the reproductive output of all individuals is equalized as much as possible through the use of studbooks and artificial insemination (McDougall et al., 2006). Pairings that would not result naturally in the wild can occur in captivity as a result of choices made by human caretakers as opposed to allowing for mate choice to occur. Random events such as inbreeding, gene-linking, or genetic drift due to small founder population size can also result in changes (Belyaev et al., 1985; Price, 1999; Trut et al., 2004; O'Regan and Kitchener, 2005; Jensen, 2006; McDougall et al., 2006).

The number of generations in captivity, as well as the extent of the differences between wild and captive environments, can increase the degree of selection that occurs in captivity (Price, 1999). Individual species also adapt to captive environments at different rates. Breeding wild cavies (*Cavia aperea*), the ancestor of the domestic guinea pig (*C. a.* f. *porcellus*), in captivity for 30 generations without intentional selection for particular traits was not sufficient time to produce animals significantly different from wild cavies, though wild cavies and cavies recently introduced into captivity differed significantly from guinea pigs in behaviour, body size and stress hormone levels (Künzl and Sachser, 1999; Künzl et al., 2003). By comparison, endangered beach mice showed reduced predator avoidance and greater variability in behaviour and mandible size after 14 and 35 generations of captive breeding, respectively (McPhee, 2003; McPhee and Silverman, 2004).

A number of reoccurring traits have been noted in captive populations, especially those maintained in captivity over many generations (Price, 1999; Künzl et al., 2003; Jensen, 2006). They include earlier development, more rapid growth, retention of juvenile characteristics, earlier sexual maturity, more frequent reproductive cycles, and a decrease in adrenal size (Price, 1999). Changes in body size or color, reduction in sexual dimorphism, and decreases in fear response, awareness of surroundings, and activity levels are also common (Price, 1984; Künzl and Sachser, 1999; Künzl et al., 2003; O'Regan and Kitchener, 2005; Jensen, 2006). These traits might be the result of convergent evolution or unintentional selection for the same traits by humans (Robison and Rowland, 2005).

3 Applications of animals in captivity

3.1 Research using wild-type animal models

Wildlife biologists study populations of wild animals to gain fundamental knowledge about ecology, evolution, physiology and life history traits (Lambrechts et al., 1999; Love et al., 2003). Access to captive populations can enhance their ability to acquire information by allowing them to test hypotheses in a controlled environment where results will not be confounded by other, unknown factors (Lambrechts et al., 1999; Caro et al., 2007). Captive breeding has been considered a solution to a variety of problems associated with disturbing or removing wild animals from their natural environment for research. Wild animals newly introduced to a captive setting can suffer ill effects such as stress and reduced breeding success compared to animals that have been held in captivity for several generations (Honess et al., 2010). Captive animals have clearly documented genealogical and clinical histories which can be useful both for research and for breeding purposes. Breeding animals in captivity can reduce impacts upon wild populations by decreasing the need for repeated harvesting for research (Santos et al., 2009; Honess et al., 2010).

Several factors make certain species good general models to explore broad ecological or behavioural concepts. Short generation times, the ability to produce offspring in high numbers or frequently, little parental care or learned abilities, and small size makes certain species simple and cost-effective to house in numbers that would allow for: 1) higher genetic variability in captivity, 2) statistically significant results in experimental tests and 3) studies of a population at multi-generational scales (McPhee, 2003; Griffiths and Pavajeau, 2008; Santos et al., 2009).

When using captive populations as models for wildlife research, it is imperative to determine what differences, if any, arise in the captive population as a result of environmental or genetic effects that might compromise the extrapolation of results to specimens living in wild environments. A recently published study on the effects of captivity on an avian model, the zebra finch, has revealed the development of significant differences between wild and captive specimens that should be taken into account when extrapolating results from captive studies to wild birds (Mainwaring et al., 2010). Zebra finches have been used in captive research for over four decades (Griffith and Buchanan, 2010). In comparisons of captive and wild nestling growth, captive birds exhibited larger asymptotic mass and size (Mainwaring et al., 2010). Captive adult finches were also up to 50 % larger than their wild counterparts, and exhibited larger hatching asynchrony than wild birds (Mainwaring et al., 2010). Significant differences were also noted in egg and nestling size between clutches produced by captive birds and wild adults brought into captivity, though the nestlings were reared under identical captive conditions (Tschirren et al., 2009).

3.2 Captive breeding in conservation and species management

Today, over 5000 known species of animals are threatened with extinction; extinction rates are two to three times higher than background rates estimated over geological history, and increasing (Nicoll et al., 2004; Cockrem, 2005). The use of captive-bred animals for reintroduction programs, or for the maintenance of populations of endangered animals in captivity until suitable environments become available in which to release them back into the wild, is becoming a very important tool in many endangered species recovery programs (White et al., 2005; Connolly and Cree, 2008). The goal of captive breeding for reintroduction is to produce animals as similar to their wild counterparts as possible, retaining all the skills necessary to survive and breed successfully in the wild (McDougall et al., 2006). Captive breeding of endangered species often means that little or no wild population remains for direct comparison to captive-bred animals, and thus it may be difficult to determine if and to what extent the reintroduced animals have been altered as a result of generations of breeding in captivity (Nicoll et al., 2004).

Currently, reintroduction programs using captive-bred animals are successful less than 50 % of the time, and are less than half as successful as programs using wild animals translocated to new regions (Nicoll et al., 2004; Brown et al., 2006; McDougall et al., 2006; Jule et al., 2008). Loss of traits such as predator avoidance, sociopositive behaviour, and the ability to forage are common causes of failure in reintroduction programs using captive animals (McPhee and Silverman, 2004; Jule et al., 2008). Originally, captive management programs for threatened or endangered species existed strictly to reintroduce animals into the wild, not making use of the opportunity to design testable hypotheses to gain information that could be used to improve future efforts (Seddon et al., 2007). Today, with the more frequent use of captive-bred animals in reintroductions, greater efforts are being made to understand more about the genetic, behavioural and habitat requirements necessary to produce animals that will successfully survive reintroduction (Seddon et al., 2007). For example, research into hacking methods of peregrine falcons (*F. peregrinus*) demonstrated that males had higher success rates when released at a younger age, while the opposite was true for females, because male falcons typically develop more quickly than females (Dzialak et al., 2006).

Conservation biologists are resorting to using model species that are ecologically, morphologically and behaviourally similar to endangered species but not threatened themselves, to create more effective captive breeding and release techniques (Whitmore and Marzluff, 1998; Nicoll et al., 2004). In Hawaii, a study was initiated using the ‘oma’o (*Myadestes obscurus*), the only species of its genus not critically endangered, as a model for a captive breeding and release program for the endangered puaiohi (*M. palmeri*; Kueller et al., 2000). The Hispaniolan parrot (*Amazona ventralis*) was used to establish breeding and pre-release training protocols for the reintroduction of its close relative, the endangered Puerto Rican parrot (*A. vittata*), a strategy that reduced post-release mortality (Collazo et al., 2003; White et al., 2005). Common ravens (*Corvus corax*), American crows (*C. brachyrynchos*) and black-billed magpies (*Pica hudsonica*) served as models to create captive breeding protocols for endangered corvids such as the Hawaiian crow (*C. hawaiiensis*) and the Mariana crow (*C. kubaryi*; Whitmore and Marzluff, 1998). Andean condors (*Vultur gryphus*) were studied to test pre-release and release programs for the endangered California condor (*Gymnogyps californianus*; Wallace and Temple, 1987). Guppies (*Poecilia reticulata*) were used to document the effects of various captive breeding regimes on morphology and survivability upon release into the wild (van Oosterhout et al., 2007). Zebra danios (*Danio rerio*) were employed for similar tests (Robison and Rowland, 2005). Lacertid lizards (*Psammodromus algirus*) were selected to test the effectiveness of a reintroduction program for small, short-generation species with little need for parental care (Santos et al. 2009). Bank voles (*Myodes glareolus*) were chosen to devise behavioural tests to determine a captive individual's suitability for release into the wild because of their relation to species such as the water vole (*Arvicola terrestris*) and dormouse (*Muscardinus avellanarius*), currently undergoing reintroduction procedures (Mathews et al., 2005).

4 Using a cross-fostering design to separate effects of origin and rearing environment

Fostering in birds, especially fostering eggs, is a useful tool for separating the effects of pre-and post-hatching variables on nestling growth (Magrath, 1992). Thus, if one is attempting to separate the effects of origin and rearing environment on nestling growth, fostering clutches of eggs of different origins into opposite rearing environments has the potential to help determine which factor has the greatest effect on nestling growth. Cross-fostering studies in birds are often conducted on large populations of synchronously nesting, colonial seabirds that provide biparental care (e.g. Reid and Boersma, 1990; Bolton, 1991; Meathrel et al., 1993; Amundsen, 1995; Amundsen et al., 1996; Hipfner and Gaston, 1999; Hipfner, 2000; Risch and Rohwer, 2000; Van de Pol et al., 2006; Silva et al., 2007). Exceptions using other avian families are rare and typically involve species with dense breeding populations, including alpine swifts (*Apus melba*; Bize et al., 2002), European starlings (Smith et al., 1995), red-winged blackbirds (*Agelaius phoencieus*; Reed et al., 1999), lapwings (*Vanellus vanellus*; Blomqvist et al., 1997), house wrens (*Troglodytes aedon*; Styrsky et al., 1999) and blackbirds (*Turdus merula*; Magrath, 1992).

Cross-fostering designs are most commonly used in studies attempting to separate the effects of parental or habitat quality and egg size on nestling growth and survival (Blomqvist et al., 1997). Most research designs involve fostering whole clutches, and all clutches are fostered in a study to remove potential differences that might arise between young reared by biological or foster parents. Exceptions do exist where only experimental clutches were fostered and control clutches were reared by biological parents (e.g. Reid and Boersma, 1990; Blomqvist et al., 1997; Silva et al., 2007).

5 The American kestrel as a wildlife model

The American kestrel (*F. sparverius*) is the smallest New World falcon, and is the most numerous and most widely distributed across the continent (Smallwood and Bird, 2002). The habitat of kestrels consists primarily of open terrain with short ground vegetation such as grasslands, pastures, fallow fields, burn-over areas, drained wetlands or deserts (Balgooyen, 1976; Smallwood and Bird, 2002). They can also be found in rural and urbanized habitat and appear to have adapted well to the presence of humans. Kestrels are sexually dimorphic and dichromatic; males are smaller than females and are most obviously distinguished from females by possessing blue-grey instead of rufous wings (Smallwood and Bird, 2002). Kestrels feed primarily on arthropods and small vertebrates including rodents and birds (Smallwood and Bird, 2002).

Kestrels are secondary cavity-nesters that make use of abandoned woodpecker holes, natural cavities, and suitable spaces in man-made structures for nests (Balgooyen, 1976; Smallwood and Bird, 2002). Nest boxes are also readily used by kestrels and have made nesting kestrels much more accessible to field researchers. As a result, the kestrel is a well-studied species (Wiemeyer and Lincer, 1987; Smallwood and Bird, 2002; Katzner et al., 2005; Smallwood and Collopy, 2009). Kestrels appear to have a relatively high tolerance for disturbance compared to other raptor species, making them desirable research subjects (Wiemeyer and Lincer, 1987).

Kestrels are able to breed in their first year and typically reproduce annually (Smallwood and Bird, 2002). Monogamous pairs are formed for a breeding season, and females typically lay five eggs per clutch, with one egg produced every second day (Smallwood and Bird, 2002). Incubation begins on the laying of the penultimate egg and lasts 26 to 32 days (Smallwood and Bird, 2002). The clutch hatches over a period of 2 to 3 days, and hatched young are semi-altricial (Balgooyen, 1976; Smallwood and Bird, 2002). The male provisions the female with food during the incubation period, and later both female and nestlings during brooding period, after which both adults provide food to the young (Smallwood and Bird, 2002). Growth rate of young increases during the first couple of days of life, and becomes almost linear until 20 days of age, at which point the asymptotic mass is reached (Smallwood and Bird, 2002). Young can be sexed by plumage colour at 12 to 14 days of age, and nestlings fledge at 28 to 31 days of age (Smallwood and Bird, 2002). Cannibalism of nestlings by parents, especially when small, is not uncommon in both wild and captive birds and is thought to be associated with stress (Bortolotti et al., 1991). Mortality rate of birds in their first year appears to be fairly high, decreasing after the birds survive into their second year in the wild (Smallwood and Bird, 2002).

Kestrels breed readily in captivity with a success rate comparable to wild pairs (Willoughby and Cade, 1964; Porter and Wiemeyer, 1970; Smallwood and Bird, 2002). Their nesting success rate, coupled with their annual reproductive cycle, their small size, their ability to be housed in same-sex groups and therefore in much larger numbers for a lower cost than other, larger raptor species, their close association with rural, urban and agricultural environments, and their close relationship to raptor species that are susceptible to environmental contaminants has made them an ideal species for captive study as a wildlife model, especially in environmental toxicology research (Porter and Wiemeyer, 1970; Wiemeyer and Lincer, 1987; Bardo and Bird, 2009).

Captive kestrels have been used as wildlife research models for decades. The value of captive kestrels as research models became evident after studies conducted on captive kestrels confirmed that 1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene (DDE), a metabolite of 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane (DDT), could cause eggshell thinning, reproductive failure and mortality in birds (Wiemeyer and Porter, 1970). Kestrels served as models to further conservation efforts of the peregrine falcon and other raptor species when their populations declined drastically due to the bioaccumulation of DDT and DDE in the environment (Bird, 1982; Wiemeyer and Lincer, 1987). Kestrels have been and continue to be used to conduct research on avian physiology, reproductive biology, behaviour, conservation management and environmental toxicology (see review by Bardo and Bird, 2009).

Recent analyses of migration counts, Breeding Bird Surveys (BBS) and Christmas Bird Counts (CBC) indicate that kestrel numbers have been steadily declining in north-eastern North America since 1974, with more recent declines in western North America and parts of the Gulf of Mexico (Bird, 2009; Farmer and Smith, 2009). In the easternmost watch sites, declines of over 50% in kestrel populations have been recorded in the last 30 years (Farmer and Smith, 2009). Numerous and as yet untested hypotheses have been proposed to explain these declines, including continued exposure to environmental DDT after the contaminant was banned (Wiemeyer and Porter, 1970; Farmer and Smith, 2009), exposure to other pesticides (Farmer and Smith, 2009), West Nile Virus (Nemeth et al., 2006; Medica et al., 2007; Farmer and Smith, 2009), predation by increasing populations of Cooper's hawks (*Accipiter cooperii*), a prolonged drought in western North America (Farmer and Smith, 2009), climate change (Smallwood et al., 2009), loss of foraging and breeding habitat to reforestation and human developments (Smallwood and Bird, 2002; Farmer and Smith, 2009), and competitors for nest cavities such as European starlings (*Sturnus vulgaris*) and screech owls (*Otus asio*; Balgooyen, 1976). Regardless of the cause(s), the declines are beginning to raise serious conservation concerns for the future of the American kestrel.

6 Research Aim and Objectives

With the increase in the number of threatened and endangered species in the world and the increased interest in conducting research on captive populations of wild-type animals, greater attention on the potential effects of captivity on animal populations is needed (Cockrem, 2005; Araki, 2007; Honess et al., 2010). More data on the effects of captive breeding on morphology, nesting success, development, and physiology are needed before captive management plans can be adjusted to maintain as much as possible a population's original wild state, either for the purposes of conservation programs or for captive wildlife research.

The overall aim of this study is to examine the effects of captivity on the morphology, nesting success and growth of a non-domestic avian species using the

American kestrel, a common wildlife research model, as a study subject. The American kestrel represents an ideal model for the following reasons:

1) Though kestrels have served as models for over 40 years of toxicological, behavioural, and reproductive research, the potential effects of captivity on the results of these studies have never been determined, and as such the continued use of captive kestrels as representative of wild-type birds should be validated.

2) Various raptor species are threatened by human activities and raptors are frequently the subjects of conservation programs; in a recent analysis of bias in reintroduction programs, raptors were found to be disproportionately represented in conservation efforts and captive breeding programs compared to other bird species (Katzner et al., 2005; Seddon et al., 2005). This would also represent the first study of the effects of long-term captive breeding on a raptor species.

3) From 1973 to 2011, the Avian Science and Conservation Centre (ASCC) housed a colony ranging annually in size from 200 to 500 pedigreed kestrels with between 10 and 20 generations of captive ancestry without intentional selection for particular traits. The birds in the colony all descended from the local wild population with which they were compared in the study, reducing risk of potential regional variation between the birds.

The specific objectives of the study were as follows. The first objective was to compare adult morphology and nesting success between captive and wild birds using a cross-fostering design intended to separate the effects of origin and rearing environment on nest success (Ch 2). The second objective was to study the effects of origin and rearing environment on nestling development (Ch 3). The third objective was to compare the nesting success and nestling development of captive-bred kestrels and wild kestrels reared in captivity from the egg-stage as part of the cross-fostering experiment (F1 birds). Adults in this component were reared under identical conditions in captivity (Ch 4). The purpose of this latter objective was to determine how F1 birds performed in captivity compared to captive-bred birds, and whether captivity affected one sex more than the other by cross-pairing captive-bred and F1 birds. The results of captive, wild and F1 birds were then compared together to determine if effects of rearing environment (implying captive-bred and F1 birds shared more similarities) or origin (implying F1 and wild birds were more similar) were more important to adult morphology, nesting success and nestling development. The final objective of the study was to compare external coloration and plasma carotenoid concentrations in adult and nestling captive, F1, and wild birds, as carotenoid pigments are known to be important to animal health and the expression of color in birds can be used to signal condition and to assess potential mates during mate selection (Bortolotti et al., 1996). Since mate selection does not occur in captivity, it is possible that the display of carotenoids for signaling quality may have changed as a result of relaxed selection.

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CONNECTING STATEMENT 1

Research into the effects of captive breeding on animal populations is still relatively new, and to date has focussed primarily on behaviour and genetic diversity. Recently it has been suggested that a more complete analysis of captive animals, encompassing traits such as their morphology, development, and nesting success, might provide greater insight into 1) the effects of captivity, and 2) where the focus should be placed in captive programs to reduce those effects. The American kestrel is a commonly used captive wildlife model, but to date no study has been conducted to determine whether multiple generations of captive breeding has rendered captive specimens significantly different and thus incomparable to their wild counterparts. To study the effects of captive breeding on kestrels, breeding pairs in the wild and in captivity were compared from 2006 to 2009 inclusive, and a cross-fostering design involving the switching of whole clutches between pairs in the two environments, was used to separate the effects of rearing environment and of origin of the nest. The following chapter presents the results of the comparisons between captive and wild adults and nesting success.

CHAPTER 2

EFFECTS OF CAPTIVITY ON ADULT MORPHOLOGY AND NESTING SUCCESS IN AMERICAN KESTRELS (*FALCO SPARVERIUS*)

Abstract

Captive breeding can provide a source of animals for conservation programs and for captive wildlife research. Goals for captive programs should include the successful reproduction of captive animals and the preservation of the wild phenotype as much as possible. Major concerns associated with captive breeding include possible changes in morphology, survival or nesting success that could render a population unfit to survive reintroduction or unsuitable for research purposes. Studies evaluating the effects of captive breeding through comparison with wild populations are rare. The American kestrel was used to study the effects of captive breeding on adult morphology and nesting success using a cross-fostering experimental design intended to separate the effects of origin and rearing environment on nesting success. Results suggested that kestrels in captivity had larger body sizes and reduced reverse sexual size dimorphism. Trends in adult mass throughout the breeding season also differed between wild and captive birds, particularly for females, and this had an impact on egg volume, clutch fertility and fledging success. Models indicated that captive birds produced larger eggs and clutches with reduced fertility and nestling survival compared to wild birds, regardless in which environment the captive clutches were reared.

Keywords: captive breeding, American kestrel, *Falco sparverius*, cross-fostering, morphology, nesting success

1 Introduction

Captive breeding can be used for wildlife conservation to produce animals for reintroduction into the wild, and for wildlife research to provide specimens which can be used to answer fundamental biological questions in a controlled environment without having impacts upon existing wild populations (Araki et al., 2007; Connolly and Cree, 2008; Honess et al. 2010). In such cases where animals reared in captivity are meant for release into the wild or to serve as representatives of wild populations, captive management programs should strive to maintain as much as possible the wild phenotype of captive populations (Connolly and Cree, 2008). Major concerns associated with captive breeding include the possible changes in behaviour, morphology, physiology, and genetic diversity as a result of captivity that could render a population unfit to survive successful reintroduction into the wild or incomparable to wild populations for research purposes (Price, 1999; Künzl et al., 2003; McDougall et al., 2006; Connolly and Cree, 2008).

As captive breeding for conservation and research becomes more common, there is a greater need for more objective-driven research into the effects of captivity on animal populations to create successful management programs (Armstrong and Seddon, 2007; Honess et al., 2010). Research on the effects of captivity on animals is relatively new and complicated by the difficulty of separating the environmental effects from genetic changes that may have resulted from selection over generations in captivity (Price, 1999; Künzl et al., 2003). Environmental factors such as housing, diet and stress can affect body size (e.g. Wisely et al., 2002; 2005), initiation of reproduction, and nesting success (e.g. Lambrechts et al., 1999; Caro et al., 2007) in captive individuals. Changes in heritable traits such as body size can also be affected by selection within a captive population (e.g. Künzl and Sachser, 1999; Künzl et al., 2003; McPhee, 2003; McPhee and Silverman 2004; Tschirren et al., 2009). The rate of these changes can be affected by species, number of generations in captivity, the extent of the differences between captive and wild environments, and selection by humans.

One method of separating the effects of origin and rearing environment would be to remove young from one environment and rear them in another. Any resulting differences in young from a common ancestry reared under separate conditions should be the result of environmental effects, and shared traits between them the result of their genetic origin (Blondel et al., 1990). The younger the animals during the transfer, the less time there will have been for parental or environmental factors to affect them before the transfer is made (Blondel et al., 1990). This method is particularly useful in birds, where eggs can be transferred between environments, providing a clear separation between pre- and post-hatching variables on nestling survival and overall nest success (Magrath, 1992). Cross-fostering of whole clutches of eggs between pairs has been used to separate the effects of egg size and adult or environmental quality on nest success (e.g. Magrath, 1992; Amundsen, 1995; Reed et al., 1999; Hipfner, 2000; Risch and Rohwer, 2000; Silva et al., 2007).

The aim of this study was to investigate the effects of captive breeding on adult morphology and nesting success in birds using the American kestrel (*Falco sparverius*) as a model. A cross-fostering experiment was conducted, transferring whole clutches between pairs of captive and wild birds and comparing results to control clutches reared in their own environments to determine if environmental or origin effects were more important to nestling survival or overall nest success.

The American kestrel is a small New World falcon that has been used in recent decades as a captive model species, particularly for research in reproductive biology, physiology and environmental toxicology (Smallwood and Bird, 2002; Bardo and Bird, 2009). Nest boxes are readily used by pairs of wild nesting kestrels, making them more accessible to researchers, and as a result the kestrel is a well-studied species (Smallwood and Bird, 2002; Katzner et al., 2005). The Avian Science and Conservation Centre (ASCC) of McGill University in Montreal, Quebec, Canada has housed a colony of captive American kestrels since 1973 (Bird, 1982). All birds in the colony have a documented pedigree and descend from stock bred in captivity for 10 or more generations. Captive birds descend from the same local Montreal population as the wild birds in this study, removing any potential differences that might have resulted from comparing birds from different geographic regions (e.g. Lambrechts et al., 1999; Caro et al., 2007).

2 Materials and methods

2.1 Wild nests

Data collection for this project was conducted in spring and summer from 2006 to 2009 inclusive. Starting the first week of April, nest boxes (0.3 x 0.3 x 0.4 m L x W x H) mounted on Hydro poles on the island of Montreal, Quebec, Canada and in the surrounding region (45˚ 01’ to 46˚ 02’ latitude north, 72˚ 44’ to 74˚ 25’ longitude west) were checked once every 5 to 7 days for the presence of nesting American kestrels. The number of boxes available to the birds ranged from 55 to 65 over the 4 years; all boxes contained wood shavings as a substrate for the eggs.

American kestrels typically lay 5 eggs per clutch, with one egg laid every two days (Sockman and Schwabl, 2001). Clutch initiation date could therefore be estimated for nests found in the egg-laying stage. Since kestrels can occasionally lay eggs at 1 or 3-day intervals and nests were not visited daily, it is possible to miscalculate clutch initiation by 1 or 2 days (Sockman and Schwabl, 2001). Clutch initiation date was used to estimate mid-incubation (14 days after the start of incubation), the point at which adult and clutch fertility measurements were taken, and hatch dates; incubation typically begins on the penultimate egg and lasts for approximately 28 days (Smallwood and Bird, 2002).

2.2 Captive work

Nine captive pairs (11 in 2009) per year were selected based on the following criteria: all pairs were unrelated for at least 5 generations and all birds used in the study had pedigrees with a minimum of 10 generations of captive breeding. No effort was made to pair birds of matching age or size, and any incompatible pairs were removed from the study and separated. Pairs were housed in visually isolated, individual pens (2.5 x 1.5 x 2.5 m L x W x H) with rope perches, a food platform, wood-shaving substrate, a screened window for natural lighting and air circulation and a nest box with the same dimensions as wild boxes filled with wood shavings. Pairs were each fed 3 to 4 frozen-thawed, day-old cockerels every day through a food door to prevent excessive disturbance. The condition of the birds could be monitored through an observation window constructed from one-way mirror glass. Captive birds were paired within days of the first sighting of a pair of wild kestrels at a nest box in an attempt to match nesting periods for comparison. Captive nest boxes were checked daily for eggs during regular morning feeding to reduce disturbance (Fernie et al., 2001). Mid-incubation and hatch date estimates were made for captive pairs once egg-laying had commenced.

