Nutritional and Physiological Constraints on Reproduction in the Endangered Swift Parrot, *Lathamus discolor*

by

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for the degree of Doctor of Philosophy (Zoology),
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DECLARATION

This thesis contains no material which has previously been accepted for a degree or diploma by the University of Tasmania or any other tertiary institution and to the best of my knowledge or belief, contains no material previously published or written by another person, except where due acknowledgment is made in the text.

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ABSTRACT

This thesis documents my investigations into the factors that constrain reproduction in the endangered swift parrot, *Lathamus discolor*. First, I have described the annual cycle of reproduction in the swift parrot, by examining changes in the gonads and reproductive hormones (oestradiol and testosterone) of both captive and wild birds. Reproductive development in wild swift parrots initiated after the birds migrated to their breeding grounds in Tasmania from the mainland of Australia. Male swift parrots commenced testes development and increased the plasma concentration of testosterone in all years of the study regardless of the foraging resources available. Reproductive activity in male birds peaked in October and November and gonadal regression occurred in late December and January associated with the development of moult. However, in this period very few female swift parrots were reproductively active. The female swift parrots' initiation of breeding appears to be the major factor limiting reproduction in wild swift parrots. Further, the female birds appear to be limited in numbers as well as opportunities to breed. There is a male sex bias (1.9:1) to the wild swift parrot population based on the examination of birds killed in window strikes and birds trapped by mist-netting. The origins of the sex bias were not determined.

In captive swift parrots, I examined the period of reproduction that encompasses egg laying, egg incubation and chick-rearing. The male swift parrot is solely responsible for the provision of resources to the female during incubation and, in the first two weeks after hatching, to the female and the chicks. This period appears to be a bottle-neck for food resources in the swift parrots' life history. To maintain the high demand for food resources, nesting must occur in close proximity to good foraging resources.

Next, I investigated links between reproduction and nutrition in swift parrots. I examined the morphological adaptations to nectarivory that have evolved in the birds, the food resources used in the breeding season and their ability to extract protein from ingested pollen; all provide evidence for the importance of the flowering of the Tasmanian blue gum, *Eucalyptus globulus,* to supporting the nutritional requirements of reproduction in swift parrots. I commenced feeding trials

in captive birds to examine links between reproduction and dietary energy and protein. However, these trials were disrupted by deaths due to renal disease, which was worsened by high protein diets. Assessment of plasma concentrations of uric acid suggests that swift parrots may have lower protein requirements than granivorous parrots.

Finally, I assessed the constraints that migration may impose on reproduction, and the causes of mortality in swift parrots during the breeding season. No physiological preparations for migration were identified in swift parrots. However, behavioural changes associated with migration were identified suggesting that migration limits the time available for reproduction and its timing within the year. Mortality related to collisions with artificial structures, such as windows, fences and automobiles was identified as a threatening process of increasing importance to the swift parrot population.

This study has major implications for the conservation of the endangered swift parrot and suggests promising avenues for future research. Further, it highlights the paucity of knowledge of the Australian avifauna and the need for scientific involvement in the management of endangered species.

PERMITS

The project was conducted with the approval of the University of Tasmania animal ethics committee under ethics permit numbers 98060, and A0005566.

The birds used in this study were held under Tasmanian Parks and Wildlife Service permit numbers 1263/98, 1263/01, FA 98122, FA 98107, FA 98108, FA 99109, and FA 00041.

Lorikeet food was imported into Australia with the approval of the Australian Quarantine and Inspection Service under Permit to Import Quarantine Material 199903800. Blue gum flowers were harvested with the permission of Hobart City Council, Kingborough Council and Clarence City Council.

Mist-netting and banding was carried out with the approval of the Australian Bird and Bat Banding Scheme. Banding authority licence number was 2348. Trapping in Victoria was carried out with the approval of the Department of Natural Resources and Environment under permits 10000498, and 10001000.

Import and export permits were obtained from relevant state authorities for the interstate movement of birds and bird products during the course of the study. Tasmanian Parks and Wildlife permit numbers were 4373/01, 3226, 3535, 3612, 4120/00, 4121/00, 4462/02, 5009/02. Victorian Natural Resources and Environment permit numbers were EX00019725, IM99017397, and EX99017398. NSW National Parks and Wildlife Service permit numbers were 15680, 11571, and 11572.

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Chapter 1. General Introduction

BACKGROUND

What constrains the initiation of breeding in the endangered swift parrot *Lathamus discolor*? The swift parrot is a small, nectarivorous bird that is one of only three migratory parrots in the world. The species breeds only in Tasmania and is listed as endangered nationally. Successful breeding occurs irregularly and is thought to be linked to the flowering of the Tasmanian blue gum, *Eucalyptus globulus*. Very little is known about the basic biology of this enigmatic parrot.

The swift parrot is the subject of a recovery plan involving state environmental agencies from all of the eastern states of Australia, including the island state of Tasmania. An action (Action 3.5) listed in the 1996 Swift Parrot Recovery Plan was to "investigate the physiological and environmental factors which initiate breeding and assess what impact these factors have on the reproductive success of swift parrots" (Brereton 1996a).

The research described in this thesis aims to address Action 3.5 of the Swift Parrot Recovery Plan, and provide some scientific insight into the unique life history strategy of the swift parrot and the specific adaptations that may be threatening the species' existence.

CONSERVATION STATUS OF PARROTS

At least 90 species (~ 25 %) of the world's parrots are considered threatened (Juniper and Parr 1998; Higgins 1999). The major threatening processes to the Psittacidae, in order of global importance, include: 1) habitat clearance, degradation and fragmentation; 2) capture and mortality associated with the live bird trade; 3) the effects of introduced species (particularly rats and cats); 4) persecution and hunting, and 5) the potential effects of global climate change (Juniper and Parr 1998).

In Australasia, four species or sub-species of parrots are now extinct, and twenty-two species or sub-species are nationally threatened (Garnett and Crowley

2000). The major threatening processes in Australia include habitat clearance, degradation and fragmentation; the effects of introduced species (particularly cats and starlings); and persecution as crop pests (Higgins 1999; Garnett and Crowley 2000; Ford et al. 2001). The effects of habitat clearance, degradation and fragmentation are most pronounced in temperate woodland and forest birds; 34 % of all threatened avian species in Australia are from these habitat types, and include the swift parrot, the superb parrot, *Polytelis swainsonii*, and the turquoise parrot, *Neophema pulchella* (Higgins 1999; Garnett and Crowley 2000).

In Tasmania, the orange-bellied parrot, *Neophema chrysogaster*, is considered critically endangered, the swift parrot is endangered, and the Tasmanian eastern rosella, *Platycercus eximius diemenensis*, is near threatened (Garnett and Crowley 2000). The major threatening processes to birds in Tasmania include habitat clearance and modification through logging and agriculture, the effects of introduced species, and collisions with artificial structures (Garnett and Crowley 2000). The swift parrot and the orange-bellied parrot are the only fully migratory parrots in the world (Juniper and Parr 1998; Higgins 1999). They breed in Tasmania during the spring and migrate across Bass Strait to over-winter on the mainland (Higgins 1999). This strategy exposes them to the effects of habitat modification at several points in their life history (Ford et al. 2001). Those species which are habitat specialists or move sequentially among several habitats are at highest risk of population decline (Ford et al. 2001).

GENERAL DESCRIPTION OF THE SWIFT PARROT

The swift parrot is the only member of its genus and its classification has received considerable attention from taxonomists. In some studies, the swift parrot is positioned taxonomically with the Loriinae (lories and lorikeets) due to similarities in tongue morphology and the tight, glossy plumage (Holyoak 1973; Smith 1975). However, other authors have disputed this classification. The swift parrot also shows anatomical similarities with members of the Platycercinae subfamily (Australian broad-tailed parrots), such as the rosellas (Forbes 1879; Homberger 1980), and recent DNA hybridisation studies (Christidis et al. 1991)

have placed the swift parrot in this subfamily. Morphological similarities between swift parrots and the lorikeets may therefore be due to evolutionary convergence rather than taxonomic affinities (Christidis et al. 1991).

Study of the swift parrot presents unique opportunities to gain insight into a range of biological phenomena. The swift parrot (Figure 1) is a nectarivorous parrot from a sub-family of granivorous parrots (Christidis et al. 1991). Therefore, study of the swift parrots' morphological and physiological adaptations may allow greater understanding of the strategy of nectarivory in birds.

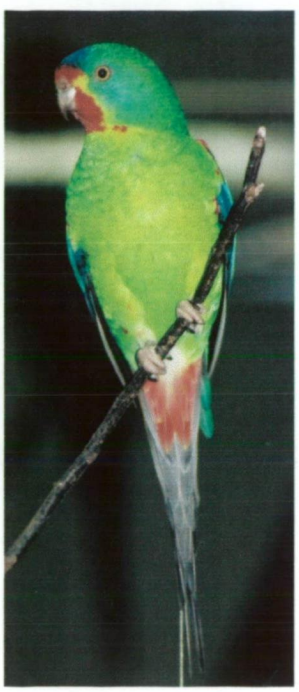


Figure 1. Captive adult male swift parrot.

The swift parrot is also one of only three migratory parrot species in the world (Dingle 1996; Higgins 1999). All three of these species breed in Tasmania and make a short distance, to and fro, migration across the Bass Strait to the mainland of Australia (Dingle 1996; Higgins 1999). There has been no previous examination of migratory behaviour or physiology in any of these species. Finally, the swift parrot is an irregular, seasonal breeder, in which successful

reproduction has been correlated to the abundant flowering of the Tasmania blue gum, *Eucalyptus globulus* (Brown 1989; Brereton 1996a). Investigation of this relationship may provide insight into the mechanisms by which environmental variables may synchronise or inhibit reproduction.

CONSERVATION STATUS OF SWIFT PARROTS

The swift parrot is listed as endangered in the Commonwealth Environment and Biodiversity Protection Act 1999 and endangered in the Tasmanian Threatened Species Protection Act 1995. The swift parrot meets the criteria as listed by the IUCN (World Conservation Union) to be considered as an endangered species (IUCN Species Survival Commision 1994). Firstly, the population is estimated to be less than 2,500 mature individuals and within the parrot's breeding range the area of occupancy is less than 500 km^2. The population has a severely fragmented distribution, there is an inferred continuing decline in the numbers of mature individuals, and an observed decline in the area and quality of habitat (Brown 1989; Brereton 1996a; 1996b; Brereton 1999). The loss of habitat has occurred in both the breeding and over-wintering ranges of swift parrots. In the breeding range it has been estimated that 70 % of the forests used by swift parrots have been cleared (Brown 1989; Brereton 1996a; Brereton 1999). In the birds' over-wintering range the loss of habitat is estimated to be greater than 85% in Victoria and greater than 70% in NSW (Traill 1993; Sivertsen 1993; Mac Nally and Horrocks 2000).

EVIDENCE OF A POPULATION DECLINE IN SWIFT PARROTS

There are inherent difficulties in surveying the population of a highly mobile, nomadic and migratory species such as the swift parrot. Estimates of the abundance of the swift parrot population are based on a census carried out in 1987/88 when the total breeding population was estimated at 1320 breeding pairs (Brown 1989) and another in 1995/96 in which the estimate was 940 breeding pairs (Brereton 1996a). The two surveys were carried out using similar methods

(Brown 1989; Brereton 1996a; 1999), although details of the methods have not been published. In 1999, an annual, repeatable, monitoring program was initiated using a fixed-stationary observer technique at 65 permanent plots. This gave a population estimate of 2,400 (± 700) swift parrots in 1999 (Brereton pers. comm.).

Prior to these surveys there are only anecdotal reports that give the impression that the birds once occurred in greater numbers. There are reports of a flock of greater than 1000 birds being seen at Lake St. Clair in central Tasmania in 1959 (Hindwood and Sharland 1964). Similar sized flocks were reported in Sydney, NSW, in 1958 (Hindwood and Sharland 1964). Some authors believe that these large flocks may be indicative of irruptions of the population (Hindwood and Sharland 1964; Brown 1989), although there is no firm evidence of this. There is a public perception that the birds are no longer as numerous as they once were. However, this is difficult to assess due to the variable distribution of birds from year to year because of their nomadic foraging behaviour and the variability of the flower resources they use. A field guide from the fifties (Sharland 1958) states that the birds were considered common at that time, but given their highly conspicuous behaviour and willingness to use urban vegetation for foraging, this may have been a mistaken impression.

There has been a reduction in the wintering range of the population. In the 1960s the swift parrots were documented as visitors in large numbers to South Australia, although even then the species was classed as an unpredictable migrant, not being seen annually (Hindwood and Sharland 1964). There have been declines in the reporting of swift parrot sightings in South Australia recently (Brereton 1996a). Similarly, there are sporadic reports of birds migrating as far north as Rockhampton in Queensland in the first half of the twentieth century (Hindwood and Sharland 1964), while recently only small numbers of birds have been found much further south near Toowoomba and Currumbin (Brereton 1999). This restriction of the wintering range may be due to the loss and fragmentation of suitable foraging habitat that has occurred in these areas (Brereton 1996a; 1999).

In summary, it is not certain whether the present population is continuing to decline or is stable. Indeed, although anecdotal reports suggest that the population is much less abundant than it was, there is no firm evidence of a

population decline during the last 50 years. Swift parrots may have been persisting in these relatively low numbers for some time. The Swift Parrot Recovery Plan suggests that the population be considered at best stable, but may still be declining (Brereton 1996a; 1999).

GENERAL CONSTRAINTS ON SEASONAL REPRODUCTION IN BIRDS

Individual organisms must be able to survive a wide variety of environmental fluctuations (Jacobs and Wingfield 2000). Some of these fluctuations are periodic (seasonal) and, therefore, coarsely predictable, particularly in temperate regions (Wingfield 1983; Jacobs and Wingfield 2000). The mechanisms that allow animals to regulate the stages of their life history to take advantage of these periodic variations include environmental cues and endogenous biological rhythms (Wingfield 1983; Jacobs and Wingfield 2000). The timing of all life history stages, in particular reproduction, is selected over generations to maximise the survival of the individual and its offspring (Farner and Wingfield 1980). The environmental factors that influence this timing are termed ultimate factors (Baker 1938; Wingfield 1983; Cockrem 1995; Jacobs and Wingfield 2000). A major ultimate factor in the timing of avian reproduction is the availability of resources for feeding young and for post-fledging survival (Wingfield 1983; Jacobs and Wingfield 2000).