2.3 Experimental methods

All measurements were taken by the primary author to reduce observer bias. At mid-incubation all nests were visited and the following data collected. The female was removed from the nest by hand. If the male was found incubating instead, data were collected on the male and the nest was revisited in the following days to capture the female. In captivity, the male was measured and sampled at the same time as the female. The incubating adult was placed into an aerated transport box while egg measurements were taken.

Eggs were removed from the nest and stored in an insulated carrier for the duration of the measurements. Eggs were marked with a non-toxic marker for identification, and length and breadth of each egg were measured using vernier callipers (accuracy 0.1 mm). The eggs were then candled to determine fertility (Fernie et al., 2001). In captivity an electric candler was used in a darkened room. For wild nests, the electric candler was plugged into an adapter in the field vehicle and the vehicle's windows were covered with opaque black plastic sheets to create a dark room. Eggs were said to be fertile if an embryo (defined as a dark shadow in the egg) and/or blood vessels were visible in the egg; it was deemed infertile if the egg was clear after 2 weeks of incubation or if the egg was cracked or punctured (Lokemoen and Koford, 1996), which only occurred in 3 eggs. The eggs were then returned to the nest within 10 min of removal.

The adult was then removed from the transport box, wild birds were banded with a US Fish and Wildlife size 3B aluminum band on the right leg, and vernier callipers were used to measure the length of the tarsus and head-bill length of all adults to the nearest 0.1 mm (Blomqvist et al., 1997; McDonald et al., 2005). Wing chord (from carpus to the tip of the longest feather, the 9th primary in kestrels; Smallwood and Bird, 2002) was measured using a ruler to the nearest mm (Hipfner and Gaston, 1999). All measurements were taken on the right side of the body (Oldendorff, 1972). Mass was measured using a Pesola spring-balance accurate to the nearest gram. The adult was then released in the vicinity of the nest in the wild or returned to its breeding pen to resume incubation. In the wild, if males were not captured during mid-incubation, bal-chatri traps with live mice (*Mus musculus*) or mist nets with a live great-horned owl (*Bubo virginianus*) lure were used to capture them during the nestling period to reduce disturbance to the pair during incubation (Steenhof et al., 1994; McDonald et al., 2005). For comparison, several captive males were recaptured and re-measured during the nestling stage.

Ideally in a cross-fostering experiment all clutches are removed from their biological parents and reared by foster parents to eliminate a potential bias, both within the control and experimental groups (Risch and Rohwer, 2000; Silva et al., 2007). Most cross-fostering experiments to date have been conducted on species that nest in concentrated or colonial breeding populations, providing sufficiently large populations with matching hatch dates to accomplish this (e.g. Magrath, 1992; Hipfner and Gaston, 1999; Reed et al., 1999; Risch and Rohwer, 2000). Kestrels are territorial birds that have been noted in densities ranging from less than one to over twenty breeding pairs per square kilometer (Smallwood and Bird, 2002); in this study no more than 15 pairs were ever studied in a given season. As a result only experimental (clutches reared in an environment different from their environment of origin) and not control (clutches reared in their environment of origin by their biological parents) kestrel nests could be cross-fostered in this study. Silva et al. (2007) compared foster and biological control groups in a cross-fostering study of thin-billed prions (*Pachyptila belcheri*) and found no differences between them for any variable measured. The resulting experimental design for this study divided nests by origin (captive or wild) and then by rearing environment (captive or wild); relation of clutch to rearing adults (biological or foster) was also considered as a variable to determine if any differences arose between clutches reared by biological or foster parents.

Nests with matching or closely matching (within 2 days) hatch dates were assigned to experimental groups (Amundsen and Stokland, 1990; Risch and Rohwer, 2000). The remainder of the nests served as control groups for their respective environments. Full clutches were switched two days after egg measurements were taken (Blomqvist et al., 1997; Risch and Rohwer, 2000; Quesada and Senar, 2009). The captive clutch was removed from its nest, replaced with dummy eggs for the female to incubate in the clutch's absence, and placed in an insulated carrier (Quesada and Senar, 2009). The clutch was immediately transported to the field site and exchanged with the designated wild clutch. Eggs from the wild nest were then brought to the ASCC and placed in the captive nest after removal of dummy eggs. Observations were made from a distance to confirm that incubation was resumed in all nests.

At the estimated hatch date, nests were checked once daily until hatching commenced. Nests were revisited on days 10, 15, 20 and 25 after hatching to monitor nestling survival and determine sex ratio within the clutch. To reduce the risk of early fledging (mean fledge date of 28 to 31 days in kestrels; Smallwood and Bird, 2002), the nest was considered a success and nestlings considered fledged on the final visit at day 25 (Silva et al., 2007).

All research was conducted with valid McGill University Animal Care Committee Animal Use Protocol, permission from the Société de la Faune et des Parcs du Québec to access birds, nests and transfer clutches, and an Environment Canada permit to capture and band migratory birds.

3 Analyses of data

Only nests reaching mid-incubation, when clutch data were first collected, were used in the analyses. Data were analysed using R 2.10.1 statistical software (R Development Core Team, 2009).

3.1 Adult morphology

Adult morphological measurements were tested for normality and homogeneity of variances using quantile-quantile plots, Shapiro-Wilk’s test for normality and Fligner-Killeen’s test for homogeneity of variances (Crawley, 2007). The data satisfied the criteria for normality after 2 birds were removed as outliers from analyses (likely the result of data recording error). Analysis of covariance (ANCOVA) was computed for each morphological measurement (mass, tarsus length, head-bill length, and wing chord) while controlling for measurement date (MD, given by the Julian date) and testing variables sex, origin of the adult (captive or wild), relation of adult to clutch it was rearing (biological or foster), and year (Crawley, 2007; Blanchet et al., 2008). Age was not used as a variable because ages of wild birds were only known in a handful of cases.

3.2 Egg volume

Egg volume in cubic centimetres was approximated from egg length and breadth using the formula $V = Kv*LB^2$, where V is volume in cm^3, L is egg length in cm, B is egg breadth at the widest diameter around the egg in cm, and Kv is a constant ($K_V = 0.51$ for eggs without high asymmetry, as in the case of kestrels; Hoyt, 1979).

Mixed modelling (LME procedure in R statistical software) fitted using maximum likelihood method was used to analyse egg volume (Crawley, 2007). Clutches were analysed with eggs as subsamples (nested) within each clutch (Reed et al., 1999; Pelayo and Clark, 2003). Covariates tested in the model included lay date of the egg (given by Julian date), biological female mass, and rearing female mass. As only a small number of wild males were captured for analyses, a subset of data was analysed with biological male mass and rearing male mass as covariates, but results were not significant and male mass was dropped as a covariate. Variables tested in the model included clutch origin (captive or wild), rearing environment (captive or wild), and relation to rearing parents (biological or foster). Stepwise comparison was used to identify the best model, and variables were considered significant if they had a p-value < 0.05.

3.3 Reproductive variables

Generalized linear models (GLM procedures in R statistical software) were used to examine clutch fertility, hatchability of fertile eggs, nestling survival, sex ratio of young in a brood, and overall nest productivity (defined as the number of fledglings produced from the initial clutch size; Clum, 1995; Reed et al., 1999; McDonald et al., 2005). Individual sample sizes were treated as weights in the weighted regression, and models were built with binomial errors and a logit link function (Bize et al., 2002). The additional covariate mean egg volume per clutch was tested in the model when looking at reproductive variables (McDonald et al., 2005). Clutch initiation date, length of hatching period, and clutch size were modelled using a generalized linear model with Poisson error family and log link function (Crawley, 2007).

4 Results

4.1 Adult morphology

A total of 72 captive and 55 wild adults from 2006 to 2009 with complete data sets were used in the analyses (Table 1). Generally females were larger than males and captive birds were larger than wild birds. A significant interaction between sex and origin ($P = 0.0005$, Table 2) was noted for adult mass at mid-incubation, with captive females a mean of 6.2 g lighter than wild females and captive males a mean of 8.5 g heavier than wild males. Males were on average 20.8 g lighter than females. Mass of birds at mid-incubation was also found to decline throughout the breeding season (mean -0.19 g / day, $P = 0.0006$); birds initiating nesting later in the season had lower mid-incubation mass compared to those nesting early in the season. Additional analysis showed that captive birds exhibited less sexual size dimorphism for mass than wild birds; captive females were 19.6 g (14.0 g – 25.1 g) heavier than captive males ($P < 0.0001$), while wild females were 34.3 g (27.5 g – 41.0 g) heavier than wild males ($P < 0.0001$).

The additive effect of sex and origin was significant for both head-bill length and wing chord; female kestrels had a larger head size (mean 1.22 mm longer, $P < 0.0001$) and longer wing chord (mean 9.19 mm longer, $P < 0.0001$)) than males, and captive birds had larger head-bill size (mean 0.81 mm, $P = 0.0007$) and longer wing length (2.37 mm longer, $P = 0.0024$) than wild birds. Captive birds had significantly longer tarsi than wild birds (mean 0.95 mm longer, P = 0.0005), though there were no differences between sexes.

4.2 Egg volume

The best-fit model for egg volume indicated an additive effect of biological female mass and origin on egg size (Fig. 1). Females with greater mass tended to produce eggs with larger volume (P = 0.0023), and captive females produced larger eggs than wild females (P = 0.0098). The second best-fit model contained only a significant effect of lay date (Fig. 2), suggesting egg volume was smaller the later the eggs were laid in the breeding season (P = 0.0026). The two models are not unrelated; female body mass was found to decline throughout the breeding season.

4.3 Reproductive variables

From 2006 to 2009, 22 captive nests reared in captivity, 14 pairs of nests switched between captive and wild environments, and 24 wild nests reared in the wild reached mid-incubation stage and were included in analyses (Table 3). No significant differences were found between the length of the laying period, between clutch sizes, or between the sex ratio of nestlings in each nest.

Of all the variables tested, only the additive effect of year and origin approached significance for clutch initiation date. Clutch initiation was generally later in the wild (mean May 3rd) than in captivity (mean April 30th, z = 1.789, P = 0.0722, df = 67), even though captive birds were only paired upon the sighting of the first wild pair. Clutch initiation in the wild occurred later from 2006 to 2009 (from mean April 30th in 2006 to mean May 9th in 2009, z = 1.895, P = 0.0581, df = 67), and consequently, captive birds were also paired later over subsequent years.

Results suggested the best model to describe clutch fertility included origin of the clutch, relation between clutch and rearing parents, and mean egg volume. Clutch fertility was found to decrease with increasing mean egg volume for all groups (z = -2.787, P = 0.0053, df = 65, Fig. 3). Captive clutches had significantly lower fertility than wild clutches (4.43 ± 0.20 and 4.74 ± 0.11 fertile eggs per clutch respectively, z = 3.111, P = 0.0019, df = 65, Fig. 4). An interaction effect was also noted between origin and relation: captive clutches fostered in the wild were significantly more fertile than captive clutches reared by biological parents, though the same was not true for wild clutches (z = -2.508, P = 0.0122, df = 65, Fig. 4).

The best model to describe egg hatchability included biological female mass and year. Eggs produced by females with a larger mass were more likely to hatch successfully (z = 3.487, P = 0.0005, df = 67). Hatchability also varied among years, with 2007 representing the year of greatest hatch success for all groups (z = 3.444, P = 0.0006, df = 67). Clutches of captive origin hatched over significantly longer periods than clutches of wild origin, regardless of rearing environment (mean 2.22 ± 0.32 and 1.72 ± 0.27 days, respectively, z = -2.062, P = 0.0392, df = 59).

The best model to describe nestling survival, the number of nestlings that survived until fledging, included origin and rearing female mass (Fig. 4). Results suggested that survival was greater in nestlings of wild origin (mean 4.13 ± 0.18 nestlings) than those of captive origin (mean 3.62 ± 0.26 nestlings), regardless of rearing environment (z = 2.358, P = 0.0184. df = 63). Rearing females with larger masses produced fewer surviving nestlings than those with smaller mass (z = -2.395, P = 0.0166, df = 63).

The best model found for overall clutch productivity, the number of fledglings produced from original clutch size, included mean egg volume and year as variables (Fig. 5). Clutches with large mean egg volume had a lower productivity than clutches with smaller mean egg volume (z = -3.841, P = 0.0001, df = 67). Overall productivity also varied among years (z = 2.276, P = 0.0229, df = 67).

5 Discussion

Results for adult morphology indicated that captive kestrels were larger than wild kestrels, and that sexual size dimorphism related to mass was reduced in captive-bred kestrels. Reduced size dimorphism and increased (or decreased) body size are common in captive populations of various wildlife species (e.g. Araújo et al., 2000; Wisely et al., 2002; Wisely et al., 2005; Forstmeier et al., 2007; Tschirren et al., 2009; Mainwaring et al., 2010).

The only exception to the larger size of captive kestrels in this study was female mass at mid-incubation, in which wild females had greater mass. This could likely be a function of foraging needs of captive versus wild birds. In the wild female raptors often accumulate large body reserves early in the breeding period against potential food shortages at critical points in the nesting cycle (Sergio et al., 2007). Males provision females during the breeding season, and as such wild males expend large amounts of energy during this period (Sergio et al., 2007). The greatest difference in mass between wild male and female raptors would therefore be during the nesting period, which could explain the large differences in wild kestrel mass found in this study. In captivity where birds have access to ad libitum diets fed on a predictable schedule, males are not required to expend any energy hunting to provide for a mate, and females do not necessarily need to increase their food intake to account for possible food shortages. This may have contributed to the reverse mass trend between the sexes captivity compared to wild kestrels, and to the marked reduction in size dimorphism.

Adult kestrel mass at mid-incubation was lower in birds nesting later in the breeding season, which may be a response to seasonal effects on reproductive cycles (Coulson, 2010). Differences in mass may also reflect adult quality; larger females produced larger eggs, and since larger eggs are often attributed to higher quality females in birds, it is possible that kestrels laying earlier in the breeding season were higher quality birds and thus in better breeding condition earlier in the season (Risch and Rohwer, 2000). Wild pairs produced clutches later in the breeding season than captive kestrels, even though captive birds were paired only upon the first sightings of wild pairs on territory. It is possible that birds could attain breeding condition more rapidly in captivity as a result of access to ad libitum diets, since access to food is a constraint in breeding female birds (Birkhead and Nettleship, 1982). It is also possible that by removing mate selection in captivity, and without the need to hunt or attract a mate, earlier nest initiation may have been encouraged. A shorter courtship period and incompatibility between mates in captive pairs may be the reason for the reduced fertility seen in captive clutches.

The best-fit model of egg volume suggested that larger females produced larger eggs, and that captive females produced larger eggs than wild females of equivalent size, which has been noted in other captive bird species (e.g. Leblanc, 1989; Mainwaring et al., 2010). Increased egg size may relate to an ad libitum diet in captivity, as food supplementation experiments in wild kestrels have shown short-term increases in the egg volumes produced by females during the period of food supplementation, though production of larger egg volume disappeared once food supplementation was stopped (Wiebe and Bortolotti, 1996). Though egg volume was related to female mass, volume was also smaller when eggs were produced later in the breeding season. Since female kestrel mass was also found to be smaller later in the breeding season, these variables may be related. Seasonal decline in egg volume has been noted in studies of other species (e.g. Birkhead and Nettleship, 1982).

When looking at nest success, results suggested that clutches produced by wild pairs were more likely to be fertile and to produce surviving fledglings than clutches produced by captive pairs, regardless of where they were raised. Large eggs had lower fertility and resulted in lower overall clutch productivity. Finally, while females with large mass appeared to be beneficial to increased egg hatchability, larger females reared fewer surviving nestlings than smaller females.

The higher fertility in fostered clutches than those incubated by their biological parents, particularly for clutches of captive origin, was an unexpected side-effect of the conditions of the cross-fostering design in this study. Captive pairs began nesting earlier than wild pairs and finished earlier; candidates for cross-fostering were therefore more likely to be found mid-way through the nesting season when both groups were initiating clutches. As a general but non-significant trend, captive clutches had greater fertility later in the breeding season, while wild clutches had higher fertility levels early in the breeding season. Clutches chosen for switching mid-way through the nesting season would likely represent those with the highest proportion of fertile eggs, which would explain the relation and origin interaction for clutch fertility.

Larger egg size is frequently associated with increased hatching success and/or nestling development and survival (e.g. Amundsen et al., 1996; Blomqvist et al., 1997; Pelayo and Clark, 2003; Silva et al., 2007; Krist, 2011). Other studies have found that egg size had no effect on nestling survival (e.g. Reid and Boersma, 1990; Meathrel et al., 1993; Smith et al., 1995; Reed et al., 1999; Styrsky et al., 1999; Risch and Rohwer, 2000; Van de Pol et al., 2006). In the present study, egg volume had a negative effect on clutch fertility and nestling survival, which is unusual. It may be the result of the egg-to-body-size ratio in American kestrels; among the Falcomiformes, kestrels lay one of the largest clutch-volume-to-female-mass ratios (Bortolotti and Wiebe, 1993). Bortolotti and Wiebe (1993) found that smaller female kestrels were more likely to hatch eggs out of laying sequence than larger females, and that the number of eggs hatched at any one time was highly variable. They proposed that smaller females were not large enough to completely cover a clutch of average size, which resulted in the variability in hatching.

The volume-to-female-mass-ratio may affect survival of embryos during development, especially during early development. Unequal incubation of eggs due to female size may have resulted in embryo failure before any detectible sign of development could be seen during candling procedures in this study. It might also help to explain why eggs of captive origin had longer hatching periods; captive birds produced larger eggs. Hatchability of eggs was also greater in clutches produced by females with larger mass, suggesting that female mass influenced hatchability rates early in egg development (before clutches were switched at mid-incubation). Overall productivity would be negatively affected by large egg volume because fewer eggs would survive to the hatching stage.

In studies of other raptor species, adults of smaller mass or size were found to be more successful in producing fledglings (e.g. Massemin et al., 2000; Krüger, 2005; Sergio et al., 2007). Smaller body size may make individuals more manoeuvrable, successful hunters and thus better able to provide for their young, or that smaller individuals have lower energy requirements than larger birds and may be able to supply more of their food to young without incurring greater costs to themselves (Massemin et al., 2000; Krüger, 2005; Sergio et al., 2007).

6 Conclusions

This study demonstrated significant differences between captive and wild kestrels, both in adult body size and in nesting success. Captive birds were generally larger than their counterparts in the wild, though effects of origin and rearing environment could not be separated for adult birds in this study. While other variables contributed to nesting success, clutch origin did have an effect on nestling survival. This suggests an effect of captivity that extends beyond the captive environment itself. Given the amount of research conducted on effects of environmental contaminants on avian nesting success using captive kestrels as models (see Bardo and Bird, 2009), further study should be conducted to determine the full extent of the differences in captive kestrels compared to their wild counterparts, and whether any of these differences could be mitigated through changes in the captive regime. Reduced fledging success in captive clutches, even when reared in the wild, could also have implications for reintroduction programs if the results from this study prove to be a general trend.

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Table 1. Adult morphological measurements of American kestrels taken at mid-incubation* (mean ± SE) from 2006 – 2009

	Female		Male	
	Captive origin	Wild origin	Captive origin	Wild origin
Mass (g)	135.75 ± 1.42	141.92 ± 1.86	116.16 ± 1.28	107.67 ± 1.61
Head-bill length (mm)	43.97 ± 0.14	43.01 ± 0.15	42.54 ± 0.20	41.92 ± 0.18
Tarsus length (mm)	33.53 ± 0.22	32.85 ± 0.24	34.05 ± 0.31	32.63 ± 0.26
Wing chord (mm)	195.11 ± 0.65	192.97 ± 0.71	185.81 ± 0.83	182.89 ± 0.88
N	36	37	36	18

* Some captive and wild males (n = 7) were measured during the brood rearing stage and were included in the analyses as they did not differ significantly from measurements taken on males at mid-incubation

Table 2. Results of ANCOVA tests for adult American kestrel morphological measurements from 2006 – 2009 (n = 127 adults)

Adult structure	Source of variation	df	SS	F	P
Mass	Sex	1	20252.10	258.13	< 0.0001 ***
	Origin	1	3.70	0.05	0.8287 n.s.
	MD	1	964.70	12.30	0.0006 ***
	Sex*Origin	1	1021.90	13.03	0.0005 ***
	Residual	122	9571.60		
Head-bill length	Sex	1	46.49	26.64	< 0.0001 ***
	Origin	1	21.01	12.04	0.0007 ***
	MD	1	2.52	1.44	0.2317 n.s.
	Sex*Origin	1	2.53	1.45	0.2306 n.s.
	Residual	122	212.90		
Tarsus length	Sex	1	4.83	2.13	0.1467 n.s.
	Origin	1	28.94	12.78	0.0005 ***
	MD	1	2.61	1.15	0.2853 n.s.
	Sex*Origin	1	6.47	2.86	0.0934 n.s.
	Residual	122	276.15		
Wing chord	Sex	1	2623.78	139.23	< 0.0001 ***
	Origin	1	181.11	9.61	0.0024 **
	MD	1	0.11	0.01	0.9399 n.s.
	Sex*Origin	1	5.21	0.28	0.6001 n.s.
	Residual	122	2299.02		

n.s., not significant
** $p < 0.01$
*** $p < 0.001$
MD, measure date (Julian date)

Table 3. Summary of reproductive variables (mean ± SE, with sample size of American kestrel nests indicated in parentheses in first column) from 2006 - 2009 *

Clutch origin	Captive		**All captive clutches**	Wild		**All wild clutches**
Clutch rearing environment	Captive	Wild		Captive	Wild	
[a] Egg volume (cm^3) (74)	14.75 ± 0.09	14.94 ± 0.11	**14.82 ± 0.07**	14.59 ± 0.15	14.43 ± 0.10	**14.49 ± 0.08**
No. eggs / clutch (74)	5.09 ± 0.06	5.08 ± 0.08	**5.09 ± 0.05**	4.85 ± 0.10	4.95 ± 0.08	**4.91 ± 0.06**
Clutch initiation date (70)	30 April ± 1.58	30 April ± 1.32	**30 April ± 1.10**	1 May ± 1.30	5 May ± 2.38	**3 May ± 1.58**
[a] No. fertile eggs / clutch (70)	4.09 ± 0.29	5.00 ± 0.00	**4.43 ± 0.20**	4.62 ± 0.14	4.82 ± 0.16	**4.74 ± 0.11**
No. hatched fertile eggs / clutch (70)	3.64 ± 0.31	4.08 ± 0.35	**3.80 ± 0.23**	3.92 ± 0.33	3.73 ± 0.37	**3.80 ± 0.26**
[a] Length of hatching period (days) (66)	1.60 ± 0.33	3.25 ± 0.54	**2.22 ± 0.32**	0.92 ± 0.26	2.26 ± 0.37	**1.72 ± 0.27**
[a] No. surviving fledglings / clutch (66)	3.62 ± 0.31	3.62 ± 0.46	**3.62 ± 0.26**	3.85 ± 0.34	4.32 ± 0.20	**4.13 ± 0.18**
No. female young / clutch (66)	1.95 ± 0.30	1.46 ± 0.24	**1.76 ± 0.21**	1.69 ± 0.31	2.05 ± 0.22	**1.91 ± 0.18**
No. successful clutches / total (74)	20 / 22	13 / 14	**33 / 36**	14 / 14	21 / 24	**35 / 38**

* Includes only nests that reached mid-incubation when eggs were measured and candled for fertility

[a] Indicates reproductive variable significantly affected by clutch origin (captive / wild)

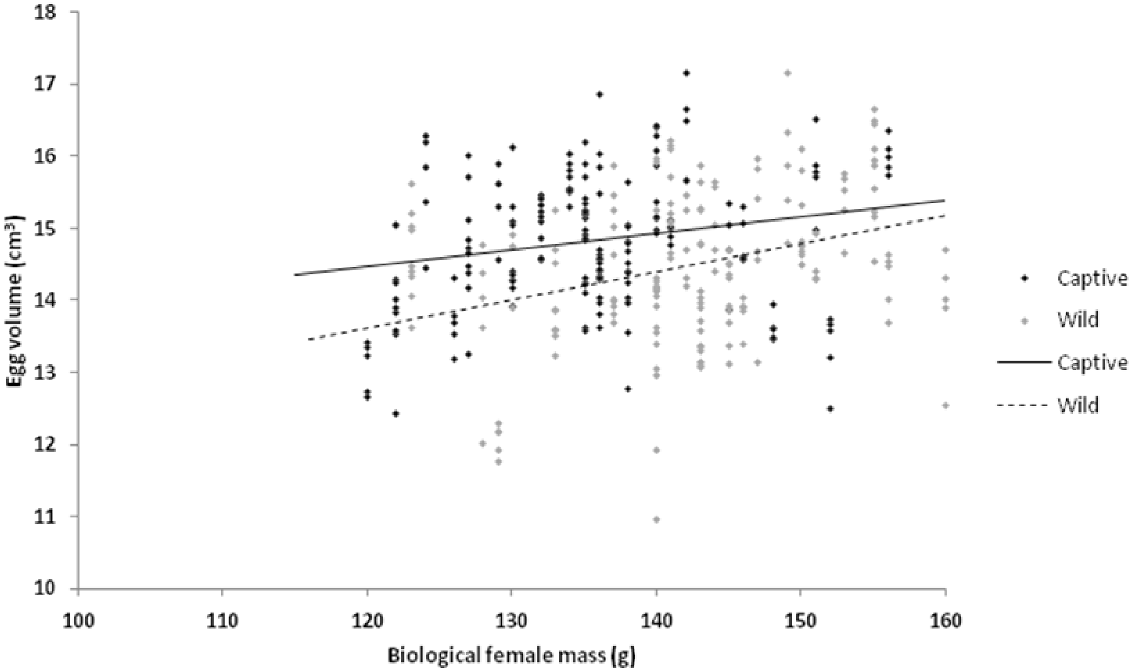

Fig. 1 Trends in egg volume from American kestrel clutches of captive and wild origin in relation to the mass of the female that produced the clutch.