Ultimate factors interact with endogenous physiological rhythms to set the annual rhythms of life history stages. The evidence for an underlying internal cycle of reproductive rhythm in avian species comes from three sources. First, captive birds, from all latitudes, which are held in constant environmental conditions (including photoperiod), demonstrate continued seasonal gonadal cycling; ie. regression and recrudescence of gonads (Wada 1983; Wingfield 1983; Gwinner and Dittami 1990; Gwinner 1996). Environmental changes are not required for these birds to go through complete and successful reproductive cycles. Interestingly, such captive birds showed varying duration of the reproductive cycle between individuals of the same species, although the cycles are constant for an individual from year to year (Wada 1983; Wingfield 1983;

Gwinner and Dittami 1990; Gwinner 1996). Most of the cycles deviate from the natural year: eg. captive African stone chats, *Saxicola torquata axillaris,* cycle on average every nine months (Gwinner and Dittami 1990; Gwinner 1996). Second, in the wild, long distance migratory species are exposed in the course of a year to very complex patterns of change in photoperiod, yet can maintain highly synchronised breeding, moult and migratory rhythms (Wada 1983; Wingfield 1983; Gwinner and Dittami 1990; Gwinner 1996). Finally, tropical species, which are exposed to little variation in photoperiod, still maintain an annual cycle, although other environmental variables may have a synchronising role (Gwinner and Dittami 1990; Gwinner 1996; Hau et al. 1998). It has been assumed that factors associated with the alternation between dry and rainy seasons are responsible for synchronising breeding (Gwinner and Dittami 1990). However, these factors vary much more between years than does the timing of the birds' seasonal activities, suggesting the involvement of endogenous rhythms (Gwinner and Dittami 1990; Cockrem 1995; Hau et al. 1998).

If the environment showed no variability apart from the predictable seasonal changes, reproduction could occur at exactly the same time each year. However, there is considerable annual variation within the seasonality of these factors. The majority of environmental fluctuations are not seasonal nor predictable. Therefore, individuals must be able to modify the progression of their own life history on a year to year basis. Environmental fluctuations that directly regulate the timing of breeding from year to year are termed proximate factors (Baker 1938). A wide range of local conditions have been identified as proximate factors, which Wingfield (1983) grouped into the four main categories described below:

i. Initial predictive factors

These factors initiate gonadal development in anticipation of the ensuing breeding season. They bring the bird into the physiological state for breeding and maintain it throughout the breeding season. On their own, these factors are not enough to initiate the nesting phase. For many birds, the main initial predictive factor is the change in daylength (Wingfield 1983; Wingfield and Farner 1993;

Cockrem 1995; Jacobs and Wingfield 2000). For arid zone birds, rainfall, or its effect on vegetation, may be more important (Wada 1983; Zann 1995).

ii. Essential supplementary factors

These factors supplement the initial predictive information and initiate the final stages of gonadal development up to nesting. These include social cues, territorial behaviour, climate and nutrition (Wingfield 1983).

iii. Synchronising and integrating factors

These factors regulate the sequence of breeding events including nest building, copulation, oviposition, incubation and rearing. They include the important social interactions between the breeding pair (Wingfield 1983).

iv. Modifying factors

These are events or conditions that can disrupt the reproductive cycle and include adverse weather, loss or disturbance of nest site, predation and loss of mate (Wingfield 1983; Wingfield and Farner 1993; Cockrem 1995; Jacobs and Wingfield 2000).

In order to understand and then investigate the variety of proximate factors that may be affecting reproduction in the swift parrot population a thorough understanding of the species' life history is required.

LIFE HISTORY OF THE SWIFT PARROT

The annual migration of the swift parrot dominates its life history (Figure 2). The birds arrive in Tasmania from early August with the bulk of the population being present by mid-September (Hindwood and Sharland 1964; Brown 1989; Brereton 1996a; 1999). Flocks of up to 70 birds are observed at this time on the flowering gums *E. globulus* and *Eucalyptus ovata*. The breeding season has been reported as September to late January (Hindwood and Sharland 1964; Brown 1989; Brereton 1996a; 1999; Higgins 1999).

Nesting occurs in tree hollows and can commence as early as late September (Hindwood and Sharland 1964; Brown 1989; Brereton 1996a; 1999). Nest-sites are sometimes re-used, but not in successive years, and it is unknown if the same birds are re-using the old sites. The choice of nest-sites appears to be determined

by the proximity of sufficient food resources (Brereton 1996b; 1999). The female bird is solely responsible for incubation while the male supplies her with food during this time (Hindwood and Sharland 1964; Brown 1989; Brereton 1996a; 1999). Clutch size varies from two to five, and the fledging period is approximated at six weeks (Brown 1989; Brereton 1996a). The number of swift parrots seen and the incidence of breeding attempts in southern Tasmania fluctuates widely, with less than 50 birds observed in some years and greater than 2000 in others (Brown 1989; Brereton 1996a). This annual fluctuation appears to be correlated with the incidence of blue gum, *E. globulus,* flowering, which is known to be sporadic but has not yet been thoroughly investigated. Reproduction in swift parrots appears to be dependent, at least during the breeding season, on this single eucalypt species, perhaps because the flowers of *E. globulus* are large and, when available, provide a large volume of nectar over a relatively long period of time. In years when *E. globulus* does not flower well, swift parrots are noted to forage primarily on the swamp gum, *E. ovata,* and introduced species of flowering plants, but the incidence of breeding attempts is suspected to be very low in these years (Brown 1989; Brereton 1996a).

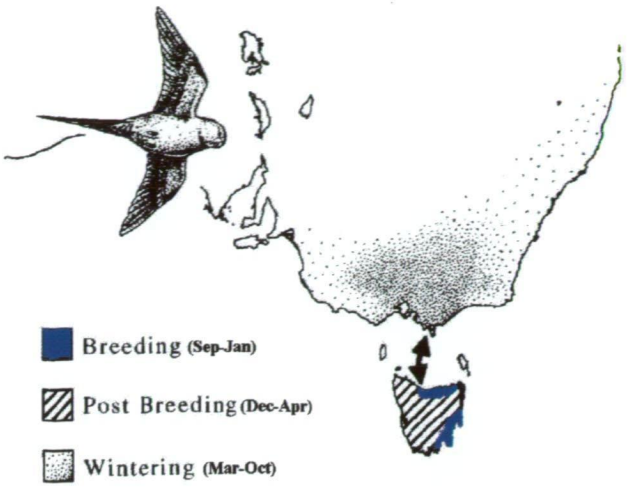


Figure 2. Distribution and annual migration of the swift parrot. Modified from Dingle (1996) with permission. The arrow represents the annual to- and fro- migration across the Bass Strait.

In late summer, there is a general movement of the population westwards within Tasmania, although these movements appear to be modified by the flowering of eucalypts of a range of species (Brereton 1996a). During this period, the movements of the swift parrots are best described as nomadic. Adult post-breeding moult begins in Tasmania, possibly commencing in January, and is usually completed before leaving Tasmania (Higgins 1999). By mid-February, birds have started to appear on the Australian mainland and the majority of the population has migrated northward across Bass Strait by the end of April (Hindwood and Sharland 1964; Brown 1989; Brereton 1996a; 1999). After migrating to the mainland, most of the population disperses throughout the remaining box-ironbark forest fragments in Victoria. Smaller numbers of birds are regularly seen in the coastal regions of NSW and near Toowoomba in Queensland (Hindwood and Sharland 1964; Brown 1989; Brereton 1996; 1999). It is unknown whether the winter distribution of swift parrots is a result of the chance nomadic wanderings of this highly mobile species, or if individual birds return to favoured foraging grounds each year. There are regularly used sites on the mainland,

however these are not used every year (Kennedy 1998). Occasional "irruptions" of large numbers of birds have occurred in a range of sites on the mainland, although these seem to have occurred more frequently in the late 1930s and the 1950s than recently (Higgins 1999).

The age structure of the swift parrot population is completely unknown. It is suspected that age to first breeding is one year, based on anecdotal reports from captive-bred birds (Brereton 1996a). Longevity and survival rates of wild birds are completely unknown. There have been no recoveries from 46 birds that were banded in Australia between 1953-1996 (Higgins 1999). There is an annual mortality of adult and juvenile swift parrots from collisions with man-made objects, in particular windows and fences. The true incidence of these events is unclear because there is no reliable method for estimating the number of dead and injured birds which are not reported to the Parks and Wildlife Service. In 1987/88 fourteen deaths were reported, with five birds being adults and nine juveniles (Brown 1989). It is difficult to assess the impact of this level of mortality on the population given the uncertainty about reporting rates and no information about the levels of recruitment back into the population. However, if the population is in severe decline, this mortality will have a progressively greater impact. Other known mortality relates to natural predation, with one study finding swift parrots remains in 57% of peregrine falcon, *Falco peregrinus,* eyries (n = 33), and also in the nests of brown goshawks, *Accipiter fasciatus,* and sparrowhawks, *Accipiter cirrhocephalus* (Brown 1989). Nesthole predation by a laughing kookaburra, *Dacelo gigas,* has been observed on one occasion, with the loss of all chicks (Brown 1989).

PROJECT AIMS

Regardless of the external causes of a decline in the population of a species, their effects are expressed as either reduced breeding success or reduced survival (Green 1994). The current project was designed to investigate both these demographic mechanisms, although the focus is predominantly upon the constraints on breeding success.

My specific scientific aims were to:

1. Describe the normal reproductive strategy of swift parrots

Only after understanding the normal reproductive biology can the constraints on reproduction and the degree of reduced breeding success be identified. This information will also help to predict the ability of the species to adapt to continued environmental change and assess the risks of extinction. Chapter 2 describes the normal reproductive biology of swift parrots by examining annual cycles of reproductive hormones; gonadal size, histology; and body weight. This information was established from captive birds and compared with data from free-living birds. Chapter 3 describes aspects of the reproductive cycle of swift parrots that relate to parental care.

2. Investigate nutritional constraints on breeding success

Nutrition is arguably the most important of the factors that constrain both the annual control of the timing of reproduction (ie. as an ultimate factor) and an individual animal's likelihood to breed within any given year (ie. as a proximate factor) (Wingfield 1983; Jacobs and Wingfield 2000). The underlying premise for this in swift parrots is that their adaptation to nectarivory, and the resulting dependence on blue gum nectar, may be a major factor in their decline. Therefore, Chapters 4 and 5 describe investigations to determine whether swift parrots are primarily nectarivorous, and to describe specific adaptations of the birds to exploit this nutritional strategy. Chapter 6 examines the dietary importance of alternative foods used by this species, particularly in regard to alternative sources of protein. Further, Chapter 9 investigates the links between nutrition and breeding success by monitoring reproductive parameters in birds on diets containing different levels of total energy and protein.

3. Investigate other physiological constraints on reproduction and survival

The breeding season of birds can be constrained both temporally and energetically by the requirements of other life history stages (Jacobs and Wingfield 2000). In swift parrots, these key stages include both moult, and, unusually for a parrot, migration. In Chapter 2, I describe the annual pattern of moult and relate its timing to that of the reproductive cycle. Chapter 10 describes the constraints that the demands of migration impose on reproduction. Chapter 11 describes the annual mortality of swift parrots due to collisions with man-made objects, particularly with the specific aim of characterising the injuries sustained in window collisions and the structure of the population involved. Chapter 11 also assesses the health of wild swift parrots to determine if disease is a significant factor in population mortality.

STRUCTURE OF THE THESIS

The thesis is divided into two parts. Part A describes the investigations of the normal reproductive biology of the species (Chapters 2 and 3). Part B is divided into two sub-sections. Chapters 4, 5, 6 and 9 describe the investigation into the nutritional constraints on reproduction in swift parrots. Chapters 4, 5 and 6 have been published and are presented in their published formats with minor changes to referencing style for the thesis format. The co-authorship on these papers is to acknowledge supervisors support in experimental design and the preparation of the manuscripts but I carried out all other work. Chapters 7, 8, 10 and 11 describe the investigations of other physiological constraints on reproduction and their implications for conservation. A general discussion (Chapter 12) follows and discusses the implications of this study for our understanding of the life history of the swift parrot and the conservation management of the species.

PART A

REPRODUCTIVE BIOLOGY
OF
THE ENDANGERED SWIFT PARROT

Chapter 2. Reproductive biology

INTRODUCTION

The normal reproductive biology of the swift parrot must be understood before the constraints on reproduction, and any degree of reduced breeding success, can be identified and characterised. In this chapter, I describe the reproductive biology of the species, focussing on the interactions of the endocrine system and reproductive organs in the initiation of breeding. The period encompassing parental care is examined in Chapter 3.

The endocrine control of seasonal reproduction in birds

In most avian species, the breeding season is timed to coincide with environmental conditions that maximise the survival of both the young and the parents (Gwinner and Dittami 1990; Harvey et al. 1990; Wingfield and Farner 1993; Cockrem 1995; Gwinner 1996). This usually means associating breeding with the period of maximal food resources and the day-lengths that permit their exploitation (Farner and Wingfield 1980; Wingfield and Farner 1993; Jacobs and Wingfield 2000). The timing of reproduction is achieved each year by a seasonal variance in gonadal activity and reproductive behaviour (Farner and Wingfield 1980; Wingfield and Farner 1993; Jacobs and Wingfield 2000), and both endogenous annual rhythms and exogenous conditions (eg. photoperiod, rainfall, and humidity) may influence the synchronisation of the events that constitute breeding (Farner and Wingfield 1980; Wingfield and Farner 1993; Jacobs and Wingfield 2000). Secretions of the endocrine glands are the primary mechanisms for the coordination and actuation of these cyclic reproductive events within an individual (Farner and Wingfield 1980; Wingfield and Farner 1993; Ottinger and Bakst 1995; Jacobs and Wingfield 2000).

The endocrine system that exerts primary control over reproduction is the hypothalamic-pituitary-gonadal (HPG) axis (Farner and Wingfield 1980; Harvey et al. 1990; Ottinger and Bakst 1995). Environmental cues and endogenous rhythms act at the level of the central nervous system via neurotransmitters and neuropeptides to stimulate or inhibit the synthesis and release of gonadotropin-releasing hormone

(GnRH) from the hypothalamus (Ottinger and Bakst 1995). The main role of GnRH is to stimulate secretion of the gonadotropins: luteinising hormone (LH) and follicle-stimulating hormone (FSH) by the anterior pituitary (Ottinger and Bakst 1995). The gonadotropins act on the gonads to regulate gamete production and the synthesis of the sex steroids, primarily testosterone and oestradiol (Ottinger and Bakst 1995). These sex steroids are responsible for the support of secondary sex structures and, possibly in conjunction with neuropeptides, sexual behaviour (Ottinger and Bakst 1995). The sex steroids also have a negative feedback role in regulating GnRH production from the hypothalamus, and weak negative feedback on the pituitary, modulating gonadotropin secretion (Ottinger and Bakst 1995).

In birds, seasonal variation in reproduction is predominantly controlled by photoperiod (Gwinner and Dittami 1990; Harvey et al. 1990; Wingfield and Farner 1993; Cockrem 1995; Gwinner 1996), even in tropical species (Hau et al. 1998; Hau 2001). Prior to the breeding season, the HPG axis must become photosensitive (Gwinner and Dittami 1990; Harvey et al. 1990; Wingfield and Farner 1993; Gwinner 1996). Photosensitivity is the readiness of the birds to be reproductively stimulated by increasing daylength. There is dramatic stimulation of gonadotropin secretion and gonadal growth in response to increasing daylength in some species (Farner et al. 1983; Cockrem 1995; Hahn and Ball 1995). At the conclusion of reproduction, avian species become photorefractory (Wingfield and Farner 1993). Photorefractoriness is the loss of response of the reproductive system to photoperiod and the regression of the gonads. There are two main types of photorefractoriness: absolute and relative (Farner et al. 1983; Wingfield and Farner 1993; Cockrem 1995; Hahn and Ball 1995). Absolute photorefractoriness involves a complete regression of the gonads and during this period the reproductive system cannot be stimulated, even by very long photoperiods (Farner et al. 1983;Wingfield and Farner 1993; Cockrem 1995; Hahn and Ball 1995). In these birds there is a complete loss of the neural activity that controls GnRH in the hypothalamus and increasing photoperiod (long days), the same factor that stimulates gonadal development in the first place, terminates breeding (Hahn and Ball 1995). Relative photorefractoriness occurs in species in which the gonads regress but are readily stimulated again by exposure to long day lengths (Farner et al. 1983;Wingfield and Farner 1993; Cockrem 1995;

Hahn and Ball 1995). These birds have a reduced level of neural activity controlling GnRH and decreasing photoperiod (short days) terminates breeding (Hahn and Ball 1995). Photosensitivity is gradually recovered after photorefractoriness, usually resulting from exposure to short day-lengths. In natural conditions most species become photosensitive in autumn. This is manifested in some species as a brief resurgence of autumnal sexuality that usually does not lead to breeding (Wingfield and Farner 1993; Cockrem 1995). For birds that normally breed in high latitudes long day-lengths are needed to induce photorefractoriness and very short day-lengths are required to regain photosensitivity in absolute photorefractory species (Wingfield and Farner 1993; Cockrem 1995). In contrast, the reproductive state of species that normally breed in low latitudes (tropical regions) can be altered by changes in photoperiod of less than one hour (Hau et al. 1998).

Some birds are short day breeders and display a reverse response to day length to that outlined above, such as the emu, *Dromaius novaehollandiae* (Malecki et al. 1998). However, most birds do not breed in autumn, and this is due to endogenous controls on the HPG axis.(Cockrem 1995; Hahn and Ball 1995). During autumn, birds that breed only in spring, have a high resistance of the pituitary to gonadal steroid feedback; this inhibits the onset of reproduction (Cockrem 1995; Hahn and Ball 1995). Gradually the 'photoperiodic drive' overcomes this inhibition as daylength increases after winter (Cockrem 1995; Hahn and Ball 1995). There may also be a gradual increase in the 'hypothalamic drive', that is, a gradual lowering of the pituitary's resistance to gonadal steroids and a progressive increase in the level of GnRH produced by the hypothalamus as spring approaches (Cockrem 1995; Hahn and Ball 1995).

These seasonal variations in the responsiveness of the central nervous system to environmental cues results in annual patterns of gonadal size and activity. Closely correlated to these gonadal changes are variations in the plasma concentrations of the reproductive hormones (Wingfield 1983; Wingfield and Farner 1993; Harvey et al. 1995; Gwinner 1996). Assessing these patterns is the first step in understanding how environmental cues interact and constrain reproduction in any avian species.

Reproduction in wild swift parrots

Swift parrots breed only in Tasmania and the recognised start of the breeding season is the arrival of birds from their over-wintering grounds on the Australian mainland. The earliest birds to return arrive in August (late winter), and the bulk of the population has returned by mid-September (early spring) (Hindwood and Sharland 1964; Brown 1989; Brereton 1996a). At this time, the birds are concentrated in south-eastern Tasmania with a smaller group of birds congregating in the north of the state between Launceston and Smithton (Figure 1) (Brereton 1996a). The reproductive status of arriving birds is not known, but they spend the first few weeks following their arrival foraging and inspecting nest hollows (Brown 1989; Brereton 1996a). Brown (1989) suggests that adults may retain pair bonds from previous seasons and return to prior nest sites. However, these assertions are based only on observations of the rapid initiation of breeding in some pairs and the re-use of nest-holes that had been occupied two years earlier (Brown 1989); identification of individual birds will be necessary to assess these hypotheses.

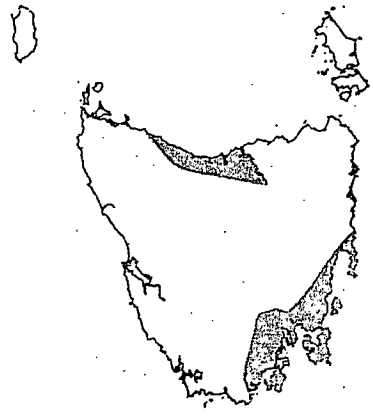


Figure 1. Distribution of swift parrots (grey areas) within Tasmania during the breeding season (September to January).

Courtship behaviour has not been well documented in wild swift parrots. However, inspection of trees for suitable nest hollows occurs in both paired and

single birds from August through to October (Brown 1989; Brereton 1996a). Swift parrots breed in the hollows of old eucalypt trees (Hindwood and Sharland 1964; Brown 1989; Brereton 1996a). Brereton (1996b) examined records of fifty-nine nest hollows and concluded that the preferred nest hollows were in old eucalypts with a mean diameter at breast height over bark of 1.3 metres. Seventy six percent of nests were located in hollows in branches and the remainder in hollows in trunks. The nest hole opening was on average 10 to 11 cm in diameter, with the entrance at a height ranging from six to twenty-eight metres from the ground. Nest hollows were not re-used annually. Pairs often nested in close proximity to each other and the nesting location was generally within eight kilometres of the coast. The nesting locations were close to foraging sites, with greater than 50% being within one kilometre of good foraging sites and approximately 90% within 6 kilometres (Brereton 1996b). The nest hollows preferred by swift parrots are not considered to be limiting to their successful breeding (Brown 1989; Brereton 1996a).

Nesting, egg-laying, incubation and the brooding of young (examined in Chapter 3) may occur as early as September, and through to January (Hindwood and Sharland 1964; Brown 1989; Brereton 1996a; b). Much remains speculative about this period. Breeding success (as measured by the appearance of juveniles) is known to show marked variation between years and appears to depend on the intensity and extent of the flowering of the Tasmanian blue gum, *Eucalyptus globulus* (Brown 1989; Brereton 1996a). The percentage of the adult population that are involved in breeding, the percentage of nest failures and the incidence of double brooding are all unknown. While the late appearance of some juveniles raises the possibility of double brooding (Brown 1989; Brereton 1996a), its occurrence in the wild remains to be confirmed. It is suspected that years of low reproductive success can be attributed to failure to initiate breeding rather than nesting failure (Brereton *pers. comm.*). There is probably little nest predation due to the high placement and small diameter of nest hollows: however, there is a single report of chicks being removed by a laughing kookaburra, *Dacelo gigas* (Brown 1989).

Once juveniles fledge, they remain in the nest vicinity, being fed by the parents, for approximately five days before they leave the area (Brown 1989). Flocks

of juveniles have been observed and it is suspected that they may migrate together (Hindwood and Sharland 1964; Brown 1989; Brereton 1996a).

The age to first breeding has not been confirmed in wild birds. Although Brown (1989) suggests that birds involved in late breeding (October to November) may be first year birds, there is no evidence provided for this assertion. Birds that do not breed are suspected to remain in the breeding areas where there is good foraging until December or January (Brereton *pers. comm*). Moulting occurs from January through to April (Higgins 1999), but there is no information available on whether moult-breeding overlap occurs. During this period, all birds appear to be nomadic in response to foraging resources (Hindwood and Sharland 1964; Brown 1989; Brereton 1996a), but with an overall movement gradually north and westwards until the northern migration occurs. The pattern of annual life history events that encompass and constrain reproduction in wild swift parrots is presented in Figure 2.

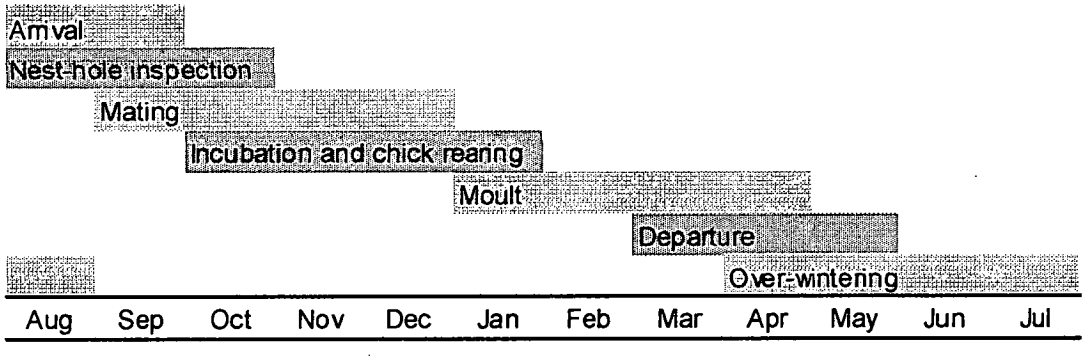


Figure 2. Time line of the annual life history events in wild swift parrots from arrival at the breeding grounds in Tasmania to departure and over-wintering on the mainland of Australia.

Reproduction in captive swift parrots

Information about reproduction in captivity is generally anecdotal. However, there are some features worth noting, in particular, the age to first breeding, the duration of the breeding season in captivity, the incidence of double clutching, and the geographic limits of successful reproduction.

The age to first breeding in captivity can be as young as 12 to 14 months for both male and female birds, but only if they were hatched early in the previous season (Laubscher 1999). Birds hatched late in the season do not seem to be able to reach maturity in their first breeding season (second year) and breed in their third year at ~ 24 months of age. Second year breeding is likely to be the minimum time in which maturity is achievable, and suggests that wild birds, which have to undergo migration and forage for their food, may not mature until their second year.

In captive swift parrots in Australia, the breeding season matches that of wild birds (Shephard 1989). This suggests that under natural photoperiods, the regular rhythm of reproduction may be retained. Laubscher (1999) states that in European aviaries breeding can take place in June. If swift parrots kept in the northern hemisphere are breeding in the European spring and summer, suggesting a synchronising effect of photoperiod on reproduction in the species.

Double, and even triple, clutching is seen regularly in captivity (Laubscher 1999; B. Cook and K. Febey *pers. comm.*). This occurs when birds are fed *ad libitum* and is more common in older pairs (B. Cook *pers. comm.*). This suggests that double clutching should be possible in the wild in periods of high food availability. However, the demands of migration, nest hole searching and foraging that occur in wild birds will temper this possibility.

The success of breeding and raising swift parrots in captivity decreases the further north in Australia the species is kept, with birds kept in Sydney rarely raising young without assistance. The consistent suggestion from bird-keepers is that temperatures over 30 °C will result in stressed birds that abandon nests and dependent hatchlings (Lendon 1979; Hutchins and Lovell 1985; Shephard 1989; Laubscher 1999). This sensitivity to heat may explain the success of European bird-keepers in breeding swift parrots as the aviaries in Europe tend to be indoors and temperature regulated (Laubscher 1999). A detrimental effect of temperature on reproduction in swift parrots may partly explain why the species has developed its migratory strategy.

Aims of this study

There are many gaps in our understanding of the reproductive cycle of the swift parrot. Before the constraints on breeding could be investigated, the following information was required:

- the proportion of adult males and females breeding each year in the wild;
- whether the timing of reproduction varies between years; and
- the endocrine and environmental correlates of successful breeding.

I used both captive and wild birds to investigate these overall questions.

1. Reproduction in captive swift parrots and musk lorikeets

Captive swift parrots were used to establish:

a) the patterns of seasonal change in gonadal size and activity, using endoscopy and testicular cytology. This allowed accurate identification of sex, and the staging and timing of spermatogenesis in male birds.

b) the patterns of seasonal change in the plasma concentrations of the reproductive steroids, testosterone in male birds and oestradiol in female birds. These changes could then be associated with changes in gonadal size and activity, and reproductive behaviours, so that differences between breeding and non-breeding birds could be established.

c) the effect of exogenous GnRH on plasma concentrations of testosterone in male birds. This gives a measure of the reproductive readiness of the HPG axis (Hirschenhauser et al. 2000) and the onset and duration of photorefractoriness (Sharp et al. 1998). In particular, I wished to examine how changes in reproductive readiness were correlated with the migratory movements of the wild population.

d) changes in behaviour associated with reproduction and the onset of moult.