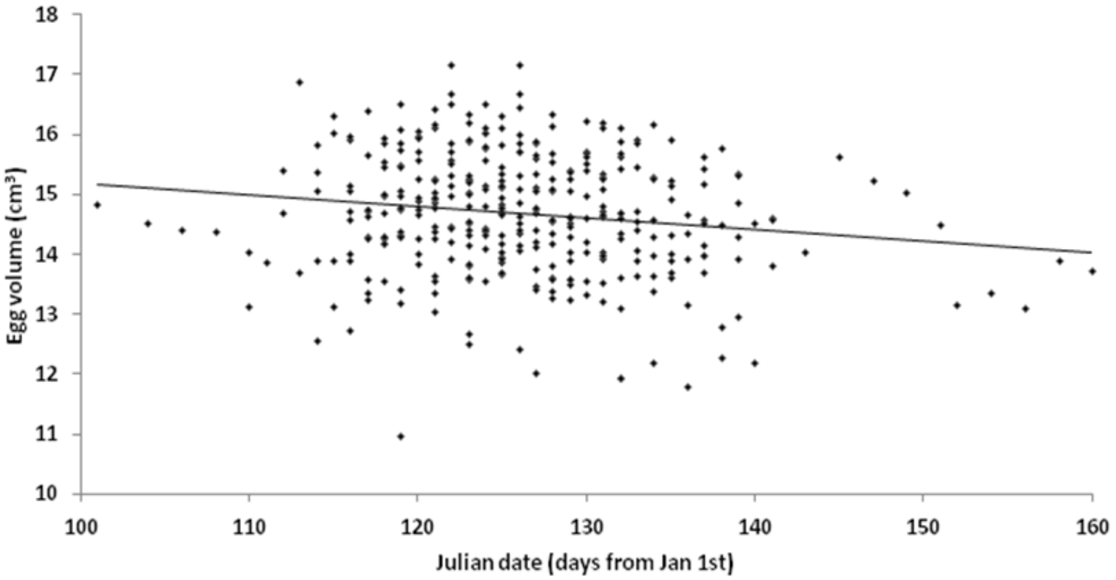

Fig. 2 Trend in egg volume for all American kestrel clutches throughout the breeding season. Represents second best-fit model after the additive effects of origin and biological female mass.

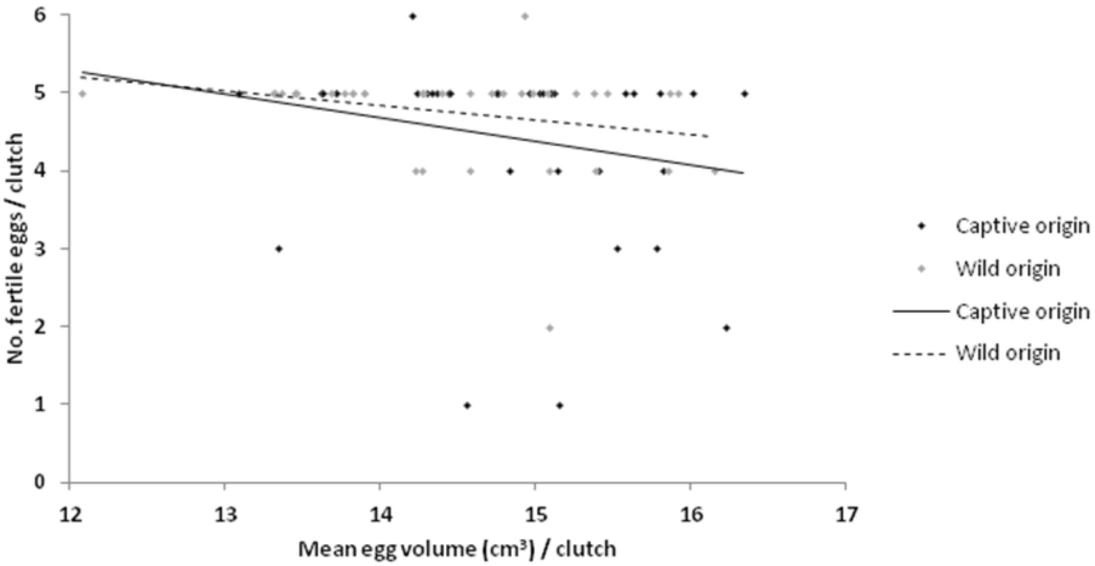

Fig. 3 Trend of clutch fertility in relation to mean egg volume per clutch in clutches of captive and wild origin. Clutch fertility tended to decline with increasing mean egg volume.

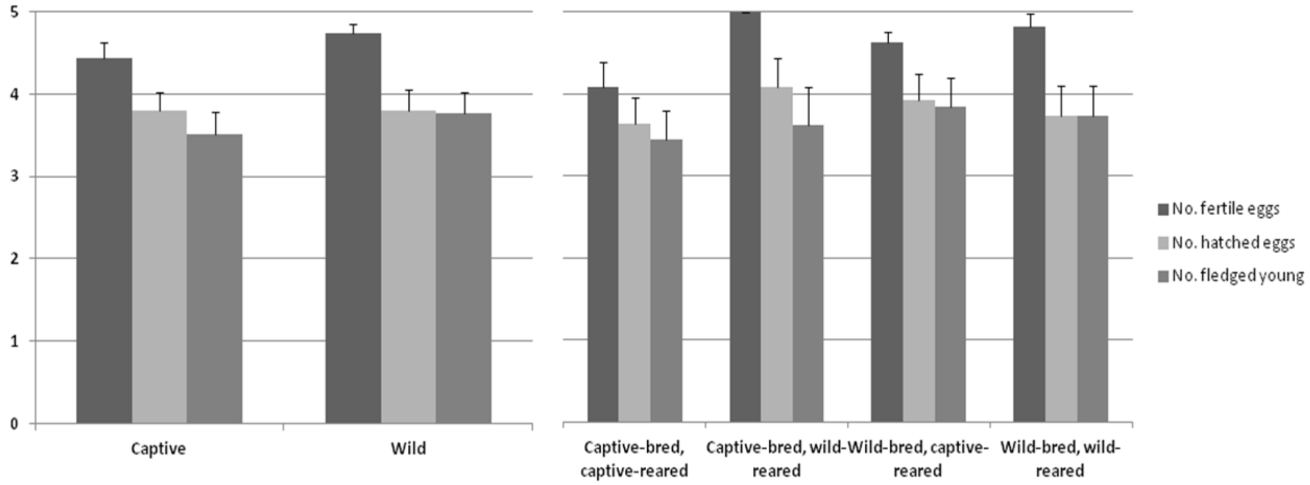

Fig. 4 Mean number of fertile eggs, hatched eggs, and fledglings produced by American kestrel nests of captive and wild origin (left). The same data are presented on the right divided by clutch origin and rearing environment.

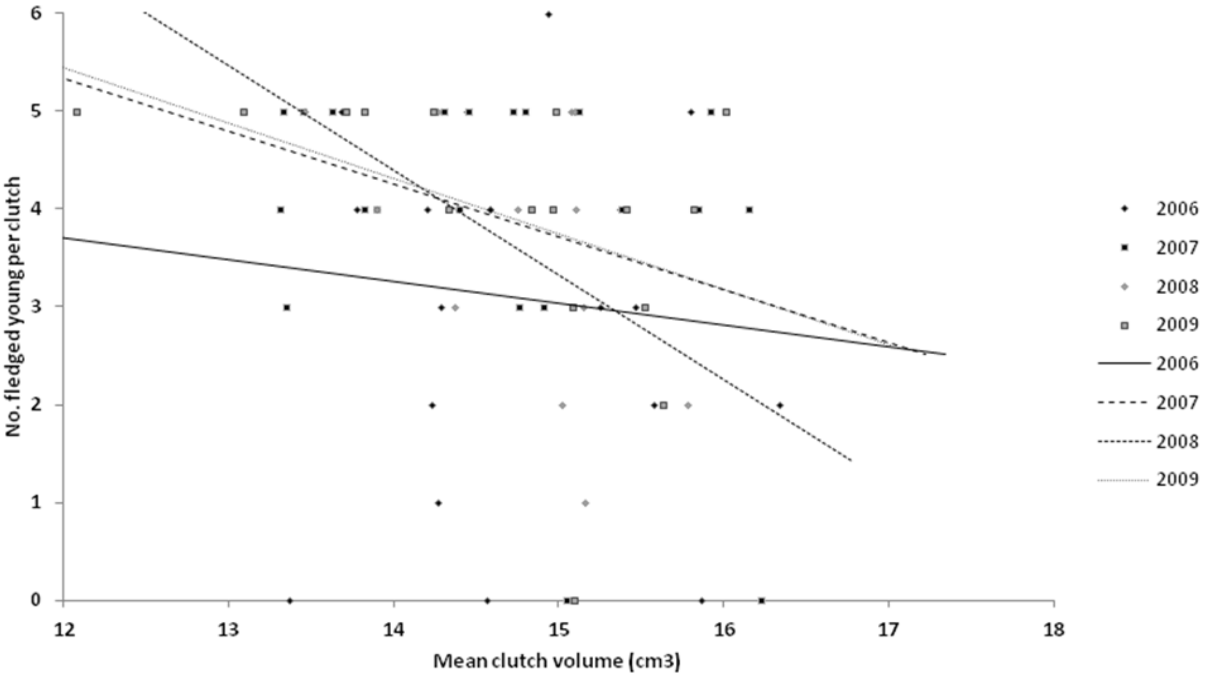

Fig. 5 Trends for overall clutch productivity in American kestrels, described as the number of fledglings produced from the total clutch size, between years in relation to mean egg volume of each clutch.

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CONNECTING STATEMENT 2

The previous chapter presented the results of a comparison of adult morphology and nesting success between captive and wild American kestrels using a whole-clutch cross-fostering design to separate rearing environment and origin effects on nesting success. To determine whether the effects of origin or rearing environment had a greater influence on nestling growth, repeated measures were taken from experimental and control nestlings during the nesting period from 2007 to 2009 inclusive. The next chapter presents the synthesis and analyses of growth models for five morphological measurements taken on the nestling kestrels.

CHAPTER 3

EFFECTS OF CAPTIVITY ON NESTLING GROWTH IN AMERICAN KESTRELS (*FALCO SPARVERIUS*): USING A CROSS-FOSTERING DESIGN TO SEPARATE THE EFFECTS OF ORIGIN AND REARING ENVIRONMENT IN CAPTIVE AND WILD NESTS

Abstract

Captive propagation is used to supply animals for wildlife research and for reintroduction programs. However, captive animals can undergo changes at the individual and population level that can render them significantly different from their wild counterparts. Studying the effects of captivity on animal populations remains a relatively new field of research, and few studies have yet attempted to differentiate between potential effects of origin and rearing environment through experimental manipulation. Fostering in birds, especially eggs, can be used to separate the effects of origin and rearing environment on nestling growth, making birds ideal subjects for research into the effects of captivity. The objective of this study was to use a cross-fostering experimental design to determine whether captive breeding resulted in significant changes in growth rates or size of nestling American kestrels, and whether those effects were the result of origin or rearing environment or a combination of both factors. Results of the study suggest that there was no lasting difference between nestling growth rates of wild- and captive-bred kestrels. Male and female nestlings attained similar sizes regardless of origin or rearing environment. The only measurement affected by rearing environment was nestling mass, with captive-reared nestlings having larger masses during their early growth, though this difference became negligible at fledgling. Based on these results, it appears that there were no significant differences between wild kestrel nestlings and kestrel nestlings produced from captive pairs with over 10 generations of captive ancestry.

Keywords: captive breeding, American kestrel, *Falco sparverius*, cross-fostering, morphology, nestling growth

1 Introduction

Captive-bred populations of wild-type animals can be used to enhance research in fields such as evolutionary ecology and physiology by allowing researchers to study animals under controlled conditions (Lambrechts et al., 1999). Using captive-bred animals as research models has the added advantage of reducing disturbance to wild populations and minimizing take of wild specimens (Honess et al., 2010). Captive breeding can also be used as a tool for wildlife conservation by providing specimens of endangered species for reintroduction into the wild (White et al., 2005). With the increased use of captive propagation for both wildlife research and conservation, greater attention is being given to the potential effects captivity can have on animal populations (Cockrem, 2005; Blanchet et al., 2008; Honess, 2010). For captive animals to serve as wildlife models or as sources for reintroduction, it is imperative that they retain their original wild state as much as possible (Price, 1984; Künzl et al., 2003; Blanchet et al., 2008).

The study of the effects of captivity is a relatively new field of research, and much of the published work on the subject consists of post hoc analyses of data collected as part of propagation or other research programs (Armstrong and Seddon, 2007). Differences noted between captive and wild populations of a species can arise from environmental effects on individuals, or from selection which can occur over generations of captive breeding (Price, 1999). These effects can be difficult to separate without experimental manipulation, however (Brown et al., 2006; Blanchet et al., 2008; Connolly and Cree, 2008). Thus there is a need for more objective-driven research into the ways animal populations can be affected by captive regimes (Armstrong and Seddon, 2007).

An individual's rearing environment can have an impact upon its condition, even into adulthood (Tschirren et al., 2009). This makes it difficult to separate effects of origin and rearing environment effects when comparing adult animals. Even if wild adult specimens were brought into captivity for comparison to captive-bred individuals, any differences between the two populations in terms of behaviour, morphology, or nesting success would be difficult to interpret as solely the result of origin effects because they were reared in different environments. An animal must be removed from its rearing environment very early in its life to remove its confounding effect on research results (Blondel et al., 1990).

Birds make ideal subjects for studies requiring the separation of environmental and genetic components on life history traits; fostering eggs from one environment into another can separate the effects of pre- and post-hatching variables on nestling growth (Magrath, 1992). Fostering of clutches has been used previously to separate the effects of egg size and parental or environmental quality on nestling growth and survival (e.g. Magrath, 1992; Risch and Rohwer, 2000; Silva et al., 2007). The use of a cross-fostering experimental design should therefore be able to separate the effects of origin and rearing environment between captive and wild populations.

The objective of this study was to determine whether captive breeding had resulted in significant changes in nestling growth and size in American kestrels (*Falco sparverius*), and if those changes were the result of origin or rearing environment. To do this, whole-clutches from captive and wild nests were fostered into opposite environments and compared to control clutches raised in their environment of origin. Nestlings were measured throughout their growth to determine whether there were greater similarities between fostered and control nestlings reared in the same environment, or between nestlings from the same origin reared in different environments.

2 Materials and methods

2.1 Study species

American kestrels are small New World falcons (Smallwood and Bird, 2002). Kestrels breed readily in captivity and have been used for over four decades as captive wildlife models, particularly in the fields of reproduction biology and environmental toxicology (Bardo and Bird, 2009). No research has yet been conducted to determine whether multiple generations of captive breeding have affected nestling growth in this species, even though a large number of toxicological studies conducted on kestrels center around nestling development in response to direct or parental exposure to contaminants (see Bardo and Bird, 2009).

The Avian Science and Conservation Centre (ASCC) of McGill University in Montreal, Quebec, Canada, where the captive component of this study was conducted, has housed a colony of captive American kestrels since 1973 (Bird, 1982). All birds in the colony descend from the local Montreal population and have at least 10 generations of captive ancestry.

2.2 Captive pairs

Nine captive pairs each in 2007 and 2008 and 11 pairs in 2009 were formed based on the following criteria: paired birds were unrelated by at least 5 generations. To minimize differences in clutch initiation dates between captive and wild pairs for clutch fostering, captive birds were paired when sightings of the first wild pairs on territory were confirmed. Captive pairs were housed in individual aviaries (2.5 x 1.5 x 2.5 m L x

W x H) with a nest box (0.3 x 0.3 x 0.4 m L x W x H) containing a wood-shaving substrate. Aviaries contained rope perches, a food platform, wood-shaving substrate, and a screened window for natural lighting and air circulation. All pairs were fed daily 3 - 4 frozen-thawed, day-old cockerels through a food door to prevent excessive disturbance. The birds could be monitored through an observation window constructed from one-way mirrored glass. Nest boxes were checked once daily during regular morning feeding to minimize disturbance (Fernie et al., 2001).

2.3 Wild nests

From April to August each year 55 to 65 nest boxes with the same dimensions as captive boxes mounted on Hydro poles in and around Montreal, Quebec, Canada (45˚ 01’ to 46˚ 02’ North, 72˚ 44’ to 74˚ 25’ West) were visited at 5 to 7-day intervals to check for the presence of kestrel pairs. Nesting was confirmed when eggs were found in the box. Clutch initiation date was determined based on an average laying rate of one egg every second day until clutch completion (average clutch size is 5 eggs, Sockman and Schwabl, 2001). Once eggs were found in a nest box the pair was not disturbed again until mid-incubation, approximately 14 days after the laying of the penultimate egg, to reduce disturbance to the breeding birds (Reid and Boersma, 1990; Sockman and Schwabl, 2001; Smallwood and Bird, 2002).

2.4 Cross-fostering of clutches

All measurements were taken by the primary author to eliminate inter-observer variability. Using the clutch initiation dates, the mid-incubation and hatch dates for each nest were estimated (Smallwood and Bird, 2002). At mid-incubation, each nest was visited and the incubating adult was removed from the nest by hand and placed in an aerated transport box. If the incubating adult at the time of the visit was male, the nest was re-visited two days later to measure the female. Eggs were then removed from the nest, individually marked using a non-toxic marker and measured for length and breadth to the nearest 0.1 mm using vernier callipers (Silva et al., 2007). Eggs were candled for fertility. Clutches were returned to the nest box within 10 minutes of removal. The adult was then banded and mass was taken using a Pesola spring-balance accurate to the nearest gram. The adult was then released in the vicinity of the nest for wild birds or returned to its breeding pen to resume incubation in the case of captive birds.

Most avian cross-fostering studies have been conducted on species with high densities of breeding pairs and synchronous nesting patterns (e.g. Meathrel et al., 1993; Amundsen et al., 1996; Reed et al., 1999). This allowed researchers to foster out both control and experimental clutches to remove any potential bias that might arise between pairs rearing biological or foster young. Kestrels are territorial birds and do not exhibit synchronous nesting; pairs in this study initiated laying from mid-April to the first week of June, and researchers had access to no more than 25 pairs per year. Exchanges between the two environments were limited to pairs of clutches with matching hatch dates. Control clutches were therefore reared by biological parents. Silva et al. (2007) found no differences in nestling development between control young reared by foster or biological parents in the thin-billed prion (*Pachyptila belcheri*).

The resulting experimental design sorted nests by origin (captive or wild) and rearing environment (captive or wild), with captive-bred broods reared by captive parents or fostered into the wild and wild-bred broods reared by wild parents or fostered into captivity. Relation of rearing adults to brood was also considered as a variable to determine if differences were noted between clutches reared by biological or foster parents in each environment.

Whole clutches were switched two days after mid-incubation measurements between captive and wild nests with matching hatch dates (Quesada and Senar, 2009). A captive clutch was removed from the nest, replaced with dummy eggs for the female to incubate in the clutch's absence, and placed in an insulated carrier (Quesada and Senar, 2009). The clutch was transported to the field site and exchanged with the wild clutch, which was then brought to the ASCC and placed in the nest box of the captive pair. An individual clutch was typically out of the nest for less than an hour throughout the transfer procedure. At the approximate hatch dates nests were checked daily for hatch initiation, which was considered day zero for nestling age (Reid and Boersma, 1990; Styrsky et al., 1999; Bize et al., 2002). It was not always possible to determine which eggs hatched or to match nestlings to particular eggs in a clutch; when using egg volume as a variable in nestling growth models, mean egg volume for an individual clutch was used.

2.5 Nestling measurements

Nestling morphological measurements were taken every 5 days, at ages 10, 15, 20 and 25 days of the oldest nestling in the box, though age ranges between siblings in each box varied between 0 and 5 days (Gard and Bird, 1992; Risch and Rohwer, 2000). Age could be verified using photographic field guides for aging nestling kestrels (e.g. Griggs and Steenhof, 1993; Klucsarits and Rusbuldt, 2007).

Nestlings were banded on day 10 of the oldest nestling in the nest (if any nestlings in the brood were too young to band upon the first visit, they were marked on the head using a non-toxic marker to allow for individual identification), and on each visit to the nest five morphological measurements were taken from each nestling, including mass using a Pesola spring balance accurate to the nearest gram. Vernier callipers were used to measure tarsus length and head-bill length to the nearest 0.1 mm (Blomqvist et al., 1997; Sockman and Schwabl, 2001; McDonald et al., 2005). A ruler with millimeter increments was used to measure wing chord and the length of the 9th primary feather (longest feather on the wing of a kestrel; Lacombe et al., 1994). All measurements were taken on the right side of the body (Olendorff, 1972). Nestlings were sexed based on plumage color after 12 days of age. A nestling was considered successfully fledged if it survived until the final day 25 measurements to reduce the risk of early fledging in nestlings beyond this age.

All research was conducted with valid McGill University Animal Care Committee, permission from the Société de la Faune et des Parcs du Québec to access birds, nests and to transfer clutches, and a permit from Environment Canada to capture and band kestrels.

3 Data analyses

Only nestlings for which all four measurements were taken were used in the analyses. Only three out of four measurements of primary feather growth were used for analyses, since primary feathers in kestrels begin to emerge after 12 days of age. Data were analysed using R 2.10.1 statistical software (hereafter ‘R’; R Development Core Team, 2009).

Variables examined in the models included origin of clutch (captive or wild), rearing environment (captive or wild), relation of clutch to rearing parents (biological or foster), and sex of the nestling. Covariates included masses of biological and rearing parents, mean egg volume per clutch, calculated using Hoyt (1979), hatch date (given by Julian date), clutch size, and year.

3.1 Models for linear growth

Wing length and primary feather growth exhibited a linear growth pattern and did not complete growth before fledging; they were represented by a linear growth model (Negro et al., 1994). A mixed-effects model (LME in R) of length by age was fitted using maximum likelihood method to analyse primary feather growth and wing growth (Crawley, 2007). Repeated measures were nested within each nestling, and each nestling was nested within its respective clutch to account for the hierarchal structure of the data during analyses (Reed et al., 1999; Pelayo and Clark, 2003).

3.2 Models for nonlinear growth patterns

Growth models for animals often display a sigmoidal pattern over sufficiently long time intervals (Ricklefs, 1967; Sockman et al., 2008). The Gompertz function is often used to model growth in birds, especially when hierarchical data structure requires the use of mixed-effects modelling (Whitmore and Marzluff, 1998; Sockman et al., 2008). Mass, tarsus length and head-bill length were analysed using a Gompertz function (Lacombe et al., 1994; Sockman et al., 2008).

The formula: morphological measurement = $a_0\exp(-b_0b_1^{age})$ from the self-starting Gompertz function, SSgompertz (NLME in R), which contains three parameters a_0, b_0, and b_1, was used to model growth rates in nestlings according to the methods of Sockman et al. (2008). In the formula, a_0 is the asymptotic size of the morphological feature (mass, head-bill or tarsus length) being measured, b_0 is the growth rate constant or the growth rate during the linear portion of nestling growth, b_1 is the location of the inflection point (point when maximum growth rate was achieved) and growth rate, and age served as the model predictor (Ricklefs, 1967). Data were once again nested to account for hierarchical data structure. Random effects could not be added to all three model parameters because the sample size was not sufficiently large. Random effects were therefore assigned to the variables(s) that varied the most between individuals based on plots of interval estimates for each variable (Sockman et al., 2008). Models were estimated using maximum likelihood method.

3.3 Initial and final nestling measurements

Day 10 and 25 measurements were treated as the initial and final nestling sizes. Mixed effects models were used to examine the day 10 and day 25 measurements of each morphological feature. Individual nestlings were nested within clutch for the analyses, and the same variables as above were examined in the models.

4 Results

Eighteen captive-bred, captive-reared broods, 10 captive-bred, wild-reared broods, 10 wild-bred, captive-reared broods, and 13 wild-bred, wild-reared broods survived until day 25 measurements. Forty-four of these broods had complete data sets. Measurements were taken from 176 nestlings (Fig. 1).

4.1 Models for linear growth

No significant effects of origin or rearing environment were noted for growth of wing or primary feathers. A weak interaction effect between sex and relation was noted for wing growth; female nestlings reared by biological parents had longer wing lengths than those of females reared by foster parents at any given age ($P = 0.0376$; Fig. 2), but the same was not seen in males. Growth rates for wing chord also differed significantly between sexes, with female having more rapid wing growth than males (P = 0.0002).

The best linear models of primary feather growth and wing growth showed a significant effect of mean egg volume. Nestlings from clutches with larger mean egg volumes had greater primary lengths (P = 0.0026) and wing lengths (P < 0.0001) at any given age.

4.2 Models for nonlinear growth patterns

Only the model for mass growth exhibited a significant effect of rearing environment. The best-fit growth model for nestling mass contained rearing and sex as significant variables. Rearing environment had a significant effect on the inflection point, with nestlings reared in captivity attaining a steeper mass gain earlier than nestlings reared in the wild (P = 0.0097; Fig. 3). This difference between the two environments disappeared near to 15 days of age, and asymptotic size between captive and wild nestlings was indistinguishable with respect to each sex. There was a significant effect of sex on growth rate and asymptotic mass; males initially had a more rapid growth rate, but were overtaken by females between 5 and 10 days of age (P = 0.0038), and females attained an asymptotic mass on average 9.63 g larger than males (P < 0.0001). Origin and relation had no effect on growth of mass, head-bill or tarsus.