Musk lorikeets, *Glossopsitta concinna*, were used as a comparative species in the captive breeding study. They are a common nectarivorous psittacine that is a nomadic resident in Tasmania (Higgins 1999). They breed readily in captivity,

although little is known of the endocrine correlates of their reproduction (Low 1998). They were a useful species for comparison with the swift parrots because:

a) they allowed confirmation that the captive environment provided would support reproduction in a nectarivorous psittacine;
b) they follow a similar reproductive strategy; that is, they are monogamous spring breeding birds using nest-hollows and producing a small asynchronously hatching clutch (Higgins 1999). Therefore, it might be expected that their gonadal and hormonal cycles should be similar to the swift parrots.
c) they are nomadic but non-migratory (Higgins 1999). Therefore, differences in reproductive rhythms between the swift parrots and the musk lorikeets may reflect their different life histories.

The advantage of using captive birds to follow reproduction is that the same birds can be assessed repeatedly over periods of greater than one year and allow tests of hypotheses that cannot be examined in the field (Lambrechts et al. 1999). In particular, observations of breeding and parenting behaviour can be made, and factors such as diet strictly controlled. The disadvantage of captive studies is that the captive environment may produce abnormalities in behaviours, and/or reproductive and physiological traits (Lambrechts et al. 1999). Therefore, studies of wild birds are needed to validate the reliability of experiments on captive birds (Lambrechts et al. 1999).

2. Reproduction in wild swift parrots

The swift parrots are a fast-flying arboreal species in the wild and are difficult to capture (Brereton 1996b). Further, it is difficult to predict their movements because of their nomadism and irregular re-use of nest-hollows. A study of reproduction in wild swift parrots is, therefore, logistically and technically difficult, and a large effort was required to return relatively little information. This emphasises the need for the captive studies to produce baseline reproductive information. The aims in assessing reproductive parameters in wild swift parrots were to establish whether:

a) wild swift parrots showed similar patterns of gonadal size and activity to the captive birds and how these changes related to migratory movements;
b) the plasma concentrations of the reproductive steroids in wild swift parrots (testosterone in males and oestradiol in females) followed similar seasonal patterns to the captive birds and how these changes related to migratory movements;
c) the gonadal and hormone changes could be used to assess the proportion of adult male and female swift parrots in breeding condition;
d) the gonadal and hormone changes could be used to assess differences in breeding capability between years, and whether these differences were correlated to the flowering patterns of the Tasmanian blue gum;
e) there was a simple method for determining the sex and breeding condition of a bird captured in the wild.

MATERIALS AND METHODS

Captive birds

Source of birds and general husbandry

Permission to keep swift parrots in captivity was obtained from Tasmanian Parks and Wildlife Service, with the condition that birds were only obtained from registered Tasmanian aviculturists or wild birds injured from accidental collisions. There are very few Tasmanian aviculturists that are licensed to keep swift parrots as trade in this endangered species is not permitted. I was, therefore, constrained in the number and sexes of swift parrots that were available and numbers fluctuated throughout the course of the project. Wild swift parrots that had been injured in collisions with windows, fences and motor vehicles and were unable to be released were obtained from the Nature Conservation Branch of the Tasmanian Parks and Wildlife Service. I purchased four musk lorikeets from a licensed bird-keeper and obtained two more as injured wild juveniles. All birds were individually identified by numbered leg bands and the sex of each bird was confirmed by endoscopic assessment of the gonads. There was a predominance of male to female swift parrots

but equal numbers of male and female musk lorikeets were held throughout the study.

Birds were kept in mixed-sex groups of between four and six birds in roofed enclosures 1.9 x 3.4 x 2.1 m. The cages were wire fronted, allowing access to natural photoperiod. Eucalypt branches were installed as perches and artificial nest-boxes (60cm x 20 cm x 18 cm) with natural spouts were installed in August each year to simulate the birds' return to breeding grounds and stimulate the initiation of reproduction (Millam et al. 1988) (Figure 3). Fresh wood shavings were placed in the nestboxes each year as nesting material. The nest boxes were removed and cleaned in March each year.

Figure 3. Adult male swift parrot on the artificial nest boxes used in the study of reproduction of captive birds. Note the natural spout and the white inspection port.

During the spring and summer of the first two years of the trial, the swift parrots were fed once a day on nectar and pellets (see Chapters 7 and 9 for further details). Nectar powder was formulated as Lori-nectar® or Lori-start® Avesproduct B.V. (66.6% DM); Farex Original Blended Cereal® Heinz Wattie's Ltd (16.7% DM); and sucrose (16.7% DM). Pellets were either Roudybush® maintenance or breeder pellets. The diets were supplemented with a small amount of fresh apple (1/4 apple for 6 birds every second day). In the non-breeding period the maintenance pellets and nectar was fed to all birds. The birds were fed each morning and offered an equal volume of nectar and pellets. One group of birds was fed small amounts of

Tasmanian blue gum flowers daily from August to December (see Chapter 9). In the final year of the project the pelleted diet was replaced with a commercial lorikeet dry food (Avione® Lorikeets rearing and conditioning food). Musk lorikeets were maintained on the low protein nectar mix and lorikeet dry food, supplemented with apple and Tasmanian blue gum flowers in the breeding season.

Assessment of gonadal cycles

Captive swift parrots - endoscopy, testicular aspiration and cytology

I used endoscopy and fine needle biopsy to assess seasonal changes in gonadal size and testicular cytology in live captive birds. The left testis volume (LTV) of captive adult male swift parrots was assessed in September (n = 5), December (n = 5), March (n = 10) and June (n = 5). However, intra-operative bleeding caused the procedure to be abandoned in one bird in September, two in March and one in June.

Endoscopy is the insertion of a small diameter rigid fibre-optic scope through the abdominal wall musculature, which allows visualisation of the internal organs. Birds are ideally suited to endoscopic evaluation of their internal organs due to the space created by their air sacs (Taylor 1994). I used a small mask to induce anaesthesia using an inhaled mix of isoflurane and oxygen via an Isotec 3 vaporiser and a T-piece breathing system. Induction of anaesthesia was carried out using 5 % isoflurane concentration; this was reduced to between 2 - 3 % once a surgical plane of anaesthesia was attained. The birds were placed in right lateral recumbency and a small area, ~2 cm diameter, of feathers in the left lateral flank were removed. The area was aseptically prepared and a 2-3 mm incision was made through the lateral abdominal muscles. The endoscope was inserted into the abdominal air sac. The tip of the endoscope was passed over the proventriculus to identify and assess the left gonad, which is located between the kidney and adrenal gland . In female birds, no further assessment was made. In males, a small surgical calliper was passed along the endoscope to measure the length and width of the testis.

In male birds, a 26 gauge needle and syringe were used to biopsy testicular tissue by aspiration. One half ml of suction was applied once the needle had been inserted into the testis. The suction was released before removing the needle from the

testis. In some birds, the laparotomy incision had to be widened to enable sufficient access, especially when the testes were regressed. The aspirated material was floated onto a microscope slide using a drop of 0.9 % saline, dried and then stained using a commercial cytological stain (Diff-Quik®). Staging of the aspirated material was based on the structure and mitotic activity of spermatogonial cells and the presence or absence of spermatozoa (Humphreys 1975).

Wild swift parrots - dissections and histology

I also examined the gonads from wild swift parrots that had died from a range of causes. Twelve carcasses of wild swift parrots that were submitted to National Parks and Wildlife Service prior to the start of the project in June 1998 had been stored frozen, with details of date of death and location recorded. From June 1998 until January 2001, all birds that were found and submitted to the National Parks and Wildlife Service were forwarded to the School of Zoology, University of Tasmania, as soon as possible. If such transfer could be done within three days of the date of death then the birds were refrigerated at 4° C until the post mortem examination could be performed. Where immediate transfer was not possible, birds were frozen. To increase the number of carcasses retrieved, a request for assistance was mailed to all veterinary surgeons in Tasmania. A total of thirty eight carcasses were collected in this period. The gonads and gastro-intestinal tract from two additional specimens were obtained from Toowoomba in Queensland during the winter of 1998. Fifty-two wild birds were obtained overall.

The length and width of the left testis was measured using callipers (± 0.1mm). I calculated left testicular volume (LTV) as the volume of an ellipsoid, $V = 4/3\pi a^2 b$, where a = half the longest width and b = half the longest diameter. The validity of this calculation was examined by assessing the relationship between LTV and left testis mass. Testicular tissue was biopsied by aspiration using a 26 gauge needle and syringe using the same technique as for endoscopic biopsy (previous page). The gonads were then fixed in either Bouin's solution or a 10 % buffered formalin solution. Histological preparation of these samples (n = 24) was carried out at the Department of Veterinary Pathology at Murdoch University, and the stage of spermatogenesis was assessed (Humphreys 1975).

In female birds, the development of follicles on the ovary was described as: inactive (no follicles larger than 1 mm); white follicles only (white follicles larger than 1 mm); or yellow follicles present (yellow follicles larger than 1 mm). The presence of yellow follicles indicates active vitellogenesis (Joyner 1994).

Assessment of endocrine cycles

Captive birds

To assess the annual cycles in plasma concentrations of the reproductive steroids, I took blood samples from captive swift parrots and musk lorikeets on a weekly basis throughout the breeding season (September to December) and on a monthly basis from January to August. The sampling period was from August 1998 to March 2001. I always sampled between 10 and 12 am to reduce variation from diurnal rhythms. I captured birds from the aviary by hand-net and took blood samples immediately; time from beginning capture to blood sampling did not exceed five minutes in any individual. Blood was taken from the left jugular vein using a heparinised syringe, the volume of blood taken from each bird did not exceed 0.4 ml on any one occasion (~ 0.65 % of body mass). Samples were held on ice until centrifuging at 6400 rpm. Plasma was separated and stored frozen at -20 °C until analysis.

Wild swift parrots

I captured wild swift parrots by mist-netting in south-eastern Tasmania in the summers of 1998/99, 1999/2000 and in 2000/2001 (Figure 4). I also captured birds in northern Tasmania at a private bird-feeding station in Spreyton on three occasions in 1998 (Figure 4). During winter, I trapped in Victoria within the box-ironbark forests near Chiltern, Tunstall's State Forest and Maldon State Forest (Figure 5). Wild musk lorikeets were not captured as priority was given to the intensive demands of mist-netting swift parrots.

To select mist-netting sites in south-east Tasmania, I surveyed the breeding range of swift parrots in August and September of each year. Mist-netting sites were chosen on the basis of: the presence of birds, sufficient amounts and expected duration of flowering resources, and sufficient space to set up mist-nets (Figure 6).

While netting in south-eastern Tasmania, I used two of my captive birds as lures. The lure birds were placed in a small cage between two mist-nets (~ 4 m height) angled at 90 ° to each other. Two additional nets were placed near foraging trees. In the north of Tasmania and in Victoria I used mist-netting without lures. I monitored the nets continually, and removed and sampled birds as soon as possible after capture. However, simultaneous multiple captures, and the need to free by-catch, meant that not all birds were sampled in less than five min after capture, even with assistants present. Time to bleeding was recorded as: less than 5 min, 5-10 min, 10-15 min and greater than 15 min. Blood sampling was otherwise as for captive birds. Morphological measurements and feather characteristics were recorded to categorise the birds' likely sex and age class (see Appendix A).

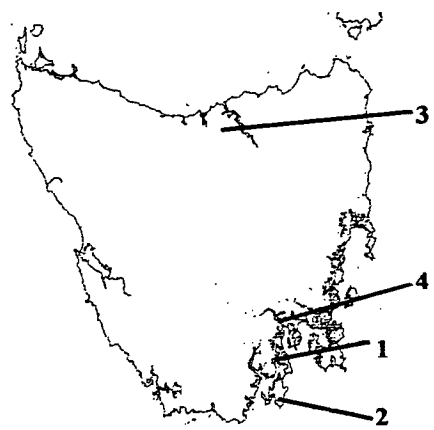


Figure 4. Location of Tasmanian sites of successful wild swift parrot mist-netting. 1. 'Peppermint Point', Woodbridge, 1998/99 (S 43° 09' E 147° 14'). 2. 'Inala', South Bruny Island, 1998/99 (S 43° 26' E 147° 13'). 3. 'The Tea Gardens', Spreyton,1998 (S 41° 13' E 146° 21'). 4. 'Seismic station', Mt Nelson, 1999/2000 (S 42° 52' E 147° 19').

Figure 5. Location of Victorian sites of swift parrot trapping. 1. Chiltern Box-Ironbark State Forest, June 1999 (S 36° 07' E 146° 33'). 2. Tunstall's State Forest, August 1999 (S 36° 45' E 143° 30'). 3. Maldon, July 2000 (S 37° 05' E 144° 04').

Figure 6. Trapping site at 'Seismic Station', Mount Nelson, Hobart. Note the white cage containing two adult swift parrots used as lure birds.

Radioimmunoassay - testosterone

I measured plasma concentrations of testosterone (T) using an established radioimmunoassay (RIA) technique. Initially, samples of 25 to 50 µl of plasma were extracted using 2 mls of 2 % ethanol in hexane. However, the extraction efficiency of this method for swift parrot plasma was only 37 % and most of the swift parrot and musk lorikeet samples assayed had no detectable T by the RIA. Extraction

efficiencies were calculated by adding labelled steroid to plasma prior to extraction and then the recovered levels were compared to those added. Detectable levels of T were extracted from between 75 to 100 µl of plasma using one ml dichloromethane with an extraction efficiency of 49 % (Standard Deviation [SD] ± 3.3 %). This necessitated combining the weekly plasma samples for each bird into monthly samples. The plasma extracts were combined with 50 µl [^{3}H]-T in ethanol (~15,000 dpm), and then evaporated to dryness. The samples were then reconstituted with 100 µl phosgel buffer (5.75 % Na_2HPO_4, 1.3 % $NaH_2PO_4.2H_2O$, 0.1 % thimerosal and 1 % gelatin in distilled water) and 100 µl antiserum (1:100, Endocrine Sciences T3-125 in phosgel buffer) and incubated overnight at 4°C. The unbound fraction was removed with 500 µl dextran-coated charcoal (0.125 %) followed by centrifugation at 4 °C. The radioactivity remaining in the supernatant was measured using a Beckman LS 5801 liquid scintillation system for radioactive counting as outlined in Swain and Jones (1994) and the results adjusted for the extraction efficiency and sample volume. The inter-assay coefficient of variation was 9.6 % (n = 9) and the intra-assay coefficient of variation was 6.7 % (n = 11). Serial dilutions of swift parrot plasma ran parallel to the standard curve ($r^2 = 0.97$). The minimum detectable limit of the assay was 3 pg (0.06 ng.ml^{-1} plasma with a 100 µl sample).

[^{3}H]-Testosterone was purchased from Amersham Life Sciences (UK) and T antiserum from Endocrine Sciences. Analytical reagent grade ethanol and dichloromethane were purchased from Biolab Scientific Pty, Ltd (Victoria, Australia). Scintillation fluid (Ecolite +) was puchased from ICN (Costa Mesa, CA).

Radioimmunoassay - oestradiol

I measured plasma concentrations of oestradiol (E2) using an established RIA technique. Initially, extraction was attempted using absolute ethanol and between 25 to 50 µl of plasma. However, most of the swift parrot and musk lorikeet samples assayed using this method did not contain detectable amounts of E2 by RIA. Similarly, poor results were obtained using a 3:2 mix of ethyl acetate: hexane as the solvent. Column extraction (Chromosorb-W [Alltech] packed columns topped with acid-washed sand) of 50 µl swift parrot and musk lorikeet plasma with 30 % ethyl

acetate in iso-octane resulted in low detectable levels of E2. However, the solvent without plasma produced low false positive readings for E2 in the same range as the plasma samples.

Reliable detectable levels of E2 were extracted from between 75 to 100 µl of plasma using one ml of micro-pore filtered dichloromethane with an extraction efficiency of 59 % (SD ± 10.1 %). This necessitated the pooling of weekly plasma samples into fortnightly pooled samples. The plasma extracts were combined with 50 µl [^{3}H]-E2 in ethanol (~15,000 dpm), and then evaporated to dryness. The samples were reconstituted with 100 µl antiserum (1:200; Endocrine Sciences E26-47 in phosgel buffer) and 100 µl phosgel buffer and incubated overnight at 4 °C. The unbound fraction was removed with 500 µl dextran-coated charcoal (0.125 %) followed by centrifugation. The radioactivity remaining in the supernatant was measured using a Beckman LS 5801 liquid scintillation system for radioactive counting as outlined in Swain and Jones (1994) and the results adjusted for the extraction efficiency and sample volume. The inter-assay coefficient of variation was 13.2 % (n = 6), and the intra-assay coefficient of variation was 10.8 % (n = 7). Serial dilutions of swift parrot plasma ran parallel to the standard curve ($r^2 = 0.93$). The minimum detectable limit of the assay was 2 pg (30 pg.ml^{-1} plasma with a 100 µl sample).

[^{3}H]-Oestradiol was purchased from Amersham Life Sciences (UK) and E2 antiserum from Endocrine Sciences. Analytical reagent grade ethanol and dichloromethane were purchased from Biolab Scientific Pty, Ltd (Victoria, Australia). Scintillation fluid (Ecolite +) was puchased from ICN (Costa Mesa, CA).

Effects of exogenous gonadotropin releasing hormone on concentrations of plasma testosterone in captive adult male swift parrots

I examined the response of six captive swift parrots to exogenous gonadotropin releasing hormone (GnRH) on a monthly basis from September 2000 to March 2001. An additional trial was carried out in July 2001 to include the winter period. I took baseline blood samples from twelve adult male swift parrots to measure luteinising hormone (LH) and T. The birds were then injected intravenously

with either 1.5 μg of gonadotropin releasing hormone ([Gln 8]-LHRH (chicken) [Auspep] in 30 μl of 0.9 % saline (treatment, n = 6) or 30 μl of 0.9 % saline only (controls, n = 6). Five minutes after injection a second blood sample was taken to assess the production of LH in response to GnRH. Fifteen minutes after injection a third blood sample was taken to assess the production of T in response to GnRH. These doses and time intervals were chosen based on experience using this GnRH in honeyeaters (L. Astheimer pers. comm.) and adjusted on a $mg.kg^{-1}$ for the body weight of the swift parrots. Total blood sampling did not exceed 0.4 ml in any bird. Samples were held on ice until centrifuging at 6400 rpm. Plasma was stored frozen at -20 °C until analysis. Testosterone analysis was carried out using the methods described previously, with the exceptions that plasma sample volumes ranged from 50 to 75 μl, and no pooling of samples occurred.

Samples taken for LH assessment have been stored as the assay has proven unreliable to date. The results, when available, will be used for later publication of this experiment.

Statistics

I used $\alpha = 0.05$ as the level of significance for all analyses and carried out all statistical analyses using Systat for Windows 7.0 (1997 SPSS Inc.). All results are presented as means ± one standard error unless otherwise stated.

Assessment of gonadal cycles

I examined the relationship between calculated gonadal volumes and masses using linear regression. I examined changes in gonadal volume over time using one way analysis of variance (ANOVA). The study of left testicular volume (LTV) assessed by endoscopy required log transformation of volumes prior to analysis. No transformations were otherwise necessary to satisfy the assumptions of normality and homogeneity of variances. *Post hoc* examination of results was carried out using Tukey's HSD tests.

Assessment of endocrine cycles

All plasma hormone concentrations were log transformed prior to analysis to satisfy the assumptions of normality and homogeneity of variances. Occasional missing data points resulted from insufficient plasma remaining for hormone analysis. These were assigned the mean value for birds in that period, although no more than one such value was assigned to any sample set or individual bird (Mundry, 1999). This resulted in complete data sets for the captive birds for monthly periods during the breeding season (spring and summer - from September to January). However, monthly samples had to be combined for autumn and winter to create a full data set for each respective season. Changes in plasma hormone concentrations between months and between species of captive birds were analysed by multi-variate repeated measures analysis of variance, (M)ANOVA. The Pillai's trace statistic was used to determine the significance of any differences because the uni-variate output of the SYSTAT programme is unsuitable due to a lack of independence of the data through time (C. Johnson, pers. comm.).

One way ANOVA was used to assess the effect of time to bleeding on the plasma T concentrations of wild swift parrots. A two-way ANOVA was used to analyse plasma T concentrations in wild swift parrots with respect to the effects of month of sampling and trapping site. A two-tailed Student t test using pooled variances was used to compare the concentration of plasma T between wild and captive male swift parrots in October only.

Effects of exogenous gonadotropin releasing hormone

A two-way ANOVA was used to assess the effects of treatment group and month on the baseline (0 mins) concentrations of plasma T in adult male swift parrots. The change in plasma T concentration (ΔT) 15 minutes after treatment with either saline or GnRH was calculated by subtracting the 15 minute plasma T concentration from the baseline (0 mins) plasma T concentration. The effect of treatment group, month examined and the interaction between these variables on ΔT was assessed using a two-way ANOVA. *A posteriori* t-tests were used to assess individual months for treatment effects. The monthly pattern of plasma T

concentrations of adult male swift parrots 15 minutes after treatment with GnRH (n = 6) was compared with the monthly pattern of plasma T concentrations of all adult captive male swift parrots (n = 17) over the three years of the study (1998-2000) using (M)ANOVA.

RESULTS

Reproduction in captivity

Swift parrots

In the first breeding season (1998), there were only three adult female and twelve adult male swift parrots in captivity and no musk lorikeets. Nestboxes were installed in August. The first evidence of investigation of the nestboxes occurred in October and was limited to occasional disturbance of the nesting material. In late November, a captive-bred female was seen regularly emerging from the nestbox. Eggs were laid on the 2nd and the 4th December. (For details on subsequent egg incubation and chick growth and fledging, see Chapter 3). No reproductive activity was seen in the other aviaries, which contained predominantly wild-bred birds. A single chick hatched on the 21st December. At this stage, many of the adult swift parrots had begun moulting primary wing feathers and no further evidence of reproductive activity was seen. The single chick fledged at the end of January 1999. The nestboxes were removed in March.

Over the autumn and winter of 1999, three more adult female swift parrots were obtained. Nestboxes were installed in late August 1999. Nestbox disturbance in the aviaries began in September and continued to be noted daily throughout September. Three of the female swift parrots were noted to have slightly swollen abdomens at this time, which was also noted in the female musk lorikeets in the week prior to oviposition. This is associated with the enlargement of follicles on the ovary prior to ovulation and the preparatory swelling of the oviduct (Hoefer 1997). However, this subsided in October and none of the swift parrots showed any further evidence of reproductive activity. This abrupt disappearance of the signs of reproduction occurred at the same time as an outbreak of neurological and renal disease, and deaths in the swift parrots (see Chapters 7 and 8 for details). In late December some of the adult swift parrots began moulting their primary wing

feathers. By the first week in January all of the adult swift parrots had commenced primary feather moult. Nestboxes were removed in March 2000. At the end of this season, all the adult female swift parrots had died (see Chapter 7), leaving only two juvenile female birds.

In the final year (2000) of the study, two adult female swift parrots were obtained from a private aviculturist and two wild adult female swift parrots injured in window strikes were obtained prior to the breeding season. The nestboxes were placed in September. Nestbox disturbance was noted in the swift parrot aviaries in late October and continued through November. One male swift parrot began aggressively defending a nestbox in mid-November and a captive bred female laid a clutch of five eggs in this nestbox from the 20th November through to the 28th November. Four of these eggs hatched from the 12th December through to the 15th December. The remaining chick was malpositioned at pipping and died attempting to hatch. No other reproductive activity was seen. 38 % (13/34) adult swift parrots had began moulting in the last week of November, with all adult swift parrots having commenced moulting primary feathers by the first week in December. 79.4 % (27/32) of adult birds had completed the moult by the last week in March 2001.

Musk lorikeets

Four adult musk lorikeets (two males and two females) were moved into two adjoining aviaries in March 1999. In August, nestboxes were installed and one pair of lorikeets immediately inspected and began defending a nestbox. Both pairs of lorikeets laid two eggs at the end of September. The musk lorikeets pairs were unaffected by the neurological and renal disease seen in the swift parrots in this year, although three out of the four eggs that were laid were infertile. The remaining musk lorikeet chick hatched on the 17th October and fledged in mid-December 1999.

In the year 2000, nestboxes were installed in the musk lorikeet aviaries in September. Both pairs of musk lorikeets were seen investigating the nestboxes on the day of installation and the females laid two eggs each in the first week of October. All four of these eggs failed to hatch, with embryos dying in the final stages of incubation. The lorikeet pairs both laid fertile second clutches of two eggs in early November, and these hatched in early December. All adult musk lorikeets had

commenced moulting primary feathers by the first week in December and had finished by March 2001.

Assessment of gonadal cycles

Captive birds - endoscopy, testicular aspiration and cytology

There were significant differences in left testis volume (LTV) over the period examined (n = 4,5,8,3 respectively for season, $F_{(3, 16)} = 116.63$, $P < 0.001$) (Figure 7). Left testis volume (mean ± one standard error) in September (31.3 ± 6.40 mm^3) and December (25.5 ± 1.82 mm^3) were significantly higher ($P < 0.001$) than in March (1.1 ± 0.13 mm^3) or June (2.5 ± 0.83 mm^3). Left testis volume was not significantly different between September and December ($P = 0.953$); however, LTV was significantly higher ($P = 0.032$) in June than in March.

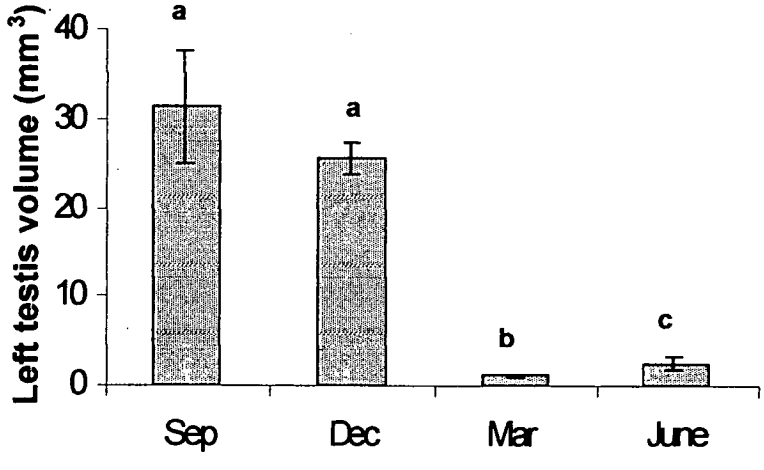


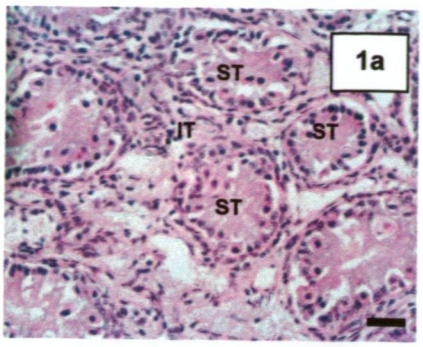
Figure 7. Left testis volume (mm^3) assessed by endoscopy of captive adult male swift parrots held in Hobart, Tasmania. Sample sizes are: September, n = 4; December, n = 5; March, n = 8; and June, n = 3. Error bars represent one standard error. Months with different letters are significantly different from each other ($P < 0.05$).

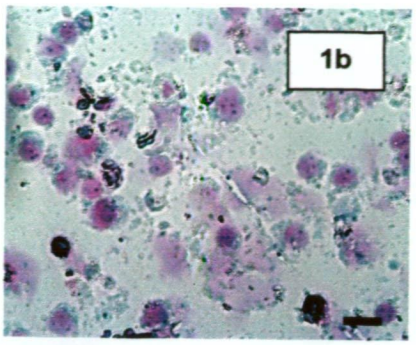
Aspiration and cytology of testicular tissue was carried out successfully in September (n = 4 out of 4 birds) and December (n = 4 out of 5 birds). However, aspiration and cytology were not successful in regressed testes [March (n = 8 birds)

or June (n = 3 birds)]. This was due to either the low cellularity of tissue aspirated or, more commonly, contamination of the aspirate with blood. I successfully aspirated the left testis of wild adult male swift parrots in November (n = 3). Testicular stages are described in Table 1 and illustrated in Figure 8. The frequency of occurrence of testicular stages as assessed by aspiration and cytology of living wild and captive birds is summarised in Figure 9. In September, captive birds were found to have testes with stage 1 and 2 development. In November, the three wild birds were in stage 3 of testis development, and in December, the birds were in stages 3, 4 and 5 of testis development.