Females attained a larger asymptotic head-bill size than males (P = < 0.0001), though growth rates were similar between the two sexes. No significant differences were found for tarsi growth between any of the nestlings.

4.3 Initial and final nestling measurements

At day 10 measurements for wing, mass, tarsus and head-bill, and at the day 15 measurement for primary growth, mean egg volume per clutch was the only variable to have a significant effect on morphological size. Clutches with larger mean egg volume were associated with larger morphological measurements at day 10 (and 15 for primary growth, Fig. 4). Only mass was significantly affected by origin at day 25, with nestlings of captive origin attaining an asymptotic mass on average 2.50 g larger than wild nestlings of each respective sex ($P = 0.0188$). The same effect of relation noted in wing growth above was noted in wing size at day 25; females reared by biological parents had wings on average 3.57 mm longer than those reared by foster parents ($P = 0.0052$), but the same difference was not found for males. Significant differences were noted between sex for mass, head-bill length and wing length at day 25; females were larger than males for all three measurements ($P = 0.0093$, < 0.0001, < 0.0001, respectively).

5 Discussion

Overall, there appeared to be no lasting differences in growth and final size between captive and wild nestlings, regardless of their rearing environment. Rearing environment had a temporary effect on mass growth, with captive-reared nestlings initially gaining mass more rapidly than wild-reared nestlings, but this difference became negligible before asymptotic size was attained. At their asymptotic size, nestlings of captive origin attained a small mass advantage over nestlings of wild origin, but the difference was very weak and might be an artefact of the sample size. There was also a significant effect of relation on wing length in female nestlings, with females reared by biological parents attaining larger wing size than female nestlings reared by foster parents.

With the exceptions above, sex and egg volume were the only other variables to have a significant effect on nestling growth. Females attained a larger overall size than males, as is expected for this species (Smallwood and Bird, 2002). Larger egg volume has been shown to increase nestling size, growth, or survival in other species (e.g. Birkhead and Nettleship, 1982; Reid and Boersma, 1990; Bize et al., 2002; Pelayo and Clark, 2003; Silva et al, 2007).

The effect of the captive rearing environment on initial growth rate for mass in nestling kestrels could be a consequence of an ad libitum diet in captivity. In fact, the overall lack of difference between captive-reared and wild-reared kestrels in this study was unexpected. Captive conditions, particularly captive diets, are often associated with faster growth rates, larger masses or larger body sizes in captive specimens of various species when compared with their wild counterparts, (e.g. Araújo et al., 2000; Santos et al., 2009; Mainwaring et al., 2010; Ritz et al., 2010). Conversely, a comparative study of nestling growth between wild, parent-reared and captive, hand-reared blue-fronted Amazons (*Amazona aestiva*) determined that both captive and wild birds attained similar asymptotic masses before fledgling, but that captive birds continued to gain mass after fledging while wild birds lost mass (Seixas and Mourão, 2003).

The lack of difference between captive and wild kestrel nestlings may relate to the biology of the species. Various studies using wild and captive kestrels have been conducted of food deprivation, starvation bouts, and brood size effects on nestling growth rates (e.g. Gard and Bird, 1992; Lacombe et al., 1994; Negro et al., 1994). Results from all studies suggest that while there were short-term effects of experimental variables on growth of mass and feather growth, overall nestling mass and structural size were unaffected (Gard and Bird, 1992; Lacombe et al., 1994; Negro et al., 1994). Kestrels in these studies appeared to exhibit compensatory growth of mass and feathers during the nestling period to attain the same final asymptotic size. The lack of effect of experimental variables on structural growth could be a consequence of the importance of limb function for mobility and prey capture; these features may be so important to survival that they do not vary greatly in response to environmental factors in the short-term (Gard and Bird, 1992; Lacombe et al., 1994; Negro et al., 1994). The same reasoning can likely be applied to this study, as kestrels have only been kept in captivity at the ASCC between 10 and 20 generations without intentional selection for particular traits such as body size, which may not have been enough time to result in significant changes to nestling growth.

The effect of relation between parents and clutch on female wing size is more difficult to explain, particularly since the effect was only noted in females, and only for wing size. Previous studies fostering clutches to separate the effect of egg size, parental quality, and environment have shown no differences between fostered young and young reared by their own parents, though most studies to date have been conducted on species with sufficiently large sample sizes to allow both control and experimental clutches to be fostered out (Silva et al., 2007). Further research into the effects of clutch cross-fostering in kestrels may reveal whether this is a common trend or an anomaly of sample size.

6 Conclusions

Results of the study suggest that for American kestrels, 10 generations of captive breeding has not significantly altered aspects of nestling growth and body size compared to wild specimens. This is not surprising, given the small number of generations of captive-breeding at the ASCC, the lack of intentional selection for particular traits in the captive colony, and the apparent lack of long-term environmental effects on American kestrel nestling growth. Any differences that were noted between nestlings in the various groups were either temporary or weak.

Thus, it can be concluded from this study that research focussing on nestling growth and using captive kestrels in place of wild birds is justified. It also suggests that, for raptors at least, captive-bred nestlings reared by wild parents result in offspring indistinguishable in morphology from wild-bred nestlings. If future research can demonstrate similar survivorship between captive-bred and wild-bred nestlings reared in the wild, fostering of whole or partial clutches in a similar manner to this study could be useful for reintroduction programs.

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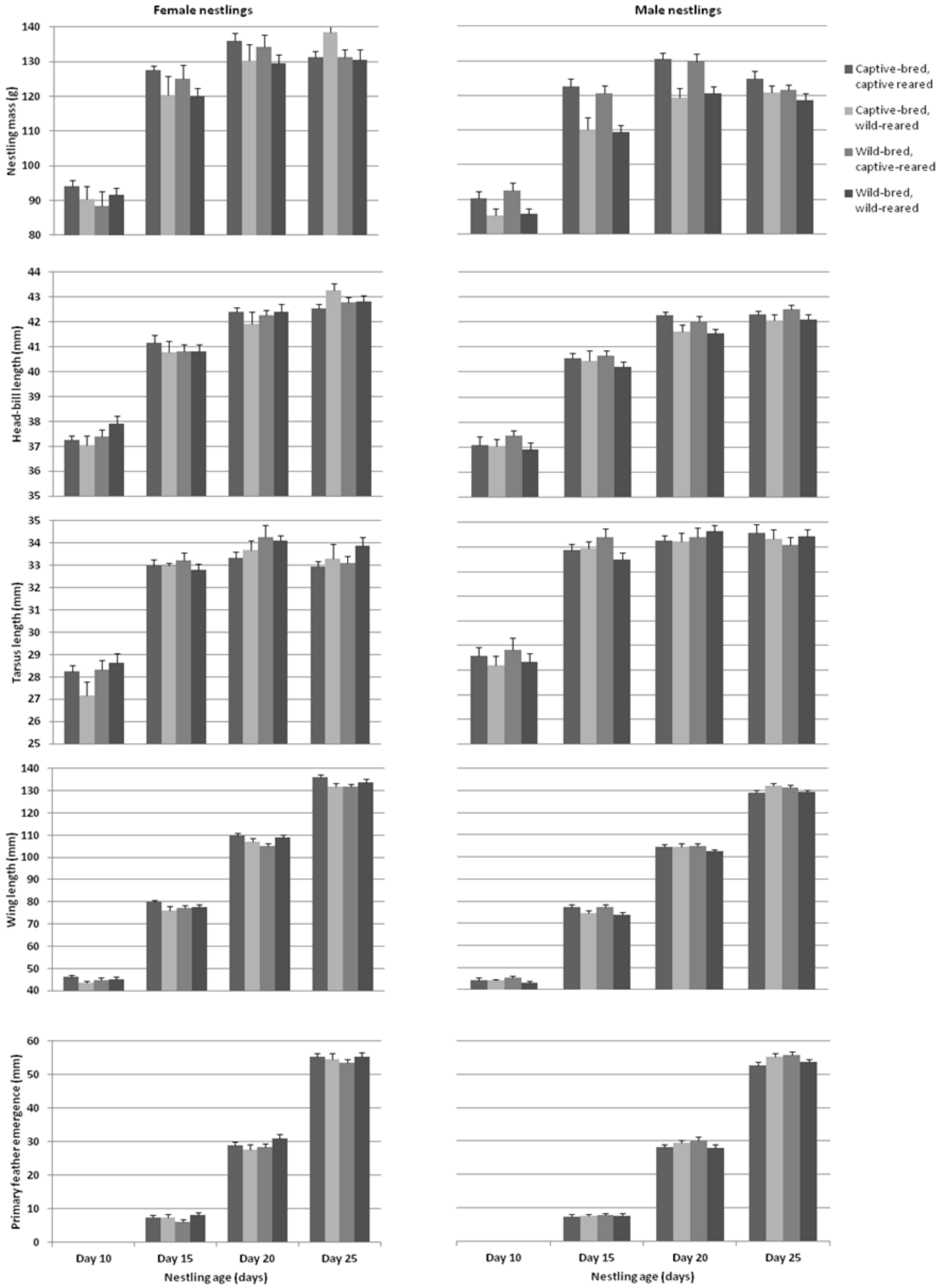

Fig. 1 Mean values and standard errors of five measurements taken from 176 nestling (44 clutches) American kestrels from 2007 to 2009, divided by sex.

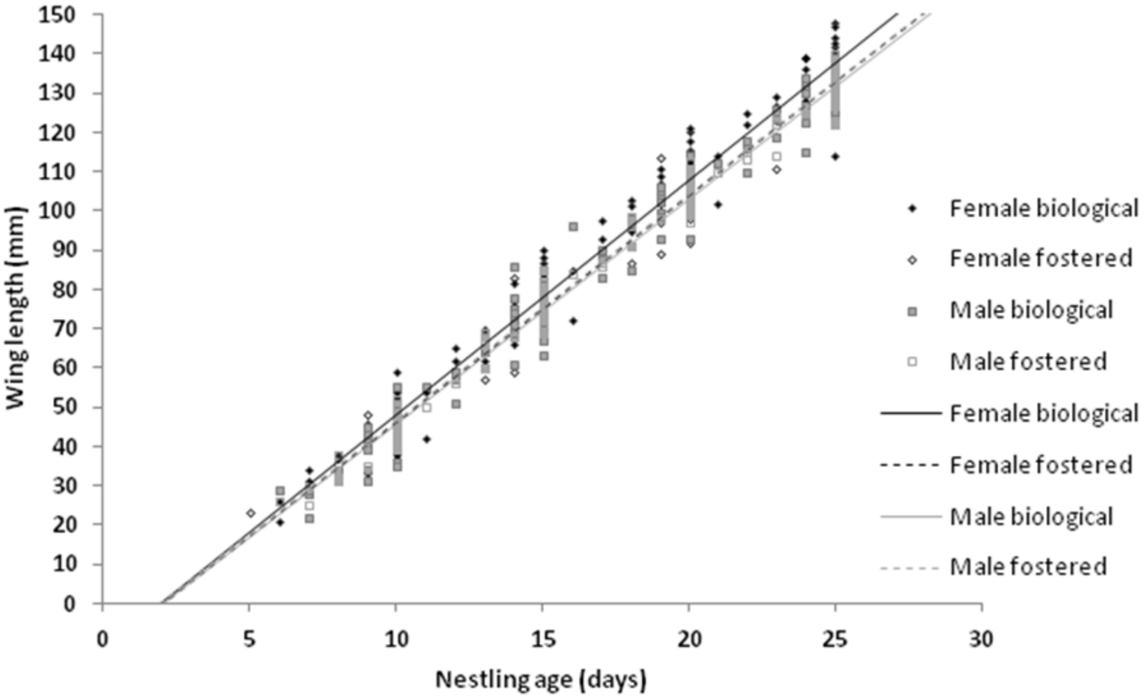

Fig. 2 Wing growth in nestling American kestrels (n = 44 clutches (176 nestlings)). Females demonstrated more rapid wing growth than males. Female young reared by biological parents exhibited more rapid wing growth than those reared by foster parents.

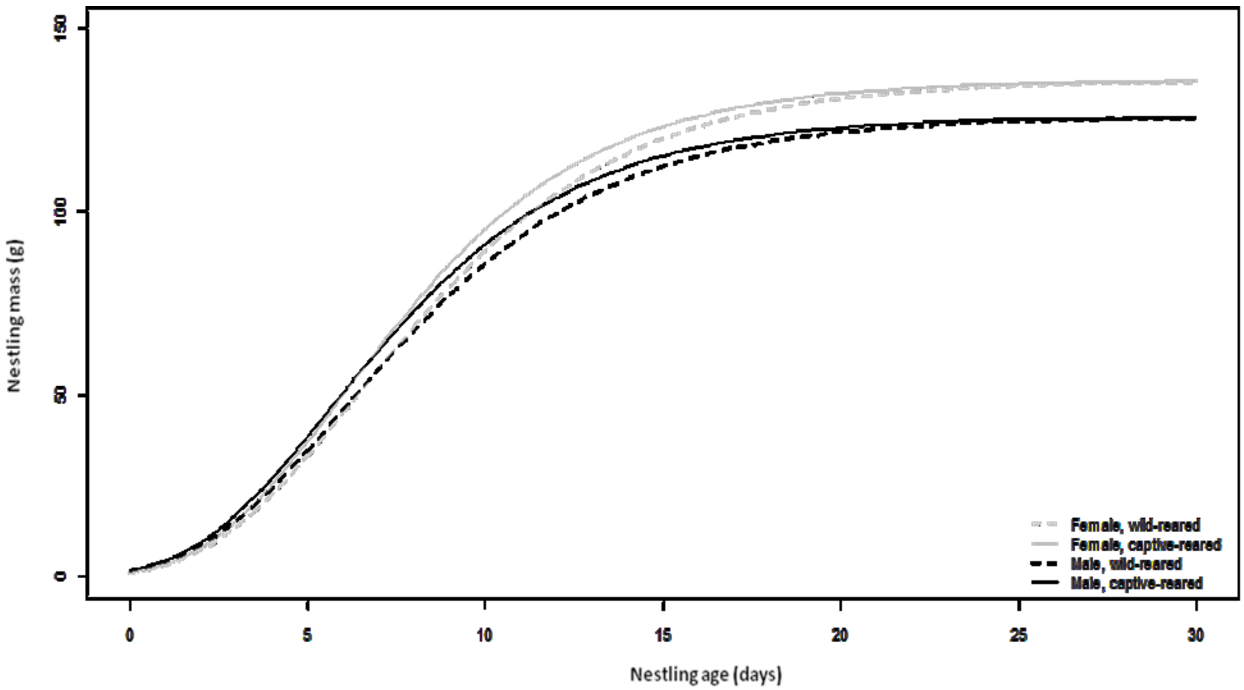

Fig. 3 Growth models for American kestrel nestling mass. Individual growth models are shown for each sex and rearing environment.

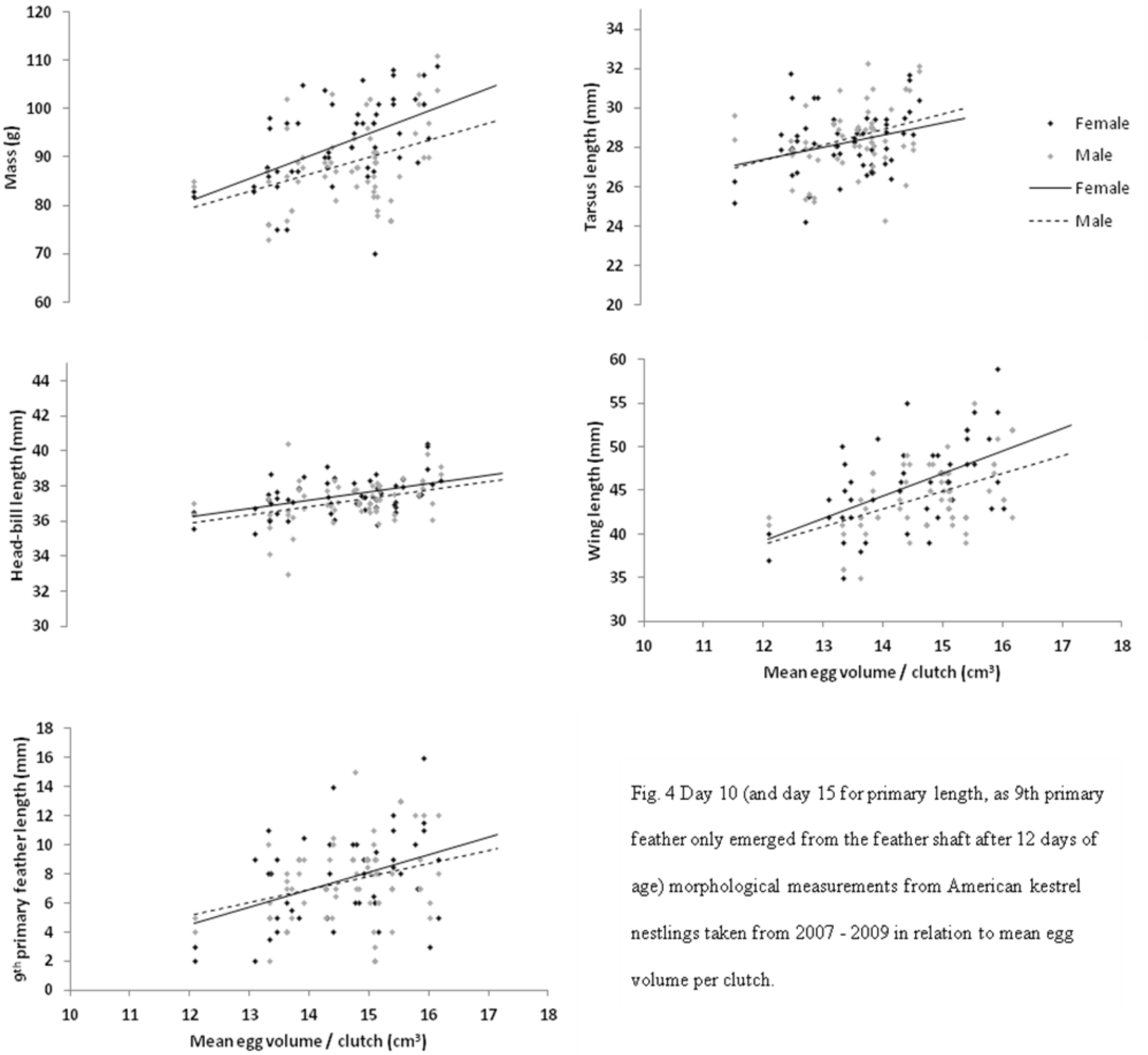

Fig. 4 Day 10 (and day 15 for primary length, as 9th primary feather only emerged from the feather shaft after 12 days of age) morphological measurements from American kestrel nestlings taken from 2007 - 2009 in relation to mean egg volume per clutch.

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CONNECTING STATEMENT 3

The previous two chapters compared adult morphology, nesting success, and nestling growth between captive and wild birds by cross-fostering clutches between the two environments to separate the effects of origin and rearing environment. The cross-fostering experiment provided a unique opportunity for further research into effects of captivity on birds: having been brought into captivity in the egg stage, wild kestrels were reared under identical conditions as captive-bred kestrels, with the only difference between them being their origin. To determine whether wild-bred birds reproduced in captivity with the same success as captive-bred birds, and whether their nestlings had similar growth patterns, in 2009 nestlings produced from 2006 to 2008 were paired in the following manner: captive / captive, captive male / wild-type female, wild-type male / captive female, and wild-type / wild-type. The results of the comparisons between the groups are presented in the following chapter. In addition, data on captive and wild nests from 2006 to 2009 were reanalysed with the data from the above study to determine which, origin or rearing environment, had the greatest impact on the overall results.

CHAPTER 4

COMPARISONS OF ADULT MORPHOLOGY, NEST SUCCESS AND NESTLING GROWTH BETWEEN CAPTIVE-BRED, FIRST-GENERATION CAPTIVE AND WILD AMERICAN KESTRELS (*FALCO SPARVERIUS*)

Abstract

Captive breeding is used to produce animals for wildlife research and to provide animals for release as part of conservation programs. To release captive-bred animals into the wild, or to make comparisons between captive and wild populations in research, it is imperative that captive-bred animals retain their wild-type traits. Research into the effects of captivity on animals is often confounded by the difficulty of separating the effects of origin and rearing environment on captive populations. In this study, comparisons were made of the morphology, nesting success and nestling growth between captive-bred and wild American kestrels reared under identical captive conditions. Results of the study suggest that captive-bred birds had greater nesting success than wild-bred birds, regardless of similar clutch sizes and fertility levels. Significant differences were noted between nestling growth rates, though final asymptotic size of nestlings did not differ between groups. A sex effect was also noted; nests produced by wild-bred males exhibited poorer nesting success than nests produced by captive males, regardless of female. Comparison to pairs in the wild revealed that most differences between captive and wild birds were the result of rearing environment, though there is some suggestion that selection is occurring in response to captivity.

Keywords: Captive breeding, American kestrel *Falco sparverius*, nestling growth, nesting success, first generation captive

Introduction

Populations of wild-type animals are bred in captivity for use as research models in place of wild animals, especially in fields such as evolutionary ecology and environmental toxicology [Bardo and Bird, 2009; Honess et al., 2010; Mainwaring et al., 2010]. Captive models can also be used to create captive breeding and release protocols for endangered species recovery programs [e.g. Wallace and Temple, 1987; Whitmore and Marzluff, 1998; Kueller et al., 2000]. Mitigating the effects of captivity on populations designated for reintroduction as part of a conservation program is important since recovery programs can fail if animals released into the wild are unable to survive and reproduce successfully [McDougall et al., 2006; Seddon et al., 2007].

The use of captive models in wildlife research is favoured over research in the wild for a number of reasons, including reducing impacts on wild populations, having knowledge of pedigrees and life histories of all individuals in a population, and being able to strictly control environmental factors [Santos et al., 2009; Honess et al., 2010]. The assumption made when working with captive models is that they represent wild-type animals, even after multiple generations of captive breeding. This has rarely been verified however, with research specifically designed to test the effects of captivity on wildlife models only recently having been published [e.g. Tschirren et al., 2009; Mainwaring et al., 2010].

With the increasing use of captive breeding for wildlife research and for conservation, greater efforts are being made to understand the effects of captivity on animals and to reduce those effects, allowing captive populations to retain as much as possible their wild-type traits [Seddon et al., 2007; Araki et al., 2008]. It can be difficult to separate the effects of origin and rearing environment on a captive population; differences could be the result of captive conditions, or of selection occurring over generations within a population [Araki et al., 2008].

A large proportion of the variation in nesting success in birds can be related to environmental factors [Clum, 1995]. To determine whether differences in nesting success and nestling growth are related to factors associated with the origin of the birds, environmental effects must be controlled for [Clum, 1995]. One method of controlling environmental effects is to compare captive and wild individuals in an identical captive setting, and this has been done with a small number of species by capturing wild adults for comparison [e.g. Künzl and Sachser, 1999; McPhee, 2003; Tschirren et al., 2009].

Capturing wild individuals to bring into captivity for comparison can have implications for research results, however. Adult condition can be affected by environmental effects during the development stage [Tschirren et al., 2009]. Differences noted between wild-caught and captive-bred birds could be related to environmental factors that influenced the earlier growth and condition of birds taken from the wild, rather than reflecting differences between genetic variables in wild-caught and captive-bred birds. The confounding effect of rearing environment on wild specimens taken for comparison can be reduced by removing them from the wild at an early age. This is especially true for egg-laying species such as birds, where eggs can be removed from one environment and placed into another before hatching occurs, thereby separating origin and rearing conditions [Magrath, 1992].

In the present study, comparisons were made between captive-bred and wild-type American kestrels (*Falco sparverius*) in captivity. Wild kestrels were taken into captivity in the egg stage and parent-reared by captive foster parents until maturity under identical conditions to captive-bred kestrels, therefore any differences between the two populations should relate to origin. The purpose of the experiment was three-fold: 1) to determine whether multiple generations of captive breeding had a significant effect on adult morphology, reproductive performance and growth of nestlings, 2) to ascertain whether effects noted were greater for one sex or the other, since sex effects in response to captivity have been seen in other species [e.g. Jones et al., 2010], and 3) to compare the above results with those of kestrels in the wild to determine whether origin or rearing environment had the greatest impact on wild kestrels reared from the egg stage in a captive environment.

Methods

Study species

The American kestrel is a small New World falcon [Smallwood and Bird, 2002]. The kestrel's small size, ability to breed yearly, relative tolerance of captivity, and relationship to other raptor species, which are often vulnerable to environmental contaminants and frequently the subjects of conservation programs, has made the species a well-known captive wildlife model for over four decades, particularly in the field of environmental toxicology [Seddon et al., 2005; Bardo and Bird, 2009]. Though it is frequently used as a model species, no research has yet determined whether multiple generations of captive breeding have rendered captive kestrels significantly different from wild kestrels.

The Avian Science and Conservation Centre (ASCC) of McGill University in Montreal, Quebec, Canada, has housed a colony of captive American kestrels since 1973

[Bird, 1982]. All birds in the colony descend from the local Montreal kestrel population, and are the result of 10 or more generations of captive breeding. All birds in the colony are descended from the local Montreal population. From 2006 to 2008 whole clutches of eggs from wild nests were fostered to captive pairs to be raised under identical conditions as captive-bred birds. Kestrels produced from wild eggs fostered in captivity were considered to be first-generation (F1) captive birds. In 2009 breeding pairs were formed using F1 and captive birds produced from 2006 to 2008 clutches (nine additional captive birds older than 3 years of age were needed to complete the pairings).

Housing

Pairs were housed in visually isolated, individual pens (2.5 x 1.5 x 2.5 m L x W x H), with rope perches, a food platform, wood-shaving flooring, a screened window for natural lighting, and a nest box (0.3 x 0.3 x 0.4 m L x W x H) filled with wood shavings as substrate. All pairs were fed daily 3 - 4 frozen-thawed, day-old cockerels through a food door to prevent excessive disturbance, and the condition of the birds could be monitored through one-way mirrored glass observation windows.

Experimental design

Thirty-six pairs were formed on April 15th, 2009. The birds were divided into four groups, with ages, breeding experience, and any siblings divided randomly between the groups: captive male paired with captive female (n=12), captive male paired with F1 female (n=7), F1 male paired with captive female (n=9) and F1 male paired with F1 female (n=8). Of these, any incompatible pairs were separated and removed from the study. Nest boxes were checked daily for eggs during morning feeding. Lay dates for each egg were recorded, and eggs were individually marked for identification with a non-toxic marker before being returned to the nest.

At mid-incubation, approximately 14 days after the penultimate egg was laid for kestrels [Smallwood and Bird, 2002] the pair was removed from the pen and placed into individual, aerated holding boxes. Eggs were removed from the nest, and length and breadth of each egg was measured using vernier callipers (accuracy 0.1 mm). The eggs were candled to determine fertility and then returned to the nest. Morphological measurements were then taken from the adults. Vernier callipers were used to measure tarsus and head-bill lengths, and wing chord was measured using a ruler to the nearest mm. Mass was measured using an electric scale to the nearest 0.1g. Adults were returned to their breeding pens and monitored until incubation behaviour resumed.

Hatch dates for all nestlings were recorded, with hatch day considered as day 0 for nestling age. Nestlings were measured 4 times during the nesting period at 10, 15, 20 and 25 days of age pertaining to the oldest nestling in each box. Nestlings were banded on the first measurement date for individual identification. Measurements were taken of the mass, tarsus length, head-bill length and wing chord of each nestling. Growth of the 9th primary feather, the longest primary on a kestrel, was taken using a ruler to the nearest mm [Smallwood and Bird, 2002]. Fledging date was recorded for each nestling, as were the final number of fledglings produced from each nest. All research was conducted with valid McGill University Animal Care Committee protocols.

Statistical analyses

Nests that failed before mid-incubation or that did not produce a clutch were dropped from analyses, as were nests or birds with missing values. Data were analysed using R 2.10.1 statistical software [hereafter R, R Development Core Team, 2009].

Adult morphology

Analysis of covariance (ANCOVA) was computed for all morphological measurements while controlling for measurement date [Crawley, 2007; Blanchet et al., 2008]. Variables tested in the analysis included sex of each bird, origin of the bird (captive or wild), origin of mate (captive or wild), and age in years.

Egg volume

Egg volume was calculated from egg length and breadth using the formula $V = Kv*LB^2$, where V is volume in cm^3, L is egg length in cm, B is egg breadth in cm, and Kv is a constant ($K_V = 0.51$ for eggs without high asymmetry) [Hoyt, 1979]. A mixed model (LME procedure in R) fitted using maximum likelihood method was employed to analyse egg volume [Crawley, 2007]. Eggs were nested within clutch to account for the hierarchical nature of the data [Pelayo and Clark, 2003]. Covariates examined in the model included lay date of the eggs (given by Julian date), female mass and male mass. Categorical variables, including female origin (captive or wild) and male origin (captive or wild), and adult age in years were then added to models containing covariates to determine whether they improved model structure.

Reproductive variables

Generalized linear models with binomial errors and logit link function (GLM procedures in R) were used to study clutch fertility, hatchability of fertile eggs, nestling survival, sex ratio of fledglings in a brood, and overall clutch productivity, defined as the number of fledglings produced from the initial clutch size [Clum, 1995; Crawley, 2007]. Variables considered in the models were adult male and female origin, and covariates included adult mass, adult age, lay date of eggs (given by Julian date), and mean egg volume per clutch. Clutch initiation date, length of hatching period, fledging date, and clutch size were modelled using generalized linear models with Poisson error family in a similar manner [Crawley, 2007].

Nestling growth

As wing chord and primary feathers exhibited a linear growth pattern and did not complete growth before fledging in kestrels, linear mixed-effects models (LME in R) were used to represent their growth [Negro et al., 1994]. Repeated measures were nested within nestling and nestlings nested within clutch for the analyses [Pelayo and Clark, 2003].

Mass, tarsus and head-bill growth were analysed using a self-starting Gompertz function (NLME in R) according to the formula: measurement = $a_0\exp(-b_0b_1^{age})$, which contains three growth parameters a_0, b_0, and b_1 [Sockman et al., 2008]. Parameter a_0 is the asymptotic size of the feature being measured, b_0 represents is the growth rate constant or the slope of the growth curve during the period of linear growth, b_1 represents the location of the inflection point, when maximum growth rate is achieved, and age is the age of the nestling [Ricklefs, 1967]. Random variables were assigned to a_0, the parameter with the greatest variability, according to the methods of Sockman et al. [2008]. Once an adequate preliminary model was achieved, predictors including female origin, male origin, and sex of the nestling were entered into the model to determine whether they improved upon the base model [Sockman et al., 2008]. Repeated measures were nested within nestling within clutch for analyses. Models were estimated using maximum likelihood.

Comparisons to wild nests – adult morphology, egg volume, reproductive variables, nestling growth

Analyses were rerun to include data collected from wild and other captive nests from 2006 to 2009. Only captive / captive and F1 / F1 crosses from 2009 were used for comparison to the new dataset because other members of pairs in the analyses shared a common origin. Rearing environment (captive / wild) was added to analyses as a variable.

Results

Adult morphology

Morphological measurements were analysed for 59 adults (Table 1). Captive birds had significantly longer wings than F1 birds by approximately 2.23 mm ($P = 0.0226$). An interaction effect between origin and age was found for head-bill length; longer head-bill lengths were noted in older captive birds, but shorter in older F1 birds ($P = 0.0090$). An interaction effect between origin, age, and breeding partner was noted for mass; F1 birds had larger masses than captive birds, and mass tended to be greater in older birds, except for F1 males ($P = 0.0019$). A disproportionately high number of birds in the captive male / F1 female pairings had larger masses than birds in other pairings. Females were significantly larger than males in all measurements except tarsus length ($P < 0.0001$ for mass, head-bill length, and wing chord). No significant differences were found between tarsi lengths for any of the variables.

Egg volume

Eleven captive / captive, 7 captive male / F1 female, 7 F1 male / captive female and 8 F1 / F1 pairs produced clutches that survived until mid-incubation (Table 2). In the best-fit model, egg volume was significantly smaller in clutches produced later in the breeding season ($P = 0.0147$).

Reproductive variables

Male mass had a significant effect on clutch fertility ($P < 0.0001$) and nestling survival ($P = 0.0051$); both clutch fertility and nestling survival were lower in clutches produced by pairs containing males with larger mass (Fig. 1). Clutches produced by pairs containing F1 adults had lower egg hatchability than clutches containing captive birds ($P = 0.0189$; Fig. 2). The number of hatchlings produced also decreased significantly with increasing male age ($P = 0.0011$).

An interaction effect between male origin, female origin, and male mass had an effect on overall clutch productivity ($P = 0.0298$). Pairs containing F1 adults produced fewer fledglings than those containing only captive adults, regardless of similar clutches sizes and fertility levels (Fig. 2), and pairs containing males with large mass had lower productivity than those containing males of smaller mass. The pairing with the worst nestling survival appeared to consist of captive males paired with F1 females, with a mean of 2.20 surviving young versus a mean of 3.75 for all other pairs (Fig. 2). This group also contained a disproportionate number of males with large mass. No significant differences were found between any of the variables for clutch initiation date, length of hatching period, fledging date, clutch size, or brood sex ratio.

Nestling growth

Nestlings produced by captive-bred males had faster wing growth than nestlings produced by F1 males ($P = 0.0168$). Female nestlings had longer wings ($P < 0.0001$) and more rapid wing growth than male nestlings ($P < 0.0001$). Primary feather growth and size did not differ between nestlings.

Male origin had a significant effect on mass, tarsus and head-bill growth (Fig. 3). Nestlings produced by captive males had a greater initial head-bill growth ($P < 0.0001$), though nestlings produced by F1males attained a larger asymptotic size ($P = 0.0312$). Tarsus growth was affected by a combination of sex, male and female origin. Tarsus growth rates were greater in nestlings produced by F1 males ($P = 0.0005$). Asymptotic tarsus length differed in size between all four groups.

Growth rate for mass was significantly affected by sex and by an interaction between sex and male origin. Male nestlings had a more rapid growth rate than females ($P = 0.0075$) until females overtook them before 10 days of age. The interaction effect suggested that male nestlings produced by captive male parents had a more rapid growth rate than male nestlings from F1 male parents, while the opposite was true for female nestlings ($P = 0.0260$).

Comparisons to wild nests – adult morphology, egg volume, reproductive variables, nestling growth

A significant difference between sex, origin, and / or rearing environment was noted for all adult measurements (Table 3; Fig. 4). Captive kestrels were the largest and wild birds the smallest, with F1 birds being intermediate in size. Female mass was the exception; wild females had a larger mass.

Egg volume was affected by an interaction of rearing environment and male mass; eggs produced by pairs reared in captivity had larger volumes than those produced by pairs in the wild, however egg volume was smaller when produced by pairs containing males with large mass in captivity, while the reverse relationship was noted in the wild (P = 0.0445).

Origin influenced various reproductive variables (Table 4; Fig. 5). Though clutches produced by wild pairs in the wild had the highest hatchability, when combined with F1 clutches, which had the lowest hatching success, clutches of wild origin had lower hatchability than captive nests (P = 0.0028). Egg hatchability also increased significantly with increasing female mass (P = 0.0005).

Rearing environment also affected reproductive variables. Clutch fertility was significantly higher in the wild than in the captive environment (P = 0.0012). Overall productivity varied significantly with origin, rearing environment, and mean egg volume. Nests reared in the wild had higher overall productivity (P = 0.0042), producing the most fledglings. Generally nests of wild origin had the highest productivity when reared in the wild, and the lowest when reared in captivity, with captive nests showing an intermediate productivity between wild and F1 nests (Fig. 5). The number of young produced was also significantly lower in clutches with larger egg volumes (P = 0.0105). No other measurements showed significant differences between groups.

When including wild nestlings in the analyses, rearing environment had a greater impact on nestling growth than origin, though asymptotic size was indistinguishable between nestlings of each respective sex (Fig. 6). Nestlings reared in captivity generally had faster growth rates than those reared in the wild for most measurements, except for male tarsus growth, which was slower in captivity. No variable effects were found for primary feather growth.

Discussion

Results suggest that most differences observed between captive, wild, and F1 birds were primarily related to environmental effects, but that small differences between captive and F1 birds indicate some level of selection may be occurring in captivity in American kestrels. Results of adult body size and egg size indicated a combined effect of rearing environment and origin on size. Captive birds were generally larger than F1 and wild birds, but F1 birds exhibited small but significant differences from both captive and wild birds, rendering them intermediate in size between captive and wild kestrels for each respective sex. For egg volume, eggs produced in a captive environment were larger than eggs produced in the wild, but captive eggs were also slightly larger than F1 eggs. Increased adult body and egg size in captive environments have been noted in other species [e.g. Leblanc, 1989; Araújo et al., 2000; Mainwaring et al., 2010].

A similar combined effect of environment and origin was seen in kestrel nesting success. Clutch fertility was higher in the wild environment than in captivity, and wild nests had higher hatchability and overall clutch productivity than captive-reared nests. Within captivity however, captive birds had higher hatchability and clutch productivity than F1 birds. When comparing the three groups, wild birds were the most successful at producing surviving young, and F1 birds the least. The decreased nesting success in captivity could relate to a lack of mate choice in the pairs; captive birds are paired according to genealogy only, and this may not be sufficient criteria to create compatibility between members of a pair. Captive conditions could also account for reduced nesting success, perhaps as a result of excessive human disturbance or close proximity of other pairs (pairs are visually but not audibly isolated).

Though there was a clear environmental difference in nesting success, significant differences were also noted between captive and F1 pairs, with F1 birds having poorer nesting success than captive birds. This suggests that captive birds have undergone some degree of selection in captivity, rendering them different from their wild counterparts even when reared in an identical environment. This difference between captive and wild-type birds is further demonstrated when looking at clutch initiation dates and hatching length; F1 birds had clutch initiation dates and hatching periods more similar to wild birds than to captive pairs. Clutch initiation dates were later and hatching periods longer in birds of wild origin regardless of rearing environment. The longer hatching period in wild and F1 nests is due in part to the later clutch initiation for these nests, as clutches produced later in the season had longer hatching periods.

Clutch initiation dates have been shown to be affected by factors related to the origin of the birds, as seen in a study of blue tits (*Parus caeruleus*), where birds brought into captivity from various geographic subpopulations initiated breeding at the same time as their respective wild subpopulations, regardless of identical captive conditions [Lambrechts et al., 1999]. The difference in timing of reproduction between captive and F1 kestrels may in part account for reduced nesting success in pairs containing F1 birds. Pairing blue tits from different subpopulations together in captivity led to higher levels of reproductive failure than pairs composed of individuals from the same subpopulation [Caro et al., 2007].

Differences in nestling growth between groups were primarily the result of rearing environment. Nestlings reared in captivity had faster initial growth rates than those reared in the wild, though asymptotic size did not differ significantly between nestlings. Likely access to ad libitum food in captivity accounted for increased initial growth rate, as seen in other studies [e.g. Santos et al., 2009; Ritz et al., 2010]. Minor differences in growth rates between captive and F1 nestlings were also noted, but they were inconsistent and short-lived. Previous studies of both captive and wild kestrel nestling growth found that structural size was relatively unaffected by experimental manipulations, and that mass exhibited compensatory growth to attain the same final asymptotic size as control nestlings [e.g. Gard and Bird, 1992; Lacombe et al., 1994; Negro et al., 1994]. The lack of variability in nestling growth in response to experimental factors could be a consequence of adaptations in wild kestrels to variable food availability and of the importance of limb function to survival in wild birds for both hunting and predator avoidance [Gard and Bird, 1992; Lacombe et al., 1994; Negro et al., 1994]. Ten generations of captive breeding may not have comprised enough time to greatly influence evolutionary adaptations in kestrel nestling growth.

Captivity appeared to have a greater effect on F1 males than on females for nesting success. Clutches produced by F1 males had smaller eggs, decreased hatching success, and lower overall clutch productivity than clutches produced by captive males. The only failed clutches in the study were produced the mates of by F1 males. Differences in growth rates of F1 and captive nestlings were related to male origin as opposed to female origin. Sex differences in response to captivity, particularly in regards to nesting success, have been noted in other species, including peregrine falcons (*F.*

peregrinus) [Clum, 1995], striped mice (*Rhabdomys* spp.) [Jones et al., 2010], blue tits [Caro et al., 2007], and black-footed ferrets (*Mustela nigripes*) [Wolf et al., 2000]. The reason for differential response to captivity between sexes is unknown, but may relate to sex roles in reproduction, at least for kestrels. Male kestrels initiate courtship during the breeding season and are responsible for provisioning both the female and later the young for a large portion of the nesting period [Smallwood and Bird, 2002]. They can therefore have an important impact on nest success and it is possible that physical or behavioural differences between F1 and captive males could account for the differences in nest success. It is possible that after over 10 generations of captive breeding the captive population has become more likely to contain males more amenable to breeding in captive conditions.

Male kestrels had an important impact on nesting success in this study regardless of origin or rearing environment. Male mass contributed significantly to clutch fertility, nestling survival and overall clutch productivity. Males of lower mass produced more fertile clutches and a higher number of surviving nestlings than males with large mass. In most Falconiformes, males initiate courtship and are the primary providers throughout the incubation and early nestling stages [Sergio et al., 2007]. In studies of other raptor species, small male body size was associated with greater reproductive output [e.g. Massemin et al., 2000; Sergio et al., 2007]. Smaller male raptors may be more effective predators or may have lower energy requirements than larger males, and may thus be able to contribute more food to the female and young, enhancing their nesting success [Sergio et al., 2007]. The fact that male kestrels initiate courtship might also be the reason why pairs containing F1 males had poorer nesting success than those with captive males, especially given the differences in timing of clutch initiation between the two groups of birds. Captive males may have undergone selection to allow them to reproduce more successfully in captivity.

Results of this study parallel those of recent comparisons between captive, wild and wild-caught adult zebra finches (*Taeniopygia guttata*), another common captive avian model [Tschirren et al., 2009; Mainwaring et al., 2010]. Captive finches were larger than wild or wild-caught birds, as were their eggs. Conversely, nestlings produced by captive finches were larger than wild-type nestlings at all stages of growth and into adulthood [Tschirren et al., 2009; Mainwaring et al., 2010]. Unlike kestrels, zebra finches have been bred in captivity for a greater number of generations (over 100, instead of 10 or more in kestrels), and have been subjected to intentional selection for particular traits such as size or color by aviculturalists [Tschirren et al., 2009]. Results of the finch study could serve as an indicator of the extent of differences that could arise in captive kestrel populations after repeated generations of captive breeding unless measures are taken to reduce effects of the captive environment on birds.

Conclusions

1 Results in this study indicate a combined effect of rearing environment and origin on kestrel size and nesting success. While environmental effects were greater, differences between F1 and captive birds and similarities shared by F1 and wild birds suggest that some level of selection has occurred in captivity.

2 Though rearing environment affected initial growth rate in nestlings, asymptotic size did not differ between captive, F1 and wild nestlings.

3 A difference between sex effects in relation to nesting success was noted in F1 birds: F1 males had lower nesting success than captive males. This suggests that captivity had a greater effect on male kestrels than females.

4 Considering results of captive and F1 nesting success, care should be taken when pairing captive and wild-type birds together in the future until further research is done on the effects of captivity on nesting success. This could have implications for re-stocking schemes intended to introduce new genes into captive colonies to reduce potential loss of genetic diversity in captivity.

5 First-generation captive birds, at least in kestrels, should be included in comparative studies of nesting success in captive populations only with caution as their success rates differ from captive-bred birds.

6 The use of wild birds reared in captivity from the egg-stage was an effective method of separating the effects of origin and rearing environment on results found for F1 birds.

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Table 1. ANCOVA table of results for mass, head-bill length, tarsus length and wing chord of adult captive and F1 American kestrels (n = 59)

Adult structure	Source of variation	df	SS	F	P
Mass	Sex	1	5704.30	110.52	< 0.0001 ***
	Origin	1	3.40	0.07	0.7986 n.s.
	Pairedwith	1	1.30	0.02	0.8767 n.s.
	Age	1	100.00	1.94	0.1700 n.s.
	Origin:Pairedwith	1	204.40	3.96	0.0521 .
	Origin:Age	1	1.40	0.03	0.8683 n.s.
	Pairedwith:Age	1	4.50	0.09	0.7690 n.s.
	Origin:Pairedwith:Age	1	557.40	10.80	0.0019 **
	Residuals	50	2580.60		
Head-bill	Sex	1	24.12	31.89	< 0.0001 ***
	Origin	1	1.49	1.97	0.1671 n.s.
	Pairedwith	1	0.27	0.36	0.5508 n.s.
	Age	1	2.42	3.20	0.0798 .
	Origin:Pairedwith	1	2.54	3.36	0.0729 .
	Origin:Age	1	5.60	7.40	0.0090 **
	Pairedwith:Age	1	2.65	3.51	0.0669 .
	Origin:Pairedwith:Age	1	0.65	0.86	0.3592 n.s.
	Residuals	50	37.81		
Tarsus	Sex	1	0.09	0.06	0.8009 n.s.
	Origin	1	1.57	1.13	0.2930 n.s.
	Pairedwith	1	0.08	0.06	0.8095 n.s.
	Age	1	2.72	1.96	0.1678 n.s.
	Origin:Pairedwith	1	0.22	0.16	0.6930 n.s.
	Origin:Age	1	0.53	0.38	0.5397 n.s.
	Pairedwith:Age	1	0.54	0.39	0.5373 n.s.
	Origin:Pairedwith:Age	1	3.06	2.20	0.1442 n.s.
	Residuals	50	69.43		
Wing	Sex	1	457.13	32.07	< 0.0001 ***
	Origin	1	78.88	5.53	0.0226 *
	Pairedwith	1	7.02	0.49	0.4862 n.s.
	Age	1	12.95	0.91	0.3452 n.s.
	Origin:Pairedwith	1	12.65	0.89	0.3507 n.s.
	Origin:Age	1	0.05	0.00	0.9537 n.s.
	Pairedwith:Age	1	0.04	0.00	0.9593 n.s.
	Origin:Pairedwith:Age	1	0.05	0.00	0.9517 n.s.
	Residuals	50	712.80		

n.s., not significant, . p< 0.10, * p < 0.05, ** p < 0.01, *** p < 0.001

Table 2. Summary of reproductive variables for clutches produced by pairs of captive and F1 American kestrels in 2009 (mean ± SE, with number of clutches indicated in parentheses in first column)

	Captive male		F1 male	
	Captive female	F1 female	Captive female	F1 female
Egg volume (cm^3) (33)	14.99 ± 0.14	14.63 ± 0.21	14.58 ± 0.12	14.49 ± 0.19
No. eggs per clutch (33)	4.90 ± 0.09	5.00 ± 0.00	4.86 ± 0.26	4.88 ± 0.13
Clutch initiation date (33)	03 May ± 2.40	30 April ± 1.50	05 May ± 2.60	04 May ± 2.40
No. fertile eggs / clutch (33)	4.40 ± 0.22	3.20 ± 0.66	4.17 ± 0.48	4.13 ± 0.61
No. hatched fertile eggs / clutch (33)	4.20 ± 0.20	2.20 ± 0.58	3.17 ± 0.75	3.71 ± 0.42
Length of hatching period (days) (29)	2.20 ± 0.42	1.29 ± 0.52	1.60 ± 0.68	1.83 ± 0.54
No. surviving hatchlings / clutch (29)	4.10 ± 0.18	2.20 ± 0.58	3.60 ± 0.51	3.83 ± 0.48
No. female young / clutch (29)	2.30 ± 0.37	0.80 ± 0.37	1.60 ± 0.40	1.33 ± 0.56
No. nests producing at least 1 fledging / total nests (33)	11 / 11	7 / 7	5 / 7	6 / 8

Table 3. Morphological measurements (mean ± SE) taken at mid-incubation from adult captive, F1, and wild American kestrels (2006 – 2009)

	Female			Male		
	Captive	F1	Wild	Captive	F1	Wild
Mass (g)	135.55 ± 1.21	136.04 ± 1.68	141.83 ± 1.91	116.50 ± 1.12	116.12 ± 2.60	107.67 ± 1.61
Head-bill length (mm)	43.96 ± 0.14	43.41 ± 0.25	43.01 ± 0.15	42.56 ± 0.18	42.39 ± 0.25	41.92 ± 0.18
Tarsus length (mm)	33.52 ± 0.19	34.02 ± 0.32	32.93 ± 0.23	34.03 ± 0.27	33.66 ± 0.34	32.63 ± 0.26
Wing chord (mm)	194.53 ± 0.59	191.35 ± 1.11	192.89 ± 0.72	186.74 ± 0.60	185.64 ± 0.92	182.89 ± 0.88
N	44	13	36	43	14	18

Table 4. Mean values of reproductive variables for captive, F1, and wild pairs of American kestrels from 2006 - 2009 (mean ± SE, with number of clutches indicated in parentheses in first column)

	Captive	F1	Wild
Egg volume (cm^3) (57)	14.74 ± 0.08	14.59 ± 0.17	14.43 ± 0.10
No. eggs / clutch (57)	5.04 ± 0.07	4.88 ± 0.13	4.88 ± 0.11
Clutch initiation date (57)	May 01 ± 1.62	May 04 ± 2.41	May 06 ± 2.34
No. fertile eggs / clutch (57)	4.16 ± 0.27	4.13 ± 0.61	4.81 ± 0.16
No. hatched fertile eggs / clutch (57)	3.72 ± 0.28	3.25 ± 0.59	3.73 ± 0.37
Length of hatching period (days) (50)	1.62 ± 0.31	1.83 ± 0.54	2.26 ± 0.37
No. surviving fledglings / clutch (50)	3.65 ± 0.29	3.29 ± 0.68	4.32 ± 0.20
No. female young / clutch (50)	1.88 ± 0.28	1.14 ± 0.51	2.05 ± 0.20
No. nests producing at least 1 fledgling / total nests (57)	23 / 25	6 / 8	21 / 24

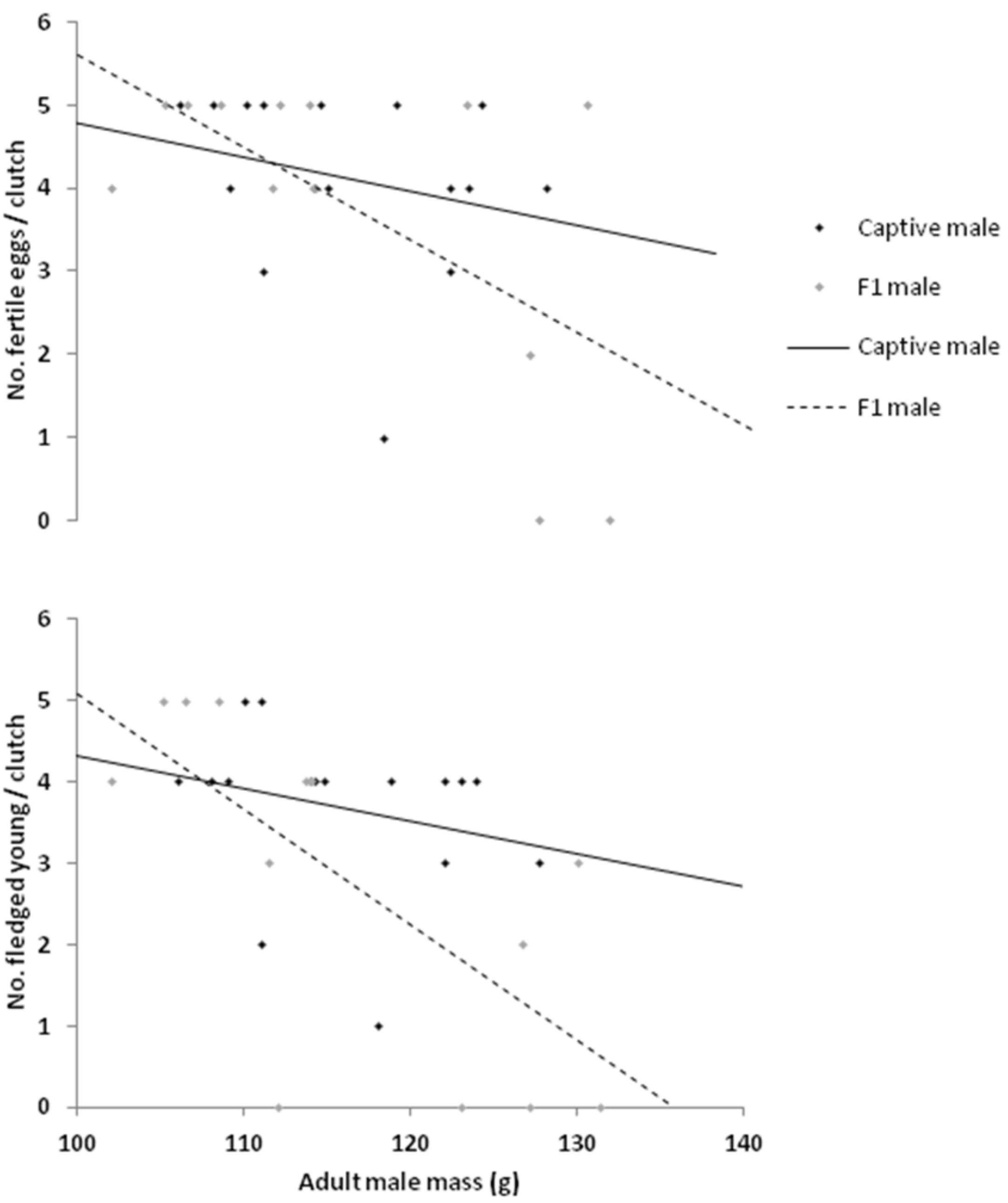

Fig. 1. Clutch fertility and nestling survival in captive and F1 pairs of American kestrels from 2009 in relation to mass of the male parent.