Table 1. Stages of testicular cycle in swift parrots characterised by changes in cross-section of interstitial and tubular cell appearance and architecture (compiled from Humpreys 1975 and this study).

	Testicular stage	Tubule (T) to Interstitium (I) diameter	No. of tubular cell layers	Spermatogonia cell structure and activity
1.	Quiescence	T < I	1-2	Large round pale nuclei Little cytoplasm No cell division
2.	Spermatagonial multiplication	T ≥ I	2-6	Increased cytoplasmic volume Active mitosis
3.	Spermatocyte division and elongation	T > I	6-10	Spermatogenesis Young spermatid nuclei present Active meiosis
4.	Regression	T ≥ I	2-10	Smaller spermatogenic cells Reduction in cytoplasm volume No cell division
5.	Rehabilitation	T < I	2-6	Disintegrating spermatocytes Reduction in cytoplasmic volume No cellular division


1a
ST
IT
ST
ST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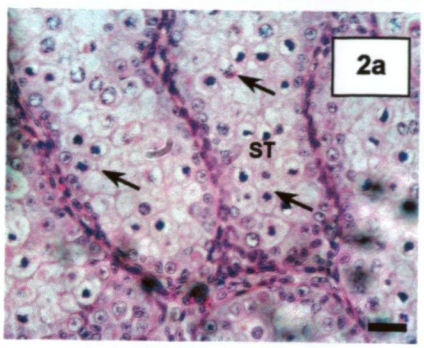
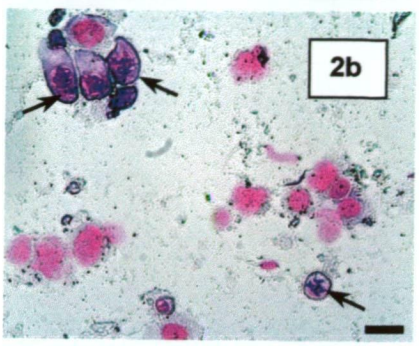

2a
ST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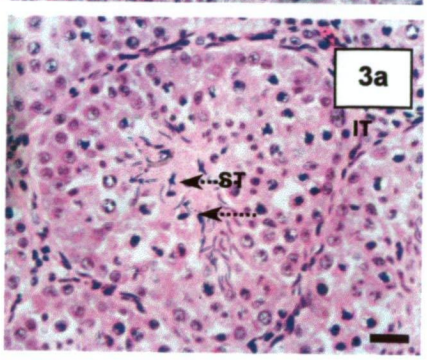
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IT
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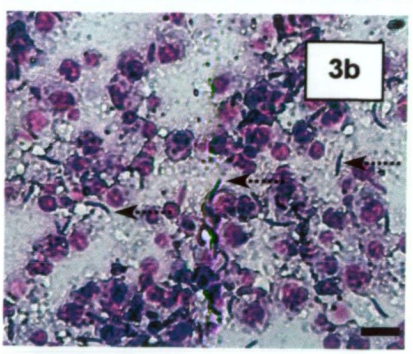
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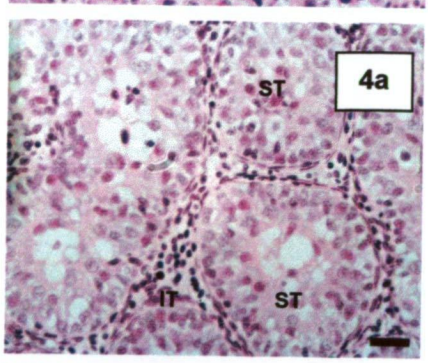
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ST
IT
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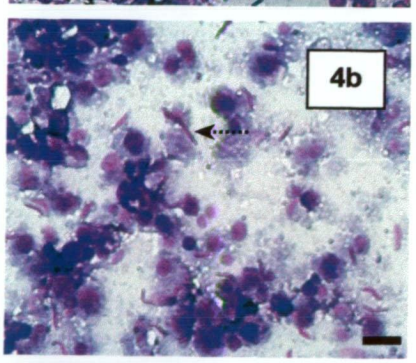
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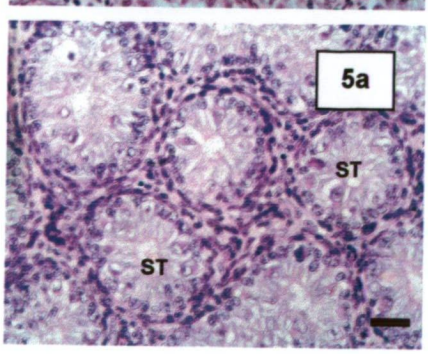

5a
ST
ST

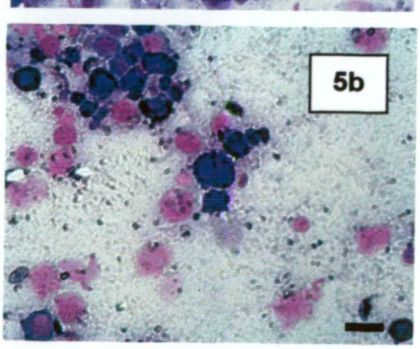

5b

Figure 8 (previous page). The appearance of testicular tissue at the different stages of spermatogenesis using a) histology (H & E), and b) aspiration and cytology (Diff Quik®). ST indicates the seminiferous tubule; IT indicates the interstitial tissue; solid arrows indicate examples of cells undergoing mitosis; dotted arrows indicate spermatazoa. Stages are numbered according to the description given in Table 1. Stage 1 = quiescence; 2 = spermatogonial multiplication; 3 = spermatocyte division and elongation; 4 = regression; and 5 = rehabilitation of the testes. Scale bars represent 10 μm.

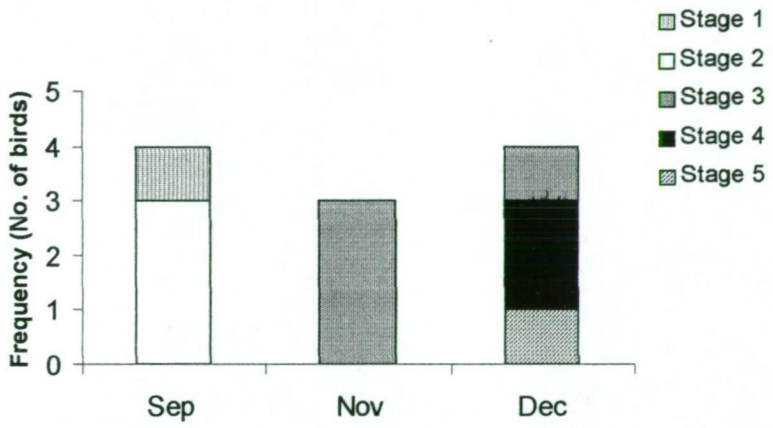


Figure 9. Frequency of occurrence of testicular stage as assessed by endoscopy, aspiration and cytology of the left testis in captive (September and December) and wild (November) swift parrots. Stages are: 1 = quiescence; 2 = spermatogonial multiplication; 3 = spermatocyte division and elongation; 4 = regression; and 5 = rehabilitation of the testes.

Wild birds - dissections and histology

a. Male birds

There was significant variation ($F_{(5, 25)} = 3.287$, $P = 0.036$) in the LTV of wild male swift parrots (n = 31) over the period examined (September to March, 1998 to 2000) (Figure 10). Left testis volume (mean ± one standard error) in September at the commencement of breeding season was relatively low (21.0 ± 10.46 mm^3, n = 2) and increased to a peak in November (59.8 ± 10.58 mm^3, n = 12).

The LTV decreased in December (25.4 ± 14.99 mm^3, n =5) and was at its lowest in January (6.6 ± 1.42 mm^3, n = 5). Only a single specimen was available from March (15.2 mm^3). *Post hoc* analysis confirmed that LTV in November was significantly greater than in January ($P = 0.037$).

As expected, there was a significant relationship between testicular volume and mass, as described by linear regression y = 0.001x + 0.007, where y = testes mass (g) and x = testes volume (mm^3): $r^2 = 0.702$. This suggests that measurement of testis volume based on length and width is a valid measure of testes development.

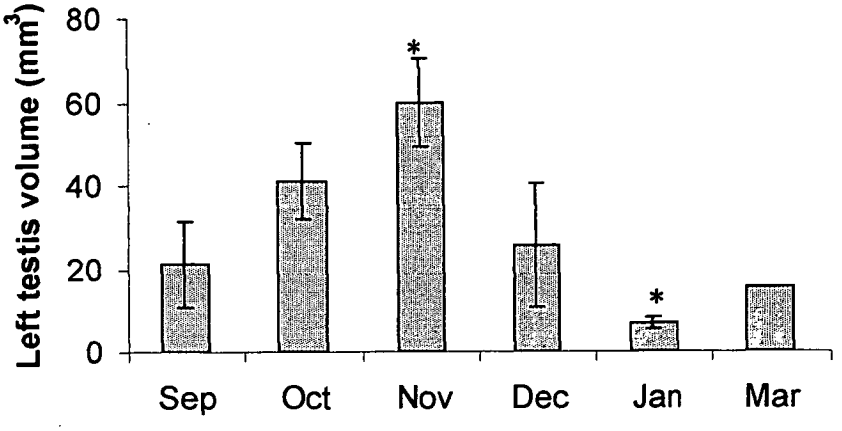


Figure 10. Variation in left testis volume (mm^3) in male swift parrots killed by window strikes in Hobart, Tasmania during the breeding seasons of 1998 to 2000. Sample sizes are: September, n = 2; October, n = 6; November, n = 12; December, n = 5; January, n = 5; and March, n = 1. Error bars represent one standard error. Months marked by * are significantly different from each other by *post hoc* tests ($P = 0.037$).

Histological staging of the testes (see Table 1) revealed a pattern of testicular development and spermatogenesis which paralleled the changes in testes volume (Figure 11), although the differences were not significantly different (n = 24, $F_{(4, 19)} = 2.552$, $P = 0.073$), probably due to low sample sizes in some months.

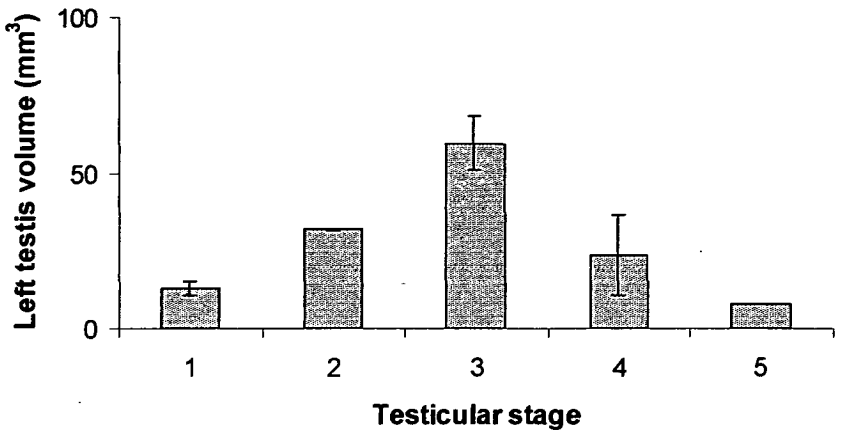


Figure 11. The relationship between left testis volume (mm^3) and testicular stage in wild swift parrots, where 1 = quiescence (n = 2); 2 = spermatogonial multiplication (n = 2); 3 = spermatocyte division and elongation (n = 14); 4 = regression (n = 5); and 5 = rehabilitation of the testes (n = 1). Error bars represent one standard error.

The frequency of occurrence of testicular stages varied throughout the reproductive season with stages 1 and 2 found in September (n = 2), stages 2, 3 and 4 (n = 6) in October and stages 3, 4 and 5 were found in November (n = 11). Stage 3 was found in December (n = 2), stage 4 seen in January (n = 3) and stage 1 seen in March (n = 1) (Figure 12).

The accuracy of testicular staging using cytological evaluation of fine needle aspirates (n = 18) was high (94.4 %) when compared to histology. The single discrepancy was a bird staged 2 on cytology and 3 on histology. For six birds, the fine needle aspirates of testes could not be interpreted, due to either low cellularity or blood contamination of the aspirates.

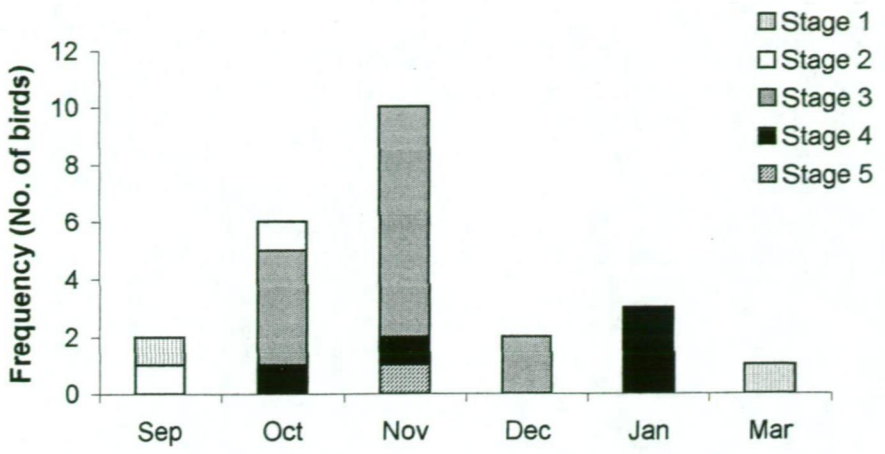


Figure 12. The frequency of occurrence of testicular stages throughout the spring and summer in adult male swift parrots. Stages are: 1 = quiescence; 2 = spermatogonial multiplication; 3 = spermatocyte division and elongation; 4 = regression; and 5 = rehabilitation of the testes.

b. Female swift parrots

Only eight wild adult female swift parrots were available for post mortem, therefore statistical analysis of gonadal staging results was not possible. The development of follicles (n = 12 birds) did vary through this period (Figure 13).