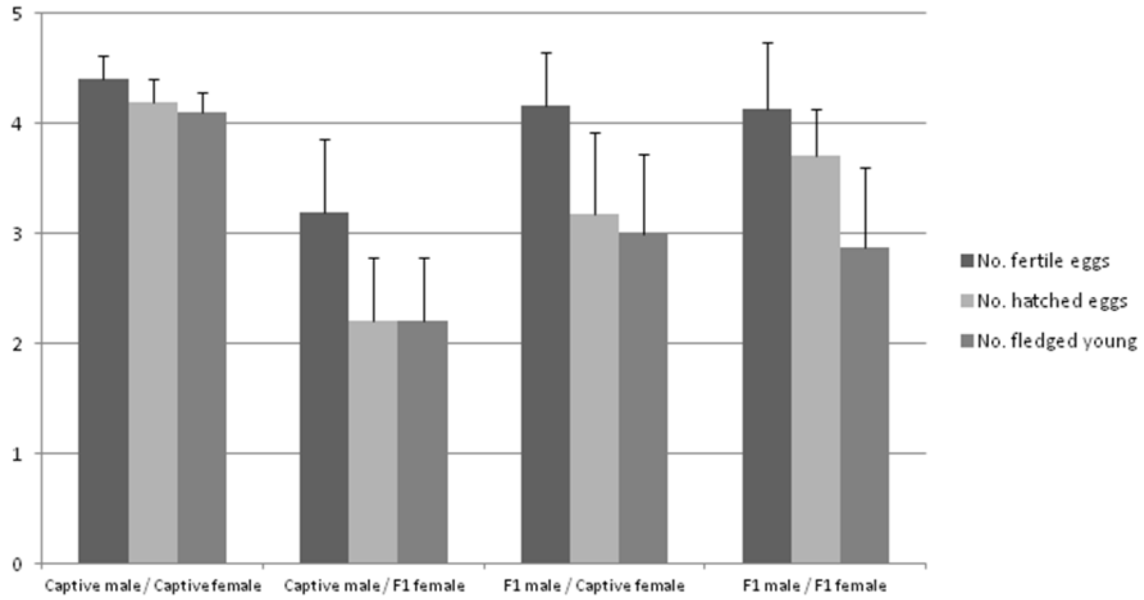

Fig. 2 Mean and standard error for clutch fertility, hatchability, and no. fledglings produced by pairs of captive and F1 American kestrels in 2009.

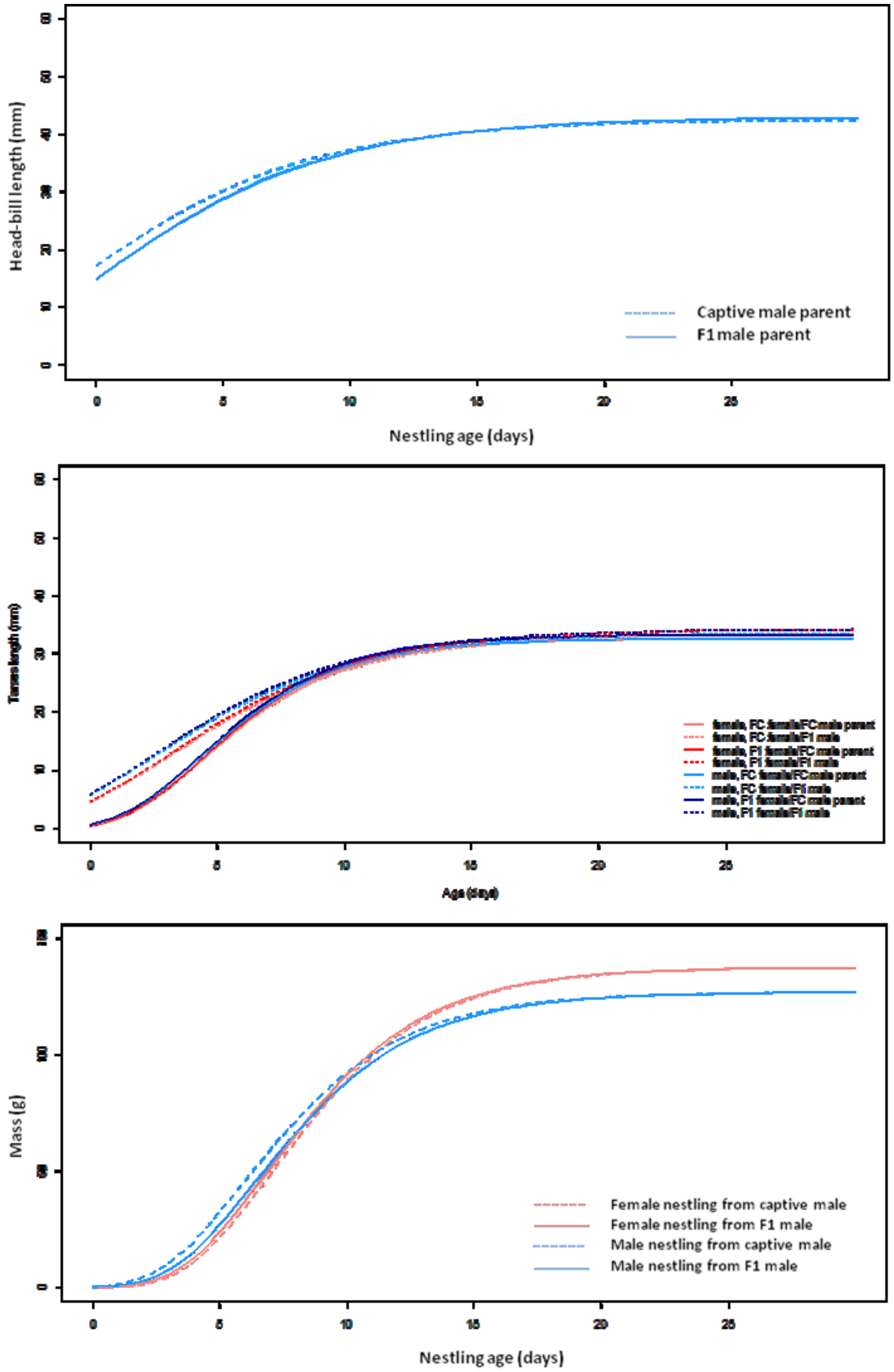

Fig. 3 Nonlinear growth models for head-bill (top), tarsus (middle), and mass (bottom) growth in American kestrel nestlings from captive and F1 pairs in 2009. Origin of male parent affected growth for head-bill and mass in nestlings, while a combination of effects from adult male and female origin were found for tarsus growth.

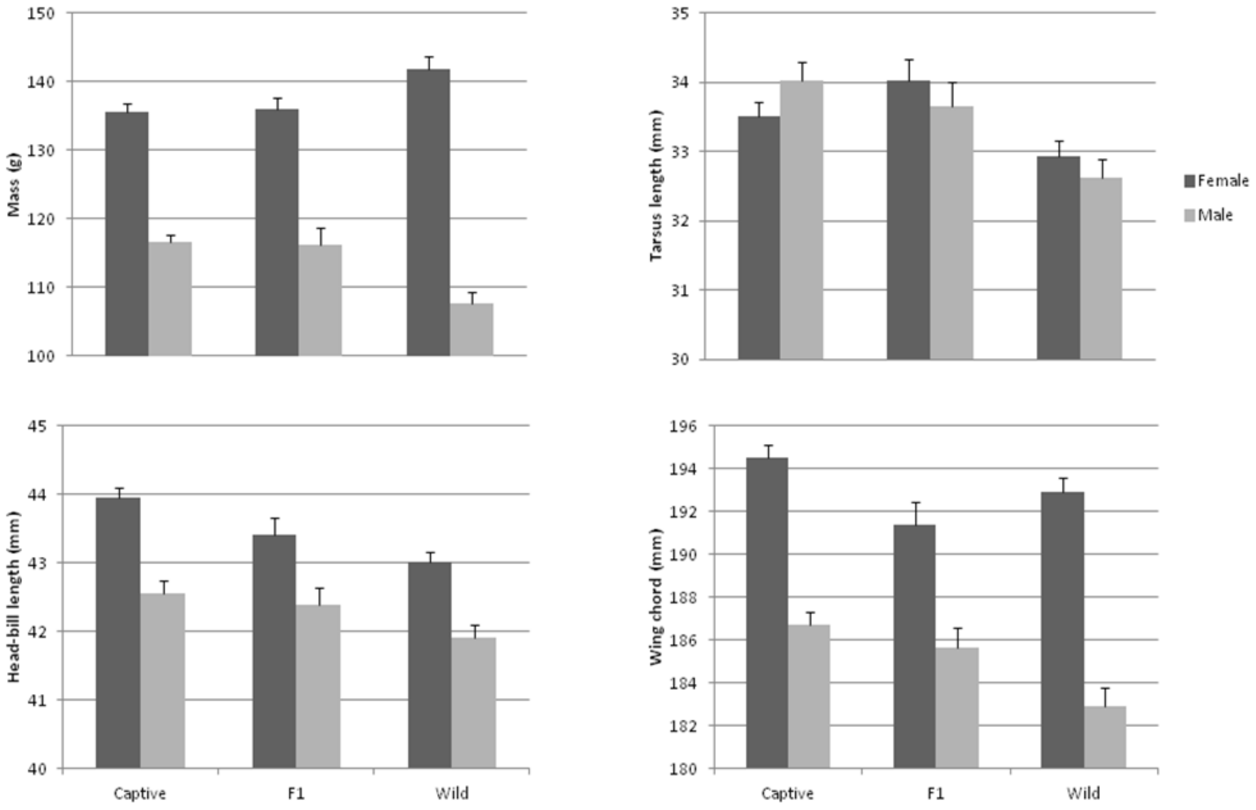

Fig. 4 Mean and standard errors of adult morphological measurements for captive, F1, and wild American kestrels (2006 - 2009). Captive birds were generally larger than wild birds for all measures except for female mass, in which wild females were larger. Measurements of F1 birds tended to be intermediate between captive and wild birds.

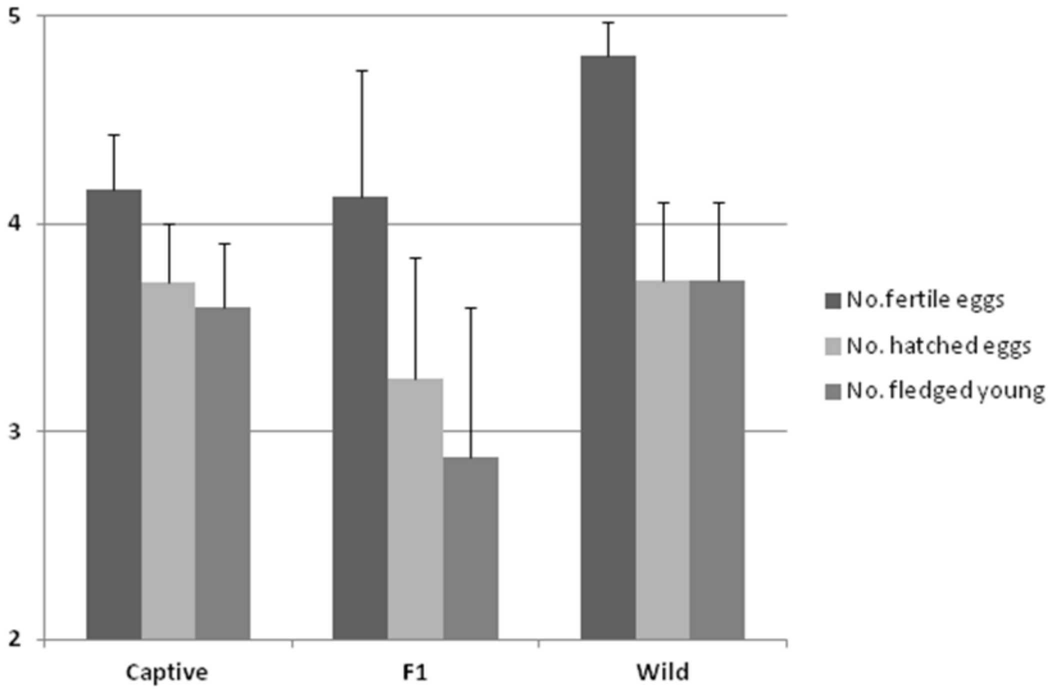

Fig. 5 Mean and standard errors of clutch fertility, hatchability and no. fledglings for captive, F1, and wild American kestrel pairs from 2006 - 2009.

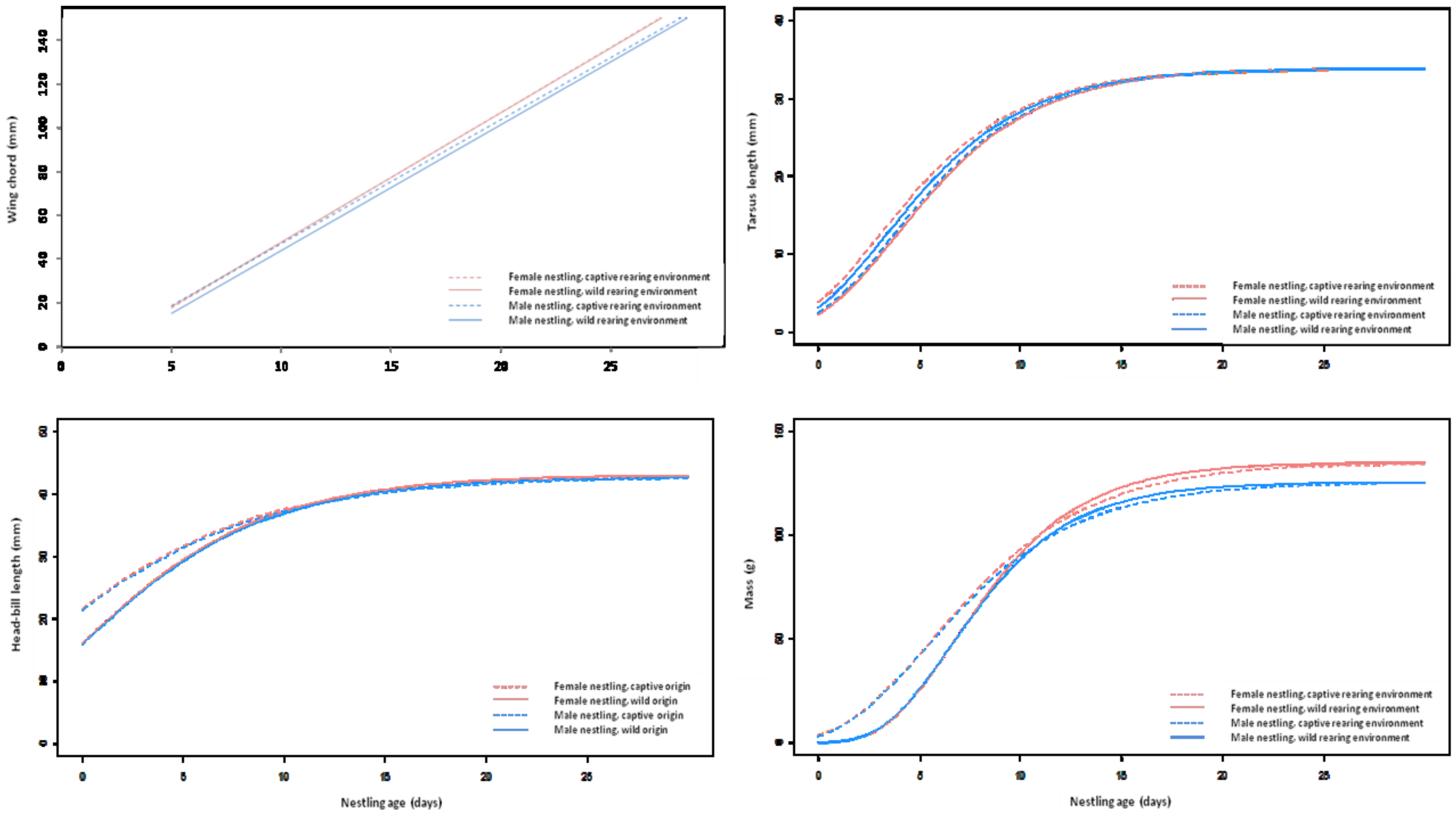

Fig. 6 Models of growth for nestlings from captive, F1, and wild American kestrel pairs from 2006 - 2009. Graphs show effects of rearing environment or of genetic origin for each sex.

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CONNECTING STATEMENT 4

The previous three chapters focussed on determining what, if any, differences existed between captive, first-generation captive, and wild American kestrels in terms of adult size, nesting success, and nestling growth, and if these differences could be attributed to origin or rearing environment. Both factors resulted in significant differences between kestrels, though rearing environmental was the predominant cause of most of the differences noted between wild and captive kestrels. In the following chapter, blood samples taken from adult and nestling kestrels throughout the study were analysed for carotenoid concentration, and were compared, along with integumentary color, between the birds to determine whether color expression in kestrels was affected by origin or rearing environment. Since carotenoids influence avian health and external expression of these colors may indicate a bird’s condition, reproductive variables were modelled using parental carotenoid levels to determine if they had any influence over nest success, and if these differences could be related to rearing environment or origin.

CHAPTER 5

PLASMA CAROTENOID CONCENTRATIONS AND EXTERNAL COLOR IN CAPTIVE-BRED, FIRST-GENERATION CAPTIVE AND WILD ADULT AND NESTLING AMERICAN KESTRELS

Abstract

Studies of physiological variables in wildlife species are often conducted in captive settings, yet few studies have addressed potential biases arising from using captive animals in such research. Carotenoids, pigments absorbed through the diet, affect an animal's health and immune function, as well as external expression of many yellows, oranges, and reds on the body. In the present study external color expression and plasma carotenoid concentrations were compared between adult captive, first-generation captive, and wild American kestrels to determine the relative effect of origin and rearing environment on these variables. Adult carotenoid levels were then tested for their effects on nestling carotenoid concentration and nest success. Rearing environment had a greater effect on adult color and carotenoid levels than origin; adults in the wild exhibited higher color scores and higher plasma carotenoid concentrations than captive birds. Conversely, captive nestlings had higher plasma carotenoid concentrations than wild nestlings, though this effect was lost as the nestlings aged. Neither parental coloration nor carotenoid levels appeared to have major effects on reproductive output or on nestling carotenoid levels.

Keywords: American kestrel *Falco sparverius*, captive, carotenoids, color, wild

Introduction

The analyses of hematological variables can be used to assess the condition of individual animals and is becoming a more frequently-used method of quantifying an animal's state of health in wildlife research (Sepp et al. 2010). However, much of the hematological research in wildlife has been conducted on wild specimens in captivity, and research into the effects of captivity on animal physiology is still relatively new (Sepp et al. 2010). Since captive populations are often used to design sampling methods for field work and to conduct research on physiological variables in a controlled setting, it is critical to have an understanding of any potential effects of captivity on animal physiology.

Determining whether reported differences between captive and wild individuals are the result of genetic or environmental factors can be difficult unless environmental effects can be controlled (Clum 1995). Birds make ideal research subjects in studies that require the separation of origin and rearing environment because they can be transferred from one environment into another during the egg-stage, thus separating pre- (origin) and post-hatching (environmental) variables (Magrath 1992).

Carotenoids are fat-soluble pigments responsible for most red and yellow colors exhibited by vertebrate animals (Negro et al. 1998; Sternalski et al. 2010). Animals cannot manufacture carotenoids and must acquire them from the environment through diet (Bortolotti et al. 1996). Carotenoids are important to animal health, as they act to stimulate immune function, exhibit antioxidant properties, scavenge free-radicals in the body and serve as a precursor to vitamin A (Negro et al. 1998; Bortolotti et al. 2000; McGraw and Ardia 2004; Sternalski et al. 2010).

Because of the effect of carotenoids on an animal's health, the expression of yellow, orange and red coloration on the body is thought to signal an animal's condition and to serve as an indicator of quality in mate selection. In zebra finches (*Taeniopygia guttata*) carotenoid supplementation experiments have shown that more colorful bills are associated with higher plasma carotenoid levels, and that birds with higher carotenoid levels also had increased immune response (Blount et al. 2003, McGraw and Ardia 2003). Female finches also preferred males with more colorful bills. In studies of house finches (*Carpodacus mexicanus*), male finches with brighter color scores exhibited faster feather growth (Hill and Montgomerie 1994), food stress reduced finches' abilities to express carotenoid-based plumage color (Hill 2000), and finches with brighter red plumage possessed fewer mites and grew in longer wing feathers during moult (Thompson et al. 1997). Male house finches with brighter red plumage were found to make more frequent feeding visits to their nests (Hill 1992).Wild male house finches with artificially brightened plumage were more likely to acquire a mate and to pair earlier in the breeding season (Hill 1992). In nestlings, which have underdeveloped immune systems and undergo rapid growth, carotenoids are important in removing free radicals (Casagrande et al. 2007).

The overall aim of this study was to examine external color and plasma carotenoid concentration in American kestrels (*Falco sparverius*) to determine whether differences existed between wild and captive kestrels and whether these differences could be attributed to origin, rearing environment, or a combination of both factors. External color was compared to carotenoid concentrations in blood plasma as blood serves as an intermediary between diet and deposition of pigment in the skin. In the past plasma concentrations of carotenoids have been shown to correlate with color scoring in kestrels (Bortolotti et al. 1996).

It has been previously shown that male kestrels exhibit brighter integument coloration and higher carotenoid levels during portions of the breeding season than females, which suggests the use of color for sexual selection in this species (Negro et al. 1998). Because the free choice of mates does not often occur in captivity, it is conceivable that multiple generations of captive breeding could result in relaxed selection for external expression of color in kestrels. Thus, in this study reproductive variables and nestling carotenoid levels were modelled to determine whether color expression or plasma carotenoid levels in adults had any effects on these results, and to investigate whether these effects differed between wild and captive pairs.

Materials and methods

Study birds

The American kestrel is a small, sexually dimorphic and dichromatic New World falcon that has been used in recent decades as a captive model species, particularly for research in reproductive biology, physiology and environmental toxicology (Smallwood and Bird 2002; Bardo and Bird 2009). American kestrels in this study came from a captive colony housed at the Avian Science and Conservation Centre (ASCC) of McGill University, Montreal, Canada since 1973 (Bird 1982), and from a wild breeding population using supplied nest boxes in the region of Montreal, Quebec. All captive birds in the colony descend from the local kestrel population, removing any potential ancestral regional differences between the birds.

Pairs of kestrels in the study belonged to one of three groups: 1) captive-bred (hereafter captive) birds from the ASCC colony with at least 10 generations of captive ancestry, unrelated to each other for at least five generations paired from 2006 to 2009, 2) wild pairs established in nest boxes in the Montreal region from 2006 to 2009, and 3) wild birds fostered to captive parents at the egg-stage from 2006 to 2008 and paired together in 2009. The third group of birds was referred to as first generation captive (hereafter F1) kestrels, as they were of wild origin but reared under identical captive conditions as captive kestrels. In an attempt to simulate the natural timing of the breeding season, birds in captivity were paired upon first local sighting of wild pairs on territory. All birds were exposed to natural lighting conditions. Captive pairs were individually housed in visually isolated breeding pens with access to a nest box of similar dimensions to wild nest boxes. Pairs in captivity were fed a daily diet of 3 – 4 frozen-thawed, day-old cockerels.