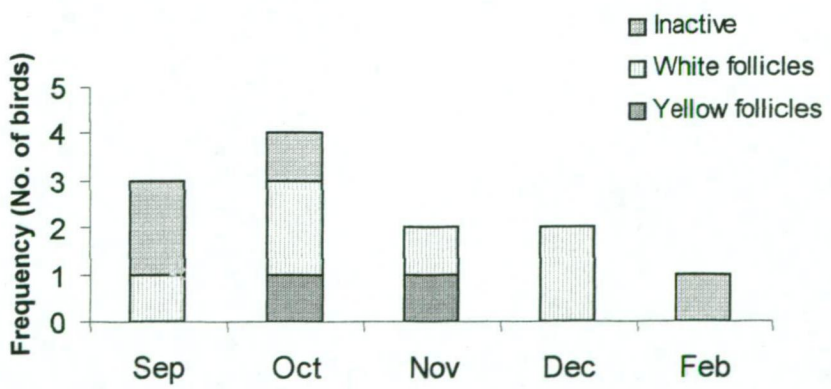


Figure 13. Frequency of occurrence of follicular development on the ovary of adult female swift parrots throughout the spring and summer. Inactive = no follicular development. White follicles = the presence of white follicles greater than one mm in diameter. Yellow follicles = the presence of yellow follicles greater than one mm in diameter.

Assessment of endocrine cycles

Captive male birds - testosterone

There was a marked annual variation in the mean monthly plasma concentration of T in adult male swift parrots in captivity (n = 17, $F_{(6, 9)} = 20.039$, $P < 0.001$) (Figure 14), which did not vary significantly between the three years of the study ($F_{(12, 20)} = 1.315$, $P = 0.284$). August plasma T concentrations were not included in the statistical analysis due to missing plasma samples (n = 9 only). Plasma T concentration (mean ± one standard error) in August was low (199 ± 88 $pg.ml^{-1}$) but increased to maximum in October (1012 ± 179 $pg.ml^{-1}$). Plasma T concentrations were at their lowest in December (74 ± 5 $pg.ml^{-1}$) and January (60 ± 8 $pg.ml^{-1}$) and had begun to increase above minimum levels by autumn.

I am unable to present the annual variation in plasma T concentration in the two male swift parrots that did participate in breeding, as insufficient plasma was available after initial hormone analyses were unsuccessful. Similarly, the annual variation in plasma T concentration of breeding male musk lorikeets is available for only one individual in two successive years (Figure 15) as no other samples remained for analysis after the initially unsuccessful hormone assays.

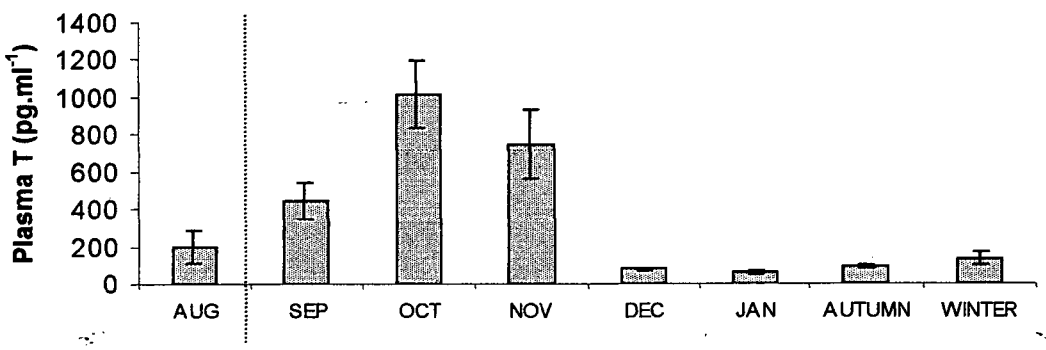


Figure 14. Annual variations in the concentration of plasma testosterone (T) in captive adult male swift parrots (n = 17). August plasma T concentrations (n = 9; left of dotted line) were not included in the statistical analysis: see text. Error bars represent one standard error.

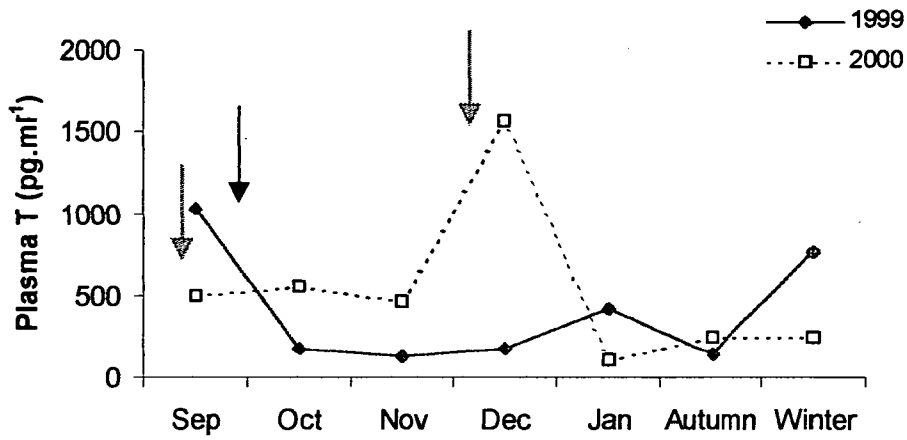


Figure 15. Annual variation in the concentration of plasma testosterone (T) in a captive breeding male musk lorikeet in two successive years. The black arrow indicates the date when the female paired to this male laid eggs in 1999. The grey arrows indicate the dates when the female paired to this male laid eggs in 2000; the first clutch was infertile, the second clutch fertile.

Wild swift parrots - trapping results

There was a high capture rate of wild birds in October and November using mist-netting and captive swift parrots as lures. Wild birds in this period showed considerable interest in the calls of the decoy birds and were often trapped after interrupting their flight or foraging to investigate. Playback of taped captive swift parrot calls did not induce the same interest. Outside of October and November, wild birds showed no interest in the calls of the decoy birds, and trapping relied on catching birds entering or leaving foraging trees. The sex ratios of all birds caught by mist-netting was 1.9: 1 (males: females) regardless of whether the birds were trapped with the aid of lure birds or not. In the three years of the project, there were no recaptures of previously trapped and banded birds.

Wild male swift parrots - testosterone

There was a significant effect of time to bleeding on plasma T concentrations in wild swift parrots (n = 51, $F_{(3, 47)} = 3.780$, $P = 0.016$) (Figure 16). The concentration of plasma T in birds sampled (in October and November) in less than

five minutes after capture (1351 ± 271 pg.ml^{-1}) was significantly higher (P = 0.010) than birds sampled between 10 to 15 minutes after capture (318 ± 141 pg.ml^{-1}).

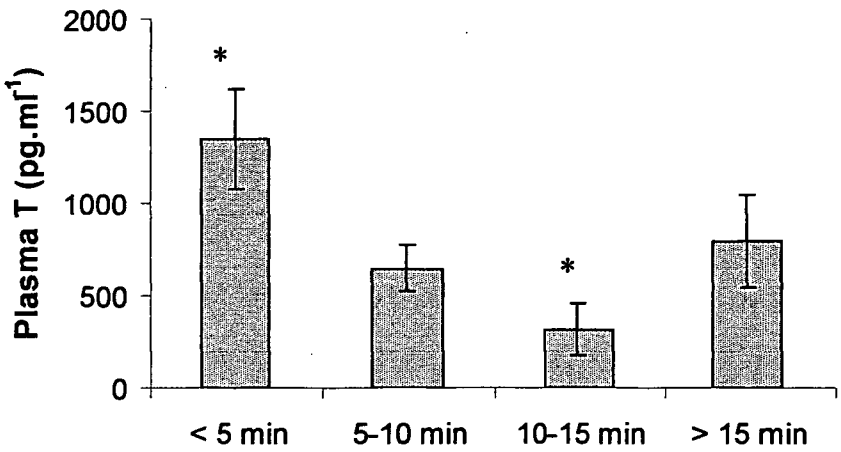


Figure 16. The effect of time to bleeding on the concentration of plasma testosterone in wild adult male swift parrots. Time periods marked with * are significantly different to each other (P = 0.01). Error bars represent one standard error.

Therefore, I analysed the concentration of plasma T in wild swift parrots for the effect of month (October and November) in separate groups depending on time to bleeding. However, this meant that samples sizes were low (< 5 min, n = 7; 5-10 min, n = 26; 10-15 min, n = 8; and > 15 min, n = 10), and no significant effect of month was detected (P > 0.05) at any level of time to bleeding.

The concentration of plasma T in wild birds sampled in less than five minutes in October (1351 ± 271 pg.ml^{-1}, n = 7) was not significantly different ($t_{(24)}$ = 0.983, P = 0.335) from the concentration of plasma T in captive birds sampled in October (1012 ± 180 pg.ml^{-1}, n = 20) suggesting that the male plasma T cycles were unaffected by captivity.

Captive female birds - oestradiol

The annual variation in plasma E2 concentration was observed in only one breeding adult female swift parrot through a full reproductive season (Figure 17). In

this bird plasma E2 concentrations remained low (< 150 pg.ml^{-1}) for most of the year. However, two marked, but short lived, elevations of plasma E2 above the basal levels of ~ 30-100 pg.ml^{-1} were observed. The first (327 pg.ml^{-1}) occurred during the time of active nest-box inspection; the second (447 pg.ml^{-1}) occurred after the hatching of the eggs. The concentration of plasma E2 in non-breeding adult female swift parrots (n = 6) did not vary significantly throughout the year ($F_{(11, 55)} = 1.682$, $P = 0.102$) and remained low (< 150 pg.ml^{-1}) (Figure 15).

The annual variations in plasma E2 concentrations in the two breeding female musk lorikeets in two years are presented for comparison in Figure 16. The elevations in plasma E2 in the musk lorikeets in October and November also preceded egg laying, and plasma E2 concentrations then remained low until the hatching of the eggs in December.

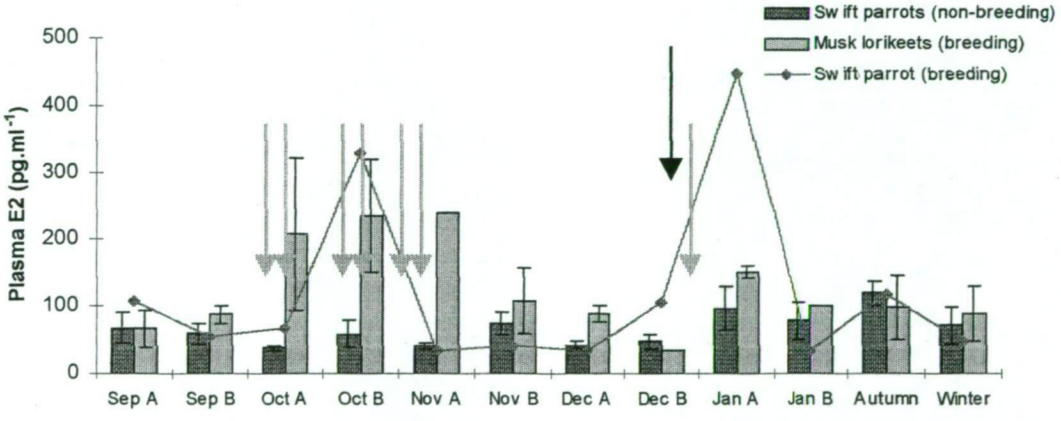


Figure 17. The annual variation in concentrations of plasma oestradiol (E2) in breeding (n = 1) and non-breeding (n = 6) adult female swift parrots, and breeding musk lorikeets (n = 2 birds x 2 seasons). From September to January samples are of fortnightly periods (A = first two weeks and B = second two weeks of the month). The black arrow indicates egg-laying in the swift parrot, grey arrows indicate egg laying in musk lorikeets. Error bars represent one standard error.

Wild female birds - oestradiol

I sampled sixteen wild adult female swift parrots throughout the study. However, due to the low plasma sample volume remaining after sub-samples of the plasma had been set aside for later LH analysis, the minimum detectable limit of the sample for oestradiol in these birds was 30 $pg.ml^{-1}$ with a 100 µl sample. Only one bird out of the sixteen sampled had detectable levels of E2 (1322 $pg.ml^{-1}$). This bird was captured at the 'Tea Gardens', Spreyton on the 17th November, 1998 and the sample was taken three minutes after capture.

Effects of exogenous gonadotropin releasing hormone

Testosterone

There was no significant difference in the baseline plasma T concentrations between the treatment (1.5 µg GnRH in 30 µl saline IV) and control groups (30 µl of saline IV) prior to each experiment ($n = 90$, $F_{(7, 74)} = 0.172$, $P = 0.680$). As expected, there was significant variation in baseline plasma T concentration between months ($n = 90$, $F_{(7, 74)} = 5.223$, $P < 0.001$) (Figure 18). The only variation from the pattern of plasma T seen in the captive male swift parrots examined previously (see Figure 16) was a highly variable elevation in plasma T in September (control group, 930 ± 794 $pg.ml^{-1}$; GnRH group, 1857 ± 818 $pg.ml^{-1}$).

The change in plasma T concentration by fifteen minutes after treatment (ΔT) differed significantly between GnRH and control birds ($F_{(1, 40)} = 4.471$, $P = 0.001$) (Figure 19). Despite some variability within groups, especially in September and November, GnRH treated birds generally responded with a rise in plasma T concentrations 15 minutes after treatment. The mean decrease in plasma T concentration in response to GnRH in September was due to two birds. Both of these birds had maximum detectable levels of plasma T at 0 mins, which decreased 15 minutes after treatment with GnRH (ΔT = -2182 $pg.ml^{-1}$ and -793 $pg.ml^{-1}$). When these birds were removed from the September data, birds treated with GnRH showed an increase in plasma T (Figure 20).