Data collection and laboratory analyses

At mid-incubation, 14 days after the production of the penultimate egg in kestrels (Smallwood and Bird 2002), incubating adults were removed from the nest by hand (wild) or captured using a hand-net (captive). In captivity, the male and female in a pair were sampled at the same time. In the wild, females as the primary incubators were captured more frequently. Any incubating males were sampled and their females were caught and samples on subsequent trips. Upon capture, the mass of each adult was taken.

A color chart was used to visually rate the color of the cere (skin above the bill), lores (anterior patch of skin to the eyes), and tarsi of each adult on a scale of 1 (dull yellow) to 6 (dark orange, Bortolotti et al. 2000, Blount et al. 2003). The color range of these unfeathered regions is typically associated with expression of carotenoids in the body, and have been used to rate color in raptors in the past (e.g. Bortolotti et al. 1996; Negro et al. 1998; Sternalski et al. 2010). These fleshy regions offer a more recent representation of carotenoid levels in the body than feathers do, as feathers only indicate carotenoid levels at the time they were grown in, and are also subject to fading and wear (Bortolotti et al. 1996). The three values were then added together for a total color score out of 18 (Bortolotti et al. 1996). High scores indicated darker colored birds and low scores dull or pale birds.

A 0.5 - 1.0 ml sample of blood was taken from the right jugular vein of each bird using a heparinized syringe, after which the bird was returned to its breeding pen or released in the vicinity of its nest box in the wild. Blood samples were stored on ice in centrifuge tubes until they could be centrifuged at 10, 000 rpm for 5 min to separate out the plasma, which was then stored at – 20^{o} Celsius for later analyses (Bortolotti et al. 1996).

Data were collected on egg fertility using an electric candler, and the sex and number of hatchlings and fledglings produced by each pair was recorded. A nestling was considered successfully fledged if it survived until the final day 25 measurement to reduce the risk of early fledging potentially caused by visiting the nests after day 25. Mass was measured for each nestling at days 10, 15, 20, and 25 of the oldest nestling in the clutch. A random male and female nestling was selected within each brood and a 0.3 ml blood sample was taken from them upon each visit. Blood samples were collected and stored in the same manner as adult samples. Nestling kestrels were not color-scored as they did not exhibit much integument coloration before fledging. Handling of the birds in this study was conducted with valid McGill Animal Care Committee Animal Use Protocols (# 3372 and # 5334).

Plasma samples were thawed and analysed for carotenoid concentrations. Samples of 50 µl of plasma were diluted in a 1:10 ratio with acetone (Bortolotti et al. 1996). Samples were then centrifuged at 10, 000 rpm for 10 min to precipitate out the flocculent. The supernatant was examined using a Pharmica Biotech Ultraspec 2000 UV / Visible Spectrophotometer set at 476 nm to determine the optical density of the carotenoid peak (Bortolotti et al. 1996). The concentration of carotenoids (µg / ml) in each sample was calculated from a standard curve of lutein (alpha-carotene-3, 3'-diol, Sigma Co., Bortolotti et al. 1996). Lutein is the dominant carotenoid found in bird plasma (Negro et al. 2001) and is deposited untransformed in fleshy parts such as the cere, lores and tarsi (Casagrande et al. 2007).

Statistical analyses

Data were analysed using R 2.10.1 statistical software (hereafter R; R Development Core Team 2009). Color scores for adult kestrels were modelled using generalized linear models (GLM in R) with a Poisson error family (Crawley 2007). Untransformed plasma carotenoid concentrations for adults were modelled using GLM with Gamma error distribution and log link function (Tella et al. 2004). Variables examined in both sets of models included sex, origin (captive or wild), rearing environment (captive or wild), plasma carotenoid concentration (for color score), adult mass, measure date (give by Julian date), and year. Relationships between plasma carotenoid concentrations and body mass and Julian measure date were also examined using Spearman correlations (Tella et al. 2004).

When examining reproductive and nestling variables, only clutches with complete data on parental mass, color score, and carotenoid concentrations were used. Clutch fertility, hatchability and overall clutch productivity (final number of fledglings produced from initial clutch size) were examined using GLM models with binomial error and logit link function to determine whether either adult color score or carotenoid concentration in the plasma was related to nesting success (Clum 1995; Bize et al. 2002; McDonald et al. 2005). Nestling carotenoid concentrations were analysed two different ways: 1) repeated measures taken throughout the nestling period were analysed using generalized linear mixed effects models, with measurements nested within nestlings and nestlings nested within each clutch, and 2) the first and last measures for each nestling were examined separately using the same method for adult plasma carotenoid levels (Sternalski et al. 2010).

Results

Adults

Combined color score of cere, lores, and tarsi was significantly different between rearing environments and sex for adult captive, F1, and wild kestrels (Fig. 1). Color scores were significantly higher in birds in the wild (df = 81, z = 3.53, P = 0.0004), and males had higher scores than females (df = 81, z = 4.21, P < 0.0001). An interaction effect between sex and rearing environment was found for plasma carotenoid concentrations; captive females had similar plasma carotenoid levels to males, but wild males had higher levels than females (df = 79, z = -1.97, P = 0.0519; Fig. 1). No correlations were found between plasma carotenoid concentrations and either body mass or measure date, and plasma carotenoid concentration had no effect on color score.

Reproductive variables

Clutches produced by pairs containing males with higher color scores were less likely to hatch successfully (df = 35, t = -2.00, P = 0.0539) or produce surviving young (df = 35, t = -2.03, P = 0.0502), though the effects were weak. Neither color score nor carotenoid concentration of either parent affected egg fertility.

Nestlings

Rearing environment was the only variable to significantly affect nestling carotenoid levels (Fig. 2). Nestlings reared in captivity had greater carotenoid concentrations in their plasma than those reared in the wild, regardless of origin (by approximately 5.5 µg / ml, df = 31, t = -3.18, P = 0.0033). However, this difference disappeared once the nestlings were older than 10 days of age.

Discussion

Rearing environment had the greatest effect on kestrel integumentary color and plasma carotenoid concentration. Adult kestrels of each respective sex in the wild exhibited higher color scores and had higher carotenoid concentrations than birds in captivity, regardless of origin. The difference between carotenoid levels of males and females was also greater in the wild than in captivity.

The higher color scores of birds in the wild and the greater difference between carotenoid levels in wild males and females may relate to diet. The captive kestrel diet, which consisted solely of day-old cockerels (*Gallus gallus domesticus*), is typically considered to be high in carotenoids since poultry feed is enriched with carotenoid supplements (Negro et al. 1998). The captive diet was uniform however, and may have lacked other supplements that serve to enhance carotenoid expression; for example, lipoprotein molecules act as carriers for carotenoids in the body (Sternalski et al. 2010). In Montagu's harrier (*Circus pygargus*) nestlings, captive birds never attained the color brightness or carotenoid concentrations of wild birds even when supplemented with carotenoids, which suggests environmental or dietary effects beyond the strict presence of carotenoids in diet (Sternalski et al. 2010). Captive conditions have also been associated with reduced expression of carotenoids in birds; small cage size and crowded conditions have been associated with reduced expression of red, orange and yellow color in passerines when compared to birds kept in less crowded conditions maintained on the same diet (Thompson et al. 1997).

Even captive birds fed uniform diets have been shown to exhibit significantly different color expression (e.g. Bortolotti et al. 1996; Negro et al. 1998; Sternalski et al. 2010). In this study, though captive-reared birds had similar carotenoid levels as a result of diet, males had significantly higher color scores than females. Carotenoid levels have been shown to fluctuate seasonally in captive kestrels; for example, carotenoid levels were found to be similar between sexes during the incubation and nestling periods in captive birds, though they differed earlier in the reproductive season (Negro et al. 1998). Blood samples in this study were drawn during the incubation period, which may explain the similarity in carotenoid levels in captive birds. It is possible this same seasonal fluctuation in carotenoid levels is not seen in the wild because of access to a more varied diet.

The difference in carotenoid levels between nestlings from captive and wild environments was not surprising; in a cross-fostering study conducted between wild kestrel nests to separate genetic effects and territory quality, rearing environment had a significant effect on carotenoid concentration in nestlings while origin did not (Bortolotti et al. 2000). Serum carotenoid levels in nestling Eurasian kestrels (*F. tinnunculcus*) were also related to rearing nest of the nestlings, and similar levels were found in both male and female nestlings (Casagrande et al. 2007). In the present study, unlike the adults nestling kestrels reared in captivity had higher carotenoid concentrations than nestlings reared in the wild, though this difference disappeared as the nestlings aged.

Carotenoid concentrations in nestlings, as well as the unexpected result that males with higher color scores had lower clutch productivity than males with lower scores, might relate to diet in wild birds. Kestrels feed on small vertebrates and insects (Smallwood and Bird 2002). Unlike food sources of granivores or frugivores, where carotenoid content is often correlated with food quality, the diet of raptorial birds often exhibits an inverse relationship between carotenoid content and caloric content (Sternalski et al. 2010). Rodents are typically considered a high-calorie but low-carotenoid food source, while birds and insects are considered a strong dietary source of carotenoids but with a lower caloric content (Bortolotti et al. 2000; Smallwood and Bird 2002; Sternalski et al. 2010). Small rodents often form a large component of the wild kestrel diet during the breeding season (Bortolotti et al. 2000; Smallwood and Bird 2002). Wild kestrels with access to large rodent populations have the ability to provide higher caloric intake for their nestlings, which may lead to an increased survivability in the young. This would help explain why males, the primary food provider during the early nesting period, with lower color scores had increased reproductive output over males with higher scores. If wild birds were more likely to provide their nestlings with rodents, it would also explain the lower carotenoid concentrations in wild nestlings.

Carotenoid levels and expression of carotenoid-based color in American kestrels appeared to be primarily the result of environmental effects rather than the result of selection occurring in captivity. This suggests that captive kestrels can serve as effective wildlife models so long as environmental factors are taken into account

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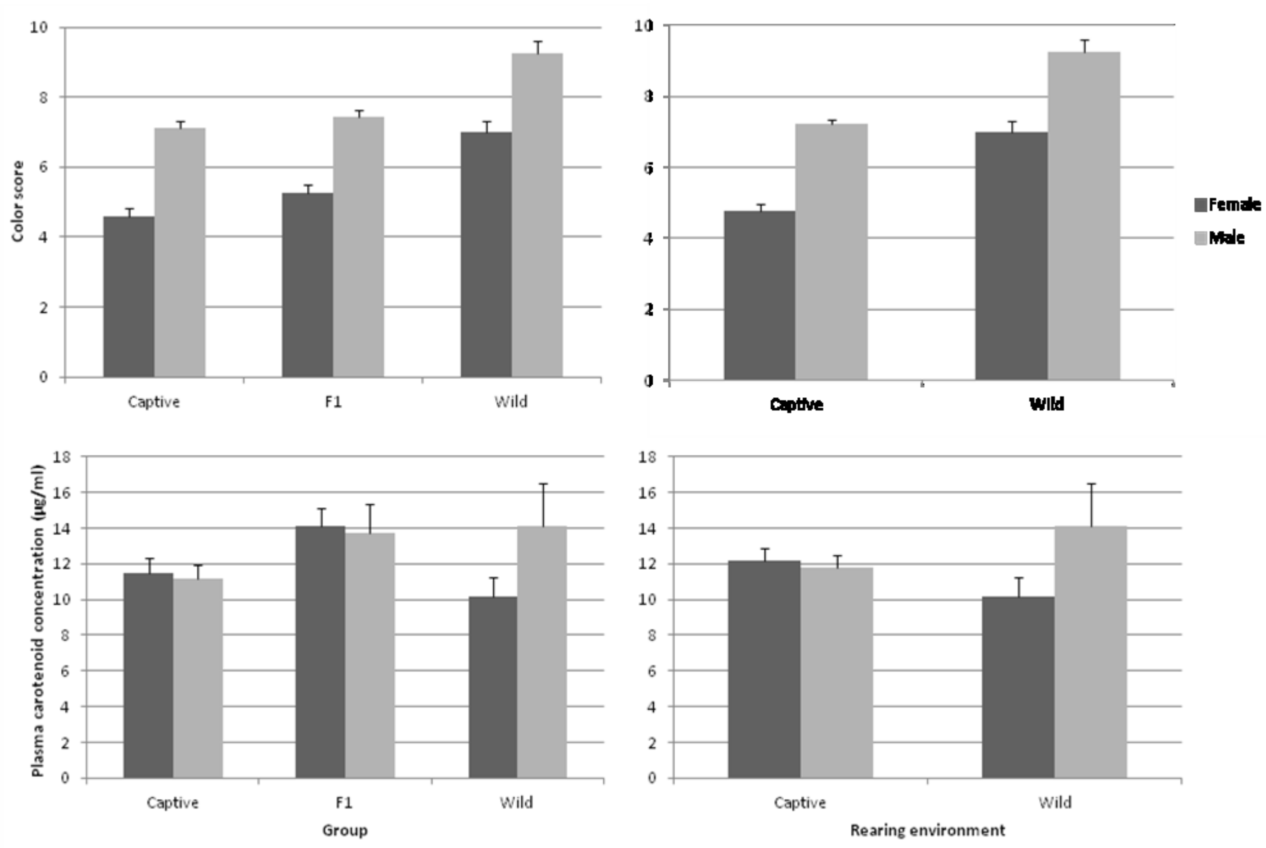

Fig. 1 Mean and standard errors for adult American kestrel color scores (a total score out of 18) and plasma carotenoid concentrations (µg / ml). Data are sorted by groups of birds in the study: captive, first-generation captive (F1), and wild (left-hand graphs). Data in the right-hand graphs are sorted by rearing environment (n = 84 adults).

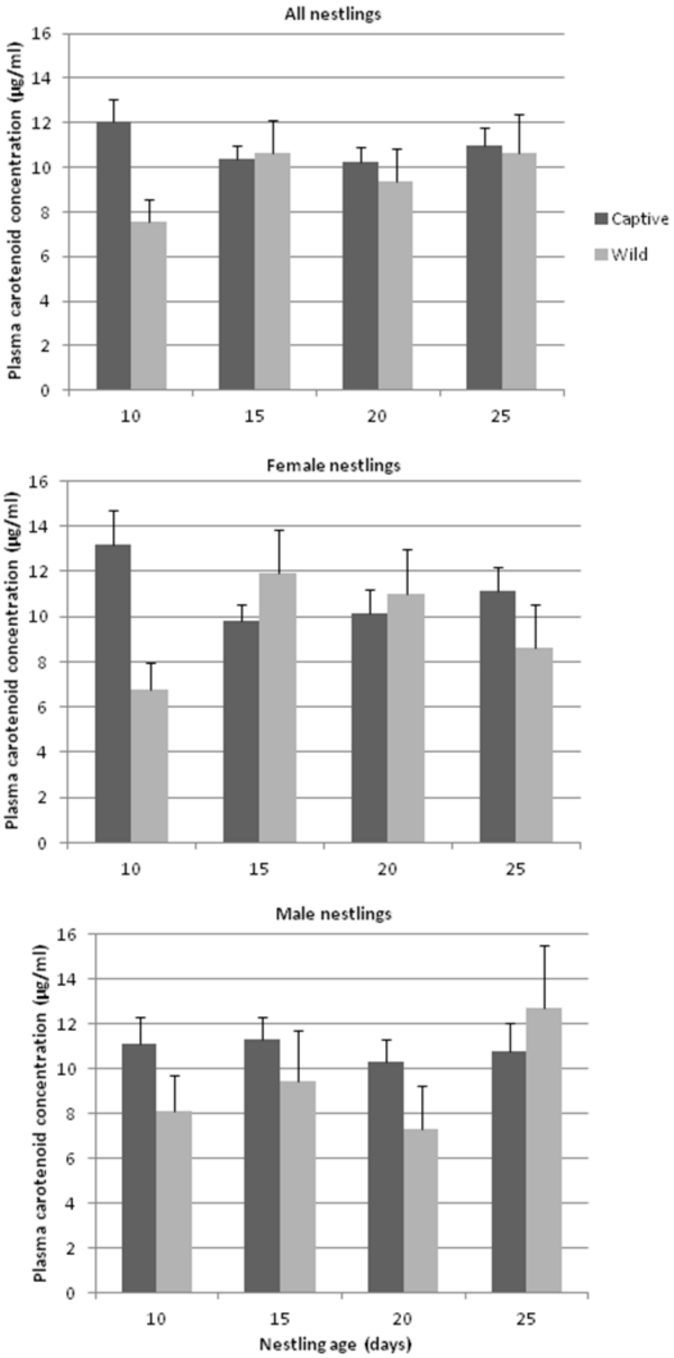

Fig. 2 Mean and standard errors for plasma carotenoid concentrations in nestling American kestrels throughout the nestling period in both the captive and wild rearing environment. Figure shows carotenoid means for all nestlings in each environment (top), for females (center), and for males (bottom) (n = 48 nestlings).

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CHAPTER 6

SUMMARY AND CONCLUSIONS

Summary

The effects of multiple generations of captivity on adult morphology, nesting success and nestling growth of the American kestrel (*Falco sparverius*) were presented in this thesis. In the first part of the study, a whole-clutch cross-fostering experiment was conducted between captive and wild pairs of kestrels. Clutches were switched between experimental captive and wild pairs to separate the effects of origin and rearing environment on nest success and nestling growth, and those results were compared to control nests reared in their native environments. In the second component of the study, wild birds reared in captivity (hereafter F1 birds) from the cross-fostering experiment and captive birds reared under identical conditions were paired in captivity to determine whether there were any differences in adult morphology, nesting success and nestling growth between them, and whether the differences were greater in one sex than the other.

In regards to adult morphology, captive birds were found to be structurally larger (i.e. wing length, head-bill length, tarsi length) than wild kestrels for each respective sex, with the exception of female mass (Ch 2). At mid-incubation, females in captivity had a significantly lower mass than females in the wild. Reverse sexual size dimorphism was also reduced in captivity, with captive females and males differing by a mean of 20 g in mass, while wild females were on average 34 g heavier than wild males (Ch 2). Mass was also smaller in birds that initiated nesting later in the breeding season. F1 birds tended to be intermediate in size between captive and wild birds of each respective sex (Ch 4).

Eggs produced by captive females had larger volumes than those produced by wild females (Ch 2). Egg volume was smaller when produced by females with smaller mass (Ch 2). Both egg volume and female mass were smaller later in the breeding season (Ch 2).

Clutch initiation dates tended to be later in wild pairs than in captive pairs, though results only approached significance (Ch 2). Wild pairs produced clutches with significantly higher fertility levels than those produced by captive pairs (Ch 2). Neither origin nor rearing environment affected egg hatchability, but nestling survival was significantly greater in clutches of wild origin, regardless of rearing environment (Ch 2). When comparing captive, F1, and wild clutches together, fertility was greatest in wild clutches and lowest in F1 clutches, with captive clutches having intermediate fertility levels (Ch 4). The same was found to be true for nestling survival (Ch 4). Nestling survival was also higher in nests reared by parents with smaller body mass (Ch 2, 4). Clutches with greater mean egg volume had lower fertility levels and lower nestling survival than those with lower mean egg volumes (Ch 2). The results for egg volume in this study are contrary to findings of other studies, which found that larger eggs were associated with increased hatchability and nestling survival (e.g. Hipfner and Gaston, 1999).

In regards to nestling growth, few differences were found between captive, F1 and wild nestlings (Ch 3, 4). The captive-rearing environment appeared to affect growth rate of captive and F1 nestlings initially, causing more rapid gains in mass and structural size than in nestlings in the wild (Ch 3, 4). Small growth rate differences were noted between captive and F1 nestlings, but the differences were not great and did not generally have a lasting effect (Ch 4). Asymptotic size for all morphological measurements did not differ between nestlings of each respective sex, regardless of origin or rearing environment (Ch 3, 4).

With regards to plasma carotenoid concentrations and external coloration, rearing environment was the only variable to result in major differences between adult and nestling kestrels (Ch 5). Wild adults, particularly males, had higher color scores and higher carotenoid levels than captive birds (Ch 5). Captive-reared birds with access to a uniform diet exhibited greater similarity in carotenoid levels between the sexes than wild adults did. There was no indication that the relationship between carotenoid levels and color expression differed between the two environments, however. In nestlings, captive birds had higher carotenoid concentrations in their plasma than wild birds, though this difference faded as the nestlings aged (Ch 5). The initial difference may relate to diet; while captive parents had only a uniform diet to offer their nestlings, wild birds had the ability to choose and diversify their offspring's diet. It is possible that given the choice, wild adults chose to provide their young with more calorie-rich food to enhance growth, which in carnivorous diets is often associated with carotenoid-poor food (Sternalski et al. 2010).

Captivity appeared to have a greater effect on F1 males than F1 females with regards to nesting success and nestling growth (Ch 4). Pairs containing an F1 male had lower fertility levels, reduced overall clutch productivity, and were more likely to suffer complete reproductive failure than pairs containing captive males (Ch 4). Growth rates also differed in nestlings produced by captive and F1 males; in general, nestlings produced by captive males had faster growth rates, though asymptotic size did not differ.

The difference in sex response to captivity has also been noted in other captive-bred species (e.g. Jones et al., 2010)

General conclusions

Results of this study suggest that rearing environment had a greater affect on kestrel morphology, nesting success and nestling growth than origin. In general, captive-reared birds were larger, exhibited reduced sexual size dimorphism, produced larger eggs, and had nestlings with greater initial growth rates than wild-reared birds. The larger size of captive-reared individuals likely relates to access to an ad libitum diet, as well as to reduced energy expenditure in captivity, where the need to hunt and avoid predators are nonexistent.

Conversely, clutches produced by wild pairs had greater fertility levels and nestling survival than clutches produced by captive pairs. Reduced nesting success in captive pairs might be a result of lack of mate choice, as captive birds are paired based solely on relation to each other and not on compatibility between members of a pair. Disturbance from human caretakers, or proximity to other nesting pairs, which may have resulted in stress (pairs were visually but not audibly isolated), may also have contributed to reduced nesting success in captivity. It is also possible that increased egg size in captive females might negatively affect a female's ability to incubate her eggs properly, leading to increased early embryo death, reduced hatching success, and prolonged hatching periods, leading to larger age gaps between nestlings which could affect their survival in the nest (see also Bortolotti and Wiebe, 1993).

Though there were significant differences in nestling growth rates, overall nestling size did not differ among any experimental groups in this study. The stability of nestling growth among groups might relate to the apparent adaptation in wild kestrels to withstand the effects of food shortage and other short-term environmental effects on growth, particularly structural growth, during the nestling period (Gard and Bird, 1992; Lacombe et al., 1994; Negro et al., 1994). It is not inconceivable that 10 or more generations of captive breeding was not sufficient time to result in significant differences in nestling kestrel growth patterns.

Though most differences between kestrels in this study could be related to environmental effects, sufficient differences were noted between captive and F1 kestrels to suggest that some level of selection has occurred over generations of captivity in the American kestrel. Differences were most noticeable in nesting success, where under identical environmental conditions captive pairs had greater overall clutch productivity than F1 pairs. This was particularly true in pairs containing an F1 male. Generations of captive breeding may have produced kestrels more amenable to reproducing under captive conditions than wild-type birds.

Several conclusions can be drawn in regards to the use of a cross-fostering experimental design to study the effects of captivity on kestrels. Cross-fostering of clutches has frequently been used to separate the effects of habitat or parental quality and egg size on nesting success or nestling growth in the wild (e.g. Magrath, 1992; Amundsen et al., 1996; Risch and Rohwer, 2000; Silva et al., 2007). Using a cross-fostering experimental setup to separate the effects of environment and origin between captive and wild birds was a logical option that proved relatively successful in this study. By cross-fostering clutches of eggs, any potential early environmental effects on nestling development that might have resulted if nestlings were fostered instead of eggs were avoided.

The use of clutch cross-fostering on kestrels led to a number of problems that did not arise in previous studies using the design, however: 1) kestrels do not nest synchronously, which created difficulties in finding clutches with matching hatch dates to switch, and 2) small sample size further increased the difficulty in finding pairs of nests with matching hatch dates. Because of these factors, only experimental nests could be fostered while control nests were reared by biological parents, which added a potential bias, relation of young to rearing parents, to be considered in the analyses. To carry out a cross-fostering study on such a species where all nests, control and experimental, could be fostered, a larger sample size would be needed to increase the likelihood of matching hatch dates. Overall however, the cross-fostering design was an effective method of comparing nesting success and nestling growth between wild and captive environments.

It can be concluded from this study that captive kestrels can serve as wildlife models, as long as certain caveats are taken into account. Caution must be exercised when evaluating nesting success in captive kestrels because this study has shown that captive birds may exhibit lower fertility levels and nestling survival than wild kestrels, and these differences appear to extend beyond environmental effects. Research using first-generation captive birds should be avoided as much as possible, as they seem to suffer high reproductive failure in captivity.

This study also has implications for captive breeding programs for species recovery plans. For birds, fostering whole or partial captive clutches to wild parents seems likely to produce offspring indistinguishable from wild nestlings in morphology.

The poor nesting success of first-generation captive birds, even when paired with captive-bred birds, suggests that, while the introduction of fresh wild genes into captivity might be beneficial to the maintenance of genetic diversity in a captive colony, both for research and propagation purposes, it does not necessarily guarantee the equal distribution of those genes to subsequent generations (also see Alcaide et al., 2010). Careful monitoring should be conducted on the success of first-generation birds in captivity as well as on their subsequent offspring to determine their overall contribution to the genetic diversity of a captive colony.

Though a complete overview of the effects of captivity on kestrels encompassing the morphology, nesting success, physiology, behaviour and genetic diversity was beyond the scope of this project, this study did serve as one of only a few to examine the effects of captivity on nesting success and nestling development on a captive wildlife model, and the only one of its kind on a raptorial species. It also demonstrated definite effects of origin and rearing environment on an avian species bred in captivity for multiple generations without being subjected to intentional anthropogenic selection for particular traits.

Recommendations for future work

The results of the present study warrant further research into the effects of captivity on wildlife models, including the American kestrel. The kestrel is a common research model, particularly in the field of environmental toxicology, where it serves as a representative of a top predator for studies on bioaccumulation of chemicals in the environment. Such studies often involve research into the impacts of chemicals on reproductive variables (see review by Bardo and Bird, 2009). In addition, given the continued rate of species decline, it is likely that the frequency of captive breeding of animals for reintroduction into the wild will only increase in the future (White et al., 2005; Connolly and Cree, 2008). The more complete our understanding of the effects of captivity on animals, the greater the possibility that those effects can be mitigated. Specific recommendations for further research as a follow-up to the present study are as follows:

1) While reproduction was studied in kestrels, behaviour during the courtship, incubation, hatchling and fledgling period, which may have had significant effect on nesting success, was beyond the scope of this investigation. A comparison between the behaviours of nesting F1, captive, and wild birds might provide insight as to why wild birds were so much more successful than captive or F1 birds.
2) Differences in nesting success may instead be related to physiological factors. In the future blood samples should be taken from the various groups of kestrels and analysed for stress and reproductive hormones such as corticosterone, testosterone, estradiol, progesterone, and prolactin.
3) Phenotypes are an expression of genotype. An effective method of determining whether birds bred in captivity for over 10 generations are significantly different from wild birds of the same regional ancestry would be to compare the genetic diversity between the two populations.
4) Given the relatively poor nesting success in captive birds compared to kestrels in the wild, examining alternative captive breeding strategies could potentially improve nesting success. Strategies that could be tested include 1) allowing for

mate choice between male and female kestrels, and 2) altering aviary design to further reduce disturbance from other pairs and from the surroundings on breeding pairs. Given that successfully breeding kestrels in the wild will often return to the same breeding site in subsequent years, and that pairing with the same mate can occur in multiple seasons (Smallwood and Bird, 2002), it would be worth examining the lifetime nesting success of 1) captive kestrels re-paired with different individuals in different aviaries yearly and 2) kestrels provided with a consistent mate and breeding local throughout their lifetime. Consistency in breeding partners has been shown to improve nesting success in other captive raptor species (e.g. Clum, 1995).

5) It would be useful, especially for reintroduction programs, to determine whether captive kestrels reared in the wild were able to survive and reproduce successfully in subsequent years. Though difficult to accomplish, with the advances being made in technology that can be used to track animals over the long term and over greater distances, it may be possible in the near future to mark and track captive-bred kestrels in the wild for at least part of their lifetime.

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APPENDIX I: SUMMARY OF MEAN AND STANDARD ERRORS FOR ADULT AND NESTLING MEASURES, CAROTENOID LEVELS AND REPRODUCTIVE VARIABLES

Table 1. Adult morphological measurements taken at mid-incubation (mean ± SE) from captive and wild American kestrels from 2006 - 2009 (n = 127)

	Female		Male	
	Captive origin	Wild origin	Captive origin	Wild origin
Mass (g)	135.75 ± 1.42	141.92 ± 1.86	116.16 ± 1.28	107.67 ± 1.61
Head-bill length (mm)	43.97 ± 0.14	43.01 ± 0.15	42.54 ± 0.20	41.92 ± 0.18
Tarsus length (mm)	33.53 ± 0.22	32.85 ± 0.24	34.05 ± 0.31	32.63 ± 0.26
Wing chord (mm)	195.11 ± 0.65	192.97 ± 0.71	185.81 ± 0.83	182.89 ± 0.88

Table 2. Adult morphological measurements taken at mid-incubation (mean ± SE) from captive and F1 American kestrels in 2009 (n = 59)

	Female		Male	
	Captive	F1	Captive	F1
Mass (g)	135.33 ± 2.18	136.04 ± 1.68	115.86 ± 1.63	116.12 ± 2.60
Head-bill length (mm)	43.97 ± 0.22	43.41 ± 0.25	42.48 ± 0.28	42.39 ± 0.25
Tarsus length (mm)	33.29 ± 0.22	34.02 ± 0.32	33.72 ± 0.33	33.66 ± 0.34
Wing chord (mm)	193.50 ± 0.93	191.35 ± 1.11	188.13 ± 0.90	185.64 ± 0.92

Table 3. Mean adult morphological measurements (mean ± SE) taken at mid-incubation for captive, F1, and wild American kestrels from 2006 to 2009 (n = 168)

	Female			Male		
	Captive	F1	Wild	Captive	F1	Wild
Mass (g)	135.55 ± 1.21	136.04 ± 1.68	141.83 ± 1.91	116.50 ± 1.12	116.12 ± 2.60	107.67 ± 1.61
Head-bill length (mm)	43.96 ± 0.14	43.41 ± 0.25	43.01 ± 0.15	42.56 ± 0.18	42.39 ± 0.25	41.92 ± 0.18
Tarsus length (mm)	33.52 ± 0.19	34.02 ± 0.32	32.93 ± 0.23	34.03 ± 0.27	33.66 ± 0.34	32.63 ± 0.26
Wing chord (mm)	194.53 ± 0.59	191.35 ± 1.11	192.89 ± 0.72	186.74 ± 0.60	185.64 ± 0.92	182.89 ± 0.88

Table 4. Summary of reproductive variables (mean ± SE, with sample size of nests indicated in parentheses in first column) for captive and wild nests from 2006 – 2009

Clutch origin	Captive		**All captive clutches**	Wild		**All wild clutches**
Clutch rearing environment	Captive	Wild		Captive	Wild	
Egg volume (cm^3) (74)	14.75 ± 0.09	14.94 ± 0.11	**14.82 ± 0.07**	14.59 ± 0.15	14.43 ± 0.10	**14.49 ± 0.08**
No. eggs per clutch (74)	5.09 ± 0.06	5.08 ± 0.08	**5.09 ± 0.05**	4.85 ± 0.10	4.95 ± 0.08	**4.91 ± 0.06**
Clutch initiation date (70)	30 April ± 1.58	30 April ± 1.32	**30 April ± 1.10**	1 May ± 1.30	5 May ± 2.38	**3 May ± 1.58**
No. fertile eggs / clutch (70)	4.09 ± 0.29	5.00 ± 0.00	**4.43 ± 0.20**	4.62 ± 0.14	4.82 ± 0.16	**4.74 ± 0.11**
No. fertile eggs hatched / clutch (70)	3.64 ± 0.31	4.08 ± 0.35	**3.80 ± 0.23**	3.92 ± 0.33	3.73 ± 0.37	**3.80 ± 0.26**
Length of hatching period (days) (66)	1.60 ± 0.33	3.25 ± 0.54	**2.22 ± 0.32**	0.92 ± 0.26	2.26 ± 0.37	**1.72 ± 0.27**
No. surviving fledglings / clutch (66)	3.62 ± 0.31	3.62 ± 0.46	**3.62 ± 0.26**	3.85 ± 0.34	4.32 ± 0.20	**4.13 ± 0.18**
No. female young / clutch (66)	1.95 ± 0.30	1.46 ± 0.24	**1.76 ± 0.21**	1.69 ± 0.31	2.05 ± 0.22	**1.91 ± 0.18**
No. successful clutches / total (74)	20 / 22	13 / 14	**33 / 36**	14 / 14	21 / 24	**35 / 38**

Table 5. Summary of reproductive variables (mean ± SE, with sample size of nests indicated in parentheses in first column) for captive and F1 pairs from 2009

	Captive male		F1 male	
	Captive female	F1 female	Captive female	F1 female
Egg volume (cm^3) (33)	14.99 ± 0.14	14.63 ± 0.21	14.58 ± 0.12	14.49 ± 0.19
No. eggs / clutch (33)	4.90 ± 0.09	5.00 ± 0.00	4.86 ± 0.26	4.88 ± 0.13
Clutch initiation date (33)	03 May ± 2.40	30 April ± 1.50	05 May ± 2.60	04 May ± 2.40
No. fertile eggs / clutch (33)	4.40 ± 0.22	3.20 ± 0.66	4.17 ± 0.48	4.13 ± 0.61
No. hatched fertile eggs / clutch (33)	4.20 ± 0.20	2.20 ± 0.58	3.17 ± 0.75	3.71 ± 0.42
Length of hatching period (days) (29)	2.20 ± 0.42	1.29 ± 0.52	1.60 ± 0.68	1.83 ± 0.54
No. surviving hatchlings / clutch (29)	4.10 ± 0.18	2.20 ± 0.58	3.60 ± 0.51	3.83 ± 0.48
No. female young (29)	2.30 ± 0.37	0.80 ± 0.37	1.60 ± 0.40	1.33 ± 0.56
No. nests producing at least 1 fledging / total nests (33)	11 / 11	7 / 7	5 / 7	6 / 8

Table 6. Mean values of reproductive variables for captive, F1, and wild pairs from 2006 - 2009 (mean ± SE, with number of clutches indicated in parentheses in first column)

	Captive	F1	Wild
Egg volume (cm^3) (57)	14.74 ± 0.08	14.59 ± 0.17	14.43 ± 0.10
No. eggs / clutch (57)	5.04 ± 0.07	4.88 ± 0.13	4.88 ± 0.11
Clutch initiation date (57)	May 01 ± 1.62	May 04 ± 2.41	May 06 ± 2.34
No. fertile eggs / clutch (57)	4.16 ± 0.27	4.13 ± 0.61	4.81 ± 0.16
No. hatched fertile eggs / clutch (57)	3.72 ± 0.28	3.25 ± 0.59	3.73 ± 0.37
Length of hatching period (50)	1.62 ± 0.31	1.83 ± 0.54	2.26 ± 0.37
No. surviving hatchlings / clutch (50)	3.65 ± 0.29	3.29 ± 0.68	4.32 ± 0.20
No. female young / clutch (50)	1.88 ± 0.28	1.14 ± 0.51	2.05 ± 0.20
No. nests producing at least 1 fledgling / total nests (57)	23 / 25	6 / 8	21 / 24

Table 7: Mean ± SE values for morphological measurements of American kestrel nestlings from captive and wild clutches (2007-2009, n = 44 clutches (176 nestlings))

Measurement	Age (Days)	10				15			
	Group	CC	CW	WC	WW	CC	CW	WC	WW
9th primary feather (mm)	Female	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	7.43 ± 0.61	7.44 ± 0.92	6.17 ± 0.70	8.02 ± 0.77
	Male	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	7.44 ± 0.58	7.50 ± 0.70	7.82 ± 0.69	7.74 ± 0.55
Wing chord (mm)	Female	46.32 ± 0.91	43.38 ± 1.13	44.80 ± 0.93	45.09 ± 1.20	79.75 ± 1.02	76.25 ± 1.66	77.17 ± 1.04	77.54 ± 1.17
	Male	44.33 ± 0.97	44.12 ± 0.69	45.50 ± 0.77	42.95 ± 0.95	77.38 ± 0.91	74.56 ± 1.31	77.41 ± 0.92	74.00 ± 1.09
Head-bill length (mm)	Female	37.25 ± 0.18	37.06 ± 0.35	37.39 ± 0.26	37.90 ± 0.31	41.15 ± 0.32	40.79 ± 0.42	40.81 ± 0.27	40.80 ± 0.29
	Male	37.07 ± 0.36	37.03 ± 0.30	37.44 ± 0.22	36.90 ± 0.27	40.56 ± 0.21	40.45 ± 0.39	40.64 ± 0.23	40.20 ± 0.21
Tarsus length (mm)	Female	28.23 ± 0.29	27.18 ± 0.59	28.31 ± 0.44	28.62 ± 0.43	32.97 ± 0.27	32.99 ± 0.09	33.22 ± 0.33	32.77 ± 0.28
	Male	28.59 ± 0.35	28.20 ± 0.38	28.81 ± 0.50	28.32 ± 0.37	32.89 ± 0.22	32.95 ± 0.28	33.41 ± 0.34	32.50 ± 0.27
Mass (g)	Female	94.18 ± 1.71	90.38 ± 3.60	88.33 ± 4.21	91.70 ± 1.80	127.45 ± 1.44	120.38 ± 5.36	125.13 ± 3.87	120.17 ± 2.10
	Male	90.28 ± 1.89	85.41 ± 1.79	92.56 ± 2.21	85.90 ± 1.50	122.53 ± 2.30	110.06 ± 3.47	120.59 ± 2.31	109.52 ± 1.86

Measurement (Cont)	Age (Days)	20				25			
	Group	CC	CW	WC	WW	CC	CW	WC	WW
9th primary feather (mm)	Female	28.98 ± 0.83	27.61 ± 1.63	28.37 ± 1.09	31.05 ± 1.06	55.20 ± 1.19	54.60 ± 1.71	53.60 ± 0.96	55.36 ± 1.09
	Male	28.11 ± 0.93	29.44 ± 0.83	30.28 ± 1.00	28.03 ± 0.94	52.67 ± 1.08	55.27 ± 0.95	55.61 ± 1.17	53.58 ± 0.90
Wing chord (mm)	Female	109.76 ± 0.97	107.06 ± 1.42	104.93 ± 1.34	108.89 ± 1.13	136.18 ± 1.15	131.90 ± 1.45	131.97 ± 1.09	133.74 ± 1.52
	Male	104.64 ± 0.95	104.41 ± 1.69	105.08 ± 0.84	102.63 ± 0.87	129.06 ± 1.15	132.15 ± 1.03	131.33 ± 0.99	129.25 ± 0.90
Head-bill length (mm)	Female	42.41 ± 0.17	41.92 ± 0.47	42.25 ± 0.22	42.41 ± 0.31	42.53 ± 0.17	43.25 ± 0.29	42.76 ± 0.22	42.81 ± 0.24
	Male	42.25 ± 0.14	41.60 ± 0.29	42.04 ± 0.19	41.56 ± 0.16	42.29 ± 0.16	42.06 ± 0.24	42.49 ± 0.19	42.11 ± 0.18
Tarsus length (mm)	Female	33.34 ± 0.24	33.67 ± 0.41	34.24 ± 0.54	34.08 ± 0.26	32.92 ± 0.25	33.29 ± 0.65	33.09 ± 0.31	33.86 ± 0.38
	Male	33.28 ± 0.18	33.22 ± 0.36	33.39 ± 0.40	33.64 ± 0.25	33.58 ± 0.34	33.35 ± 0.36	33.08 ± 0.31	33.44 ± 0.29
Mass (g)	Female	136.05 ± 2.25	130.33 ± 4.58	134.20 ± 3.50	129.58 ± 2.43	131.18 ± 1.89	138.40 ± 3.44	131.20 ± 2.26	130.57 ± 2.96
	Male	130.39 ± 1.95	119.24 ± 2.74	129.83 ± 2.25	120.50 ± 2.11	124.83 ± 2.11	120.92 ± 1.81	121.50 ± 1.65	118.60 ± 1.90

Table 8: Mean ± SE values for measurements of American kestrel nestlings (n = 28 clutches (100 nestlings)) from captive and F1 clutches in 2009

Measurement	Age (Days)	10				15			
	Male parent origin	Captive		F1		Captive		F1	
	Female parent origin	Captive	F1	Captive	F1	Captive	F1	Captive	F1
9th primary feather (mm)	Female	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	7.89 ± 0.87	3.33 ± 0.33	5.92 ± 0.93	6.92 ± 1.31
	Male	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	8.00 ± 0.87	6.42 ± 1.44	5.90 ± 0.60	7.28 ± 0.63
Wing chord (mm)	Female	47.36 ± 1.11	44.00 ± 1.53	45.00 ± 1.25	45.67 ± 2.22	80.82 ± 1.29	77.50 ± 1.04	78.00 ± 1.34	76.25 ± 2.57
	Male	46.38 ± 1.48	44.00 ± 1.51	44.60 ± 0.93	46.89 ± 0.89	79.50 ± 1.16	70.33 ± 3.62	80.20 ± 2.27	79.00 ± 0.67
Head-bill length (mm)	Female	37.15 ± 0.22	37.28 ± 0.38	37.67 ± 0.30	37.24 ± 0.42	40.82 ± 0.23	41.40 ± 0.20	41.23 ± 0.36	39.29 ± 1.10
	Male	36.65 ± 0.31	36.78 ± 0.18	37.58 ± 0.47	37.46 ± 0.28	40.43 ± 0.34	40.60 ± 0.14	40.61 ± 0.65	40.26 ± 0.11
Tarsus length (mm)	Female	28.19 ± 0.27	28.17 ± 1.07	27.82 ± 0.53	27.93 ± 0.73	32.43 ± 0.33	33.80 ± 0.82	31.33 ± 0.22	33.00 ± 0.67
	Male	27.76 ± 0.54	28.82 ± 0.53	29.00 ± 0.76	28.81 ± 0.35	32.59 ± 0.33	33.46 ± 0.86	32.58 ± 1.03	32.47 ± 0.42
Mass (g)	Female	93.64 ± 2.40	101.67 ± 3.71	86.67 ± 2.63	92.67 ± 4.63	127.71 ± 1.95	138.33 ± 2.91	125.33 ± 3.62	125.50 ± 2.59
	Male	88.38 ± 2.81	87.83 ± 4.32	85.80 ± 1.46	91.33 ± 1.92	122.75 ± 3.71	119.17 ± 3.89	122.40 ± 1.96	123.00 ± 2.17

Measurement (Cont)	Age (Days)	20				25			
	Male origin	Captive		F1		Captive		F1	
	Female origin	Captive	F1	Captive	F1	Captive	F1	Captive	F1
9th primary feather (mm)	Female	29.46 ± 1.11	28.00 ± 2.65	26.83 ± 0.80	29.83 ± 1.14	56.61 ± 1.62	59.00 ± 4.73	53.83 ± 1.19	53.33 ± 1.52
	Male	30.13 ± 1.49	29.17 ± 2.36	29.40 ± 0.93	28.94 ± 0.92	55.25 ± 1.59	51.67 ± 4.36	55.70 ± 0.30	57.22 ± 0.78
Wing chord (mm)	Female	110.54 ± 1.22	110.00 ± 2.00	108.00 ± 0.58	107.00 ± 2.32	138.00 ± 1.22	135.00 ± 1.15	134.17 ± 0.54	132.67 ± 2.30
	Male	106.38 ± 1.40	104.42 ± 2.24	106.20 ± 0.73	106.22 ± 0.60	132.13 ± 1.22	130.25 ± 2.52	131.10 ± 0.87	132.06 ± 0.69
Head-bill length (mm)	Female	42.30 ± 0.19	42.18 ± 0.44	42.38 ± 0.45	41.85 ± 0.27	42.19 ± 0.18	42.67 ± 0.22	42.45 ± 0.55	42.47 ± 0.41
	Male	41.76 ± 0.45	42.18 ± 0.22	42.11 ± 0.65	41.68 ± 0.32	42.33 ± 0.20	42.53 ± 0.26	42.14 ± 0.30	42.46 ± 0.40
Tarsus length (mm)	Female	32.67 ± 0.22	33.45 ± 0.51	32.53 ± 0.22	33.29 ± 0.78	32.25 ± 0.26	33.17 ± 0.16	33.03 ± 0.47	34.50 ± 1.02
	Male	32.93 ± 0.44	33.49 ± 0.33	32.52 ± 0.25	33.82 ± 0.40	33.43 ± 0.35	33.37 ± 0.58	32.94 ± 0.60	33.52 ± 0.52
Mass (g)	Female	136.64 ± 3.16	148.33 ± 2.33	137.33 ± 4.35	129.83 ± 1.28	130.07 ± 2.79	139.33 ± 3.76	130.50 ± 5.29	127.00 ± 2.46
	Male	129.38 ± 3.18	128.67 ± 4.65	125.80 ± 1.24	129.78 ± 2.52	123.88 ± 2.47	123.00 ± 3.79	119.20 ± 1.77	119.56 ± 2.08

Table 9: Mean ± SE values for morphological measurements of American kestrel nestlings from captive, wild, and F1clutches from 2007 – 2009

Measurement	Age (Days)	10			15		
	Group	Captive	F1	Wild	Captive	F1	Wild
9th primary feather (mm)	Female	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	7.34 ± 0.63	6.92 ± 1.31	9.14 ± 0.78
	Male	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	7.45 ± 0.51	7.28 ± 0.63	7.61 ± 0.58
Wing chord (mm)	Female	46.36 ± 0.90	45.67 ± 2.22	45.94 ± 1.44	79.84 ± 0.98	76.25 ± 2.57	78.75 ± 1.19
	Male	44.26 0.92	46.89 ± 0.89	42.84 0.95	77.39 ± 0.81	79.00 ± 0.67	73.84 ± 1.17
Head-bill length (mm)	Female	37.24 ± 0.17	37.24 ± 0.42	37.9 ± 0.31	41.15 ± 0.31	39.29 ± 1.10	40.80 ± 0.29
	Male	36.93 ± 0.34	37.46 ± 0.28	36.91 ± 0.27	40.40 ± 0.22	40.26 ± 0.11	40.20 ± 0.21
Tarsus length (mm)	Female	28.15 ± 0.29	29.93 ± 0.73	28.62 ± 0.43	32.88 ± 0.27	33.00 ± 0.67	32.77 ± 0.28
	Male	28.30 ± 0.38	28.81 ± 0.35	28.32 ± 0.37	32.84 ± 0.20	32.47 ± 0.42	32.50 ± 0.29
Mass (g)	Female	96.64 ± 1.85	92.67 ± 4.63	92.11 ± 2.18	127.59 ± 1.42	125.50 ± 2.59	120.17 ± 2.06
	Male	88.79 ± 1.96	91.33 ± 1.92	86.00 ± 1.65	122.00 ± 2.11	123.00 ± 2.17	109.42 ± 2.02

Measurement (Cont)	Age (Days)	20			25		
	Group	Captive	F1	Wild	F1	Captive	Wild
9th primary feather (mm)	Female	28.89 ± 0.80	29.83 ± 1.14	31.22 ± 1.10	55.23 ± 1.18	53.33 ± 1.52	56.14 ± 1.05
	Male	28.34 ± 0.86	28.94 ± 0.92	28.08 ± 0.99	52.95 ± 0.99	57.22 ± 0.78	53.45 ± 0.94
Wing chord (mm)	Female	109.50 ± 0.96	107.00 ± 2.32	109.28 ± 1.12	136.36 ± 1.10	132.67 ± 2.30	134.42 ± 1.67
	Male	104.84 ± 0.89	106.22 ± 0.60	102.82 ± 0.89	129.63 ± 1.07	132.06 ± 0.69	129.11 ± 0.93
Head-bill length (mm)	Female	42.40 ± 0.16	41.85 ± 0.27	42.41 ± 0.31	42.50 ± 0.17	42.47 ± 0.41	42.81 ± 0.24
	Male	41.99 ± 0.22	41.68 ± 0.32	41.56 ± 0.16	42.27 ± 0.14	42.46 ± 0.40	42.11 ± 0.18
Tarsus length (mm)	Female	33.27 ± 0.24	33.29 ± 0.78	34.08 ± 0.26	32.84 ± 0.25	34.50 ± 1.02	33.86 ± 0.38
	Male	33.11 ± 0.22	33.82 ± 0.40	33.64 ± 0.25	33.54 ± 0.31	33.52 ± 0.52	33.44 ± 0.29
Mass (g)	Female	135.95 ± 2.15	129.83 ± 1.28	129.33 ± 2.56	131.36 ± 1.91	127.00 ± 2.46	129.33 ± 3.37
	Male	130.42 ± 1.84	129.78 ± 2.52	121.11 ± 2.13	124.84 ± 1.98	119.56 ± 2.08	118.84 ± 1.98

Table 10. Mean and standard error values for color rating and plasma carotenoid concentrations in adult captive-bred, first generation captive (F1) and wild American kestrels from 2006 - 2009 (n = 84)

	Female			Male		
	Captive	F1	Wild	Captive	F1	Wild
Color score *	4.59 ± 0.24	5.25 ± 0.25	7.00 ± 0.30	7.13 ± 0.19	7.43 ± 0.20	9.25 ± 0.37
Plasma carotenoid concentration (μg / ml)	11.48 ± 0.88	14.09 ± 1.05	10.18 ± 1.06	11.16 ± 0.79	13.74 ± 1.59	14.08 ± 2.43

* Color score is based on the addition of separate scores for cere, lore and tarsi, each with a rating from 1 (dull yellow) to 6 (dark orange), to give a final score out of 18

Table 11. Mean and standard errors for plasma carotenoid concentrations (μg/ml) for captive, F1, and wild nestling American kestrels from 2006 - 2009 (n = 48 nestlings)

	Nestling age (days)	10	15	20	25
Captive	Female	13.92 ± 1.82	9.03 ± 0.76	10.23 ± 1.24	11.26 ± 1.39
	Male	11.15 ± 2.08	11.18 ± 1.51	9.71 ± 1.60	10.47 ± 1.89
F1	Female	9.64 ± 0.07	12.10 ± 1.67	10.00 ± 1.76	10.65 ± 1.00
	Male	11.00 ± 1.00	11.29 ± 0.77	11.01 ± 1.20	11.18 ± 1.65
Wild	Female	6.74 ± 1.24	11.90 ± 1.96	10.97 ± 2.02	8.63 ± 1.93
	Male	8.11 ± 1.57	9.42 ± 2.28	7.29 ± 1.92	12.69 ± 2.78