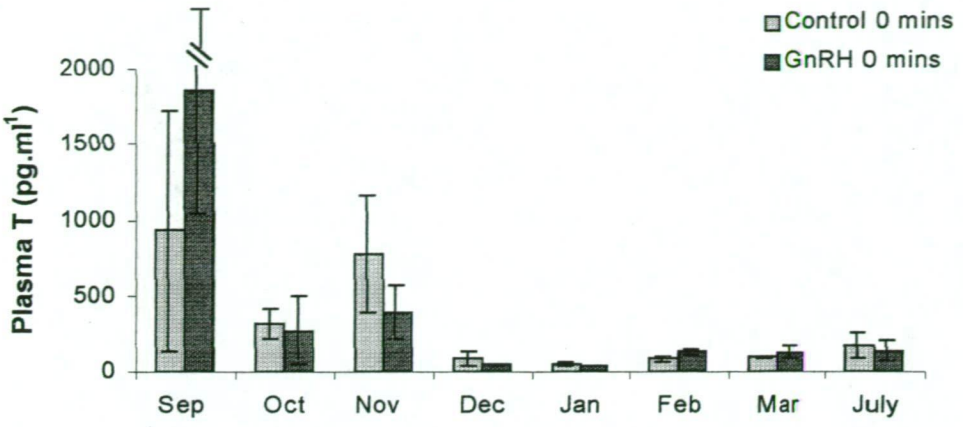


Figure 18. Variation in baseline plasma testosterone (T) over time in treatment (1.5 μg GnRH in 30 μl of saline IV, n = 6) and control (30 μl of saline IV) groups of adult male swift parrots. There were no significant differences between treatment groups within each month. Error bars represent one standard error.

Control birds showed negligible changes in plasma T concentration except in October and November (Figure 19). However there was no significant interaction effect between treatment groups and the period examined ($F_{(7, 40)} = 0.905$, $P = 0.559$). *A posteriori* t tests showed significant differences between treatment and control groups in February ($t_{(10)} = 3.094$, $P = 0.011$) and March ($t_{(10)} = 2.672$, $P = 0.023$).

There was a significant difference ($F_{(6, 14)} = 6.463$, $P = 0.002$) between the plasma T concentrations of swift parrots 15 minutes after treatment with GnRH (n = 6), and the captive male swift parrots (n = 17) over the three years of the study, 1998-2000 (Figure 21).

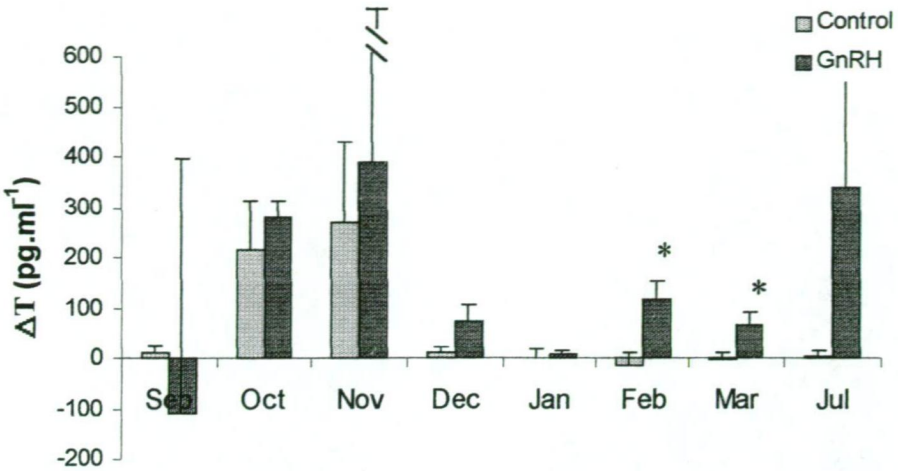


Figure 19. The change in concentration of plasma T (ΔT) in adult male swift parrots fifteen minutes after intravenous injection with either 1.5 μg GnRH in 30 μl of saline IV (GnRH, n = 6) or 30 μl of saline only (Saline, n = 6). Months marked with * show a significant difference ($P < 0.05$) between treatment groups. Error bars represent one standard error.

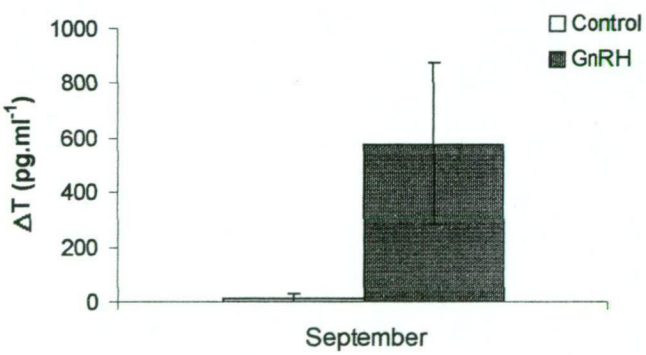


Figure 20. The change in concentration of plasma T (ΔT) in adult male swift parrots fifteen minutes after intravenous injection with either 1.5 μg GnRH in 30 μl of saline IV (GnRH, n = 4) or 30 μl of saline only (Saline, n = 6), after two birds with very high levels of plasma T at 0 minutes and a negative response to GnRH were excluded. Error bars represent one standard error.

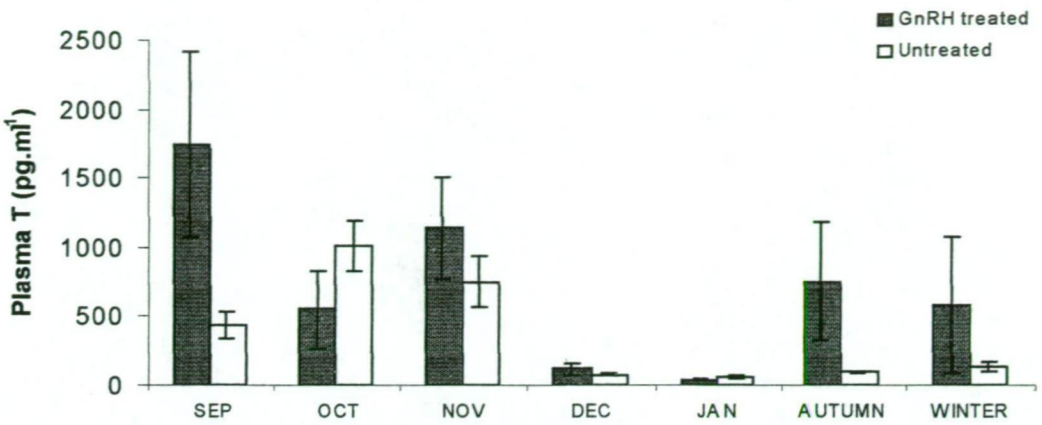


Figure 21. The plasma testosterone (T) concentrations of adult male swift parrots 15 min after treatment with GnRH (n = 6) compared to the plasma T concentrations of all adult captive male swift parrots (n = 17) combined over the three years of the study (1998-2000). Error bars represent one standard error.

DISCUSSION

As expected, the marked seasonal pattern of reproduction seen in swift parrots is paralleled by physiological changes in the gonads and plasma concentrations of the main reproductive hormones. The strongest evidence for the timing of reproductive events during the breeding season in wild swift parrots was found in the measurements of male testes volume and stage of spermatogenesis. However, the limited evidence available on ovarian volume and stage from the wild female swift parrots assessed suggests that they follow a similar pattern of gonadal development.

Despite the low breeding success of swift parrots in captivity, the results of the endoscopic assessment of left testis volume (LTV) and stage of non-breeding male swift parrots suggest that their gonadal development was following the same temporal pattern as their wild counterparts. This was strongly supported by the pattern of plasma T concentrations that peaked in October and November in captive male swift parrots, which correlated with the peak in testes volume in wild birds. Further, the concentration of plasma T in wild birds in October was not different to that of captive swift parrots, suggesting that captivity was not affecting the reproductive cycle in the male birds.

In both captive and wild male swift parrots the peak of reproductive activity, as measured by gonadal size and activity, and plasma T concentration, occurred in October and November. The most frequently observed stage of testicular development in this period was that of spermatocyte division and elongation. This stage has been correlated with peak levels of T secretion in other bird species (Wingfield and Farner 1993 for review). The peak period of male reproductive activity in October and November was associated with active nestbox inspection in the captive birds and corresponds to recorded periods of nest-hollow inspection in the wild (Brown 1989). Peaks of T and gonadal activity are associated with courtship and territorialism around the time of nest establishment in males of most avian species studied; for example; pied flycatchers, *Ficedula hypoleuca* (Silverin 1983), great tits, *Parus major* (Röhss and Silverin 1983), starlings, *Sturnus vulgarus* (Temple 1974); kiwi, *Apteryx australis mantelli* (Potter and Cockrem 1992); and white-winged crossbills, *Loxia leucoptera* (Deviche and Sharp 2001). The social behaviour of wild swift parrots, both male and female, as assessed by their response to the lure birds used in mist-netting, also appeared to alter in this period. Further study to characterise this behaviour is needed as it may indicate a period of increased intra-species interaction associated with courtship. Alternatively, it may simply indicate increased territorialism.

These results suggest that in swift parrots the development of testicular activity is delayed until after their arrival in the breeding grounds in Tasmania. This is in contrast to the migratory pied flycatcher, in which ~50 % of the males arrive at the breeding grounds with testes in the final stages of spermiogenesis (Stage 3) (Silverin 1983). However, nest-sites are limited in the breeding grounds of the pied flycatcher and males must secure and defend territory early in the breeding season (Silverin 1983). In contrast, the swift parrots are not thought to be limited by nest-hollows (Brereton 1996a), but, instead, by the unreliable and widespread nature of their foraging resources (Brown 1989; Brereton 1996a). This variability may mean that the first months spent in Tasmania will be used assessing potential nest-sites for their proximity to sufficient foraging resources. Under these circumstances there is no advantage to the early maturation of the testes, particularly given the potential detrimental effects of prolonged elevations of plasma T concentrations on immunity

and survival (Hau et al. 2000b). The male swift parrots appear to be ready to breed from September through to early December.

The measurement of the concentration of plasma T in wild birds was complicated by the effect of time to bleeding. The decrease seen in plasma T with increased time from capture is reflected by a correlating increase in plasma corticosterone concentration (Chapter 10). However, these results suggest that the measurement of plasma T in captive birds should not be affected by acute capture stress as all captive birds were sampled in less than five minutes from capture. Further, the captive swift parrots used as controls in the GnRH challenge did not show an effect of time from capture to bleeding (15 minutes). This suggests that the stress effects of handling were diminished by habituation in the captive birds. In the wild birds, the plasma T concentration of male swift parrots assessed in less than five minutes after capture in October and November was not significantly different from that found in captive birds. Short-term stress results in decreases in plasma T concentrations in some avian species (Knol 1991) but in increased plasma T in others (Heiblum et al. 2000). It is, therefore, important to understand a species' response to capture stress when assessing plasma steroid levels, particularly in wild birds that are not habituated to human contact, and where capture-to-bleeding times may be extended.

In the successfully breeding male musk lorikeets, initial rises in plasma T associated with nest establishment were followed by reduced levels during the incubation of eggs and chick raising. This is a similar pattern to that seen in many avian species, such as pied flycatchers (Silverin 1983), great tits (Röhss and Silverin 1983), starlings (Dawson 1983) and wandering albatross, *Diomedea exulans* (Hector et al. 1985). Further investigation of breeding male swift parrots through successful reproductive cycles is needed to determine if the pattern of reproductive events is similar to that in the musk lorikeets. However, all adult male swift parrots, both captive and wild, showed evidence of reproductive activity (elevated plasma T concentration, or increased testis size and activity) during the period September until early December. This suggests that male swift parrots examined in this period were physiologically able to breed. This is similar to the situation in cooperatively breeding birds such as Florida scrub-jays, *Aphelocoma coerulescens,* where the

plasma T concentrations and gonad development of non-breeding males parallels that of breeding males (Schoech et al. 1996). In the swift parrots, this male reproductive readiness, combined with the male sex bias evident in the swift parrot population suggests that constraints on reproduction in swift parrots may be acting through female, rather than male, physiology.

The main evidence that female swift parrots in captivity were following a similar reproductive cycle to their wild counterparts was the two successful episodes of breeding. On both occasions, egg laying occurred within the known breeding season for wild swift parrots. This synchrony of captive breeding with the wild breeding season is also seen in other aviaries (Laubscher 1999; B. Cook *pers. comm.*). The majority of female swift parrots in captivity did not show conclusive evidence of reproductive activity and in these non-breeding captive females, there was no recognisable annual pattern in plasma E2 concentrations throughout the year. In the single breeding female swift parrot in which plasma E2 concentrations were assessed, there was a short-lived peak of plasma E2 concentration during nest establishment and another in the fortnight prior to oviposition. A similar pattern was seen in captive female musk lorikeets that bred successfully, although nest establishment and egg laying occurred very close together in this species, and separate peaks of E2 were not distinguishable. High plasma concentrations of E2 of short duration have been associated with nest establishment and ovulation in many avian species, for example: canaries, *Serinus canaria* (Sockman and Schwabl 1999); domestic chickens *Gallus domesticus* (Kamiyoshi and Tanaka 1983); starlings (Dawson 1983); and pied flycatchers (Silverin and Wingfield 1982). Similarly, plasma E2 concentrations in the wild female swift parrots were mostly undetectable. Only a single female from sixteen wild female swift parrots trapped during the three breeding seasons of this project (1998 to 2000) had detectable plasma concentrations of E2.

Elevations of plasma E2 concentration were only seen in breeding female swift parrots. Therefore, measuring the plasma E2 of wild female swift parrots through the breeding season (September to December) may allow assessment of: a) the individual female's reproductive condition, and b) the percentage of females in the swift parrot population in breeding condition. However, the accuracy of a single

measurement of plasma E2 concentration to assess reproductive condition in wild birds is limited by the short duration of the E2 peak. In wild female pied flycatchers, circulating levels of E2 could only be detected during the first part of the egg laying period and the latter part of incubation (Silverin and Wingfield 1982). Also, no seasonal variations in plasma E2 were detected in wild female Indian rose-ringed parakeets, *Psittacula krameri,* sampled over the breeding season (Sailaja et al. 1988). Further study is needed to assess whether measurement of plasma E2 concentration can be a reliable estimate of the proportion of the wild female swift parrots that are breeding.

The low incidence of detectable reproductive activity in both captive and wild swift parrots in this study strongly suggest that it is the initiation of breeding in female swift parrots that is limiting the reproductive success of the species. The limitation of reproductive success by the failure of females to initiate breeding has been described in other endangered avian species, most notably the kakapo, *Strigops habroptilus* (Higgins 1999). The male kakapos exhibit reproductive behaviour, in the form of "booming" at leks, for three months in most years; however, female reproduction occurs only once every three to four years (Higgins 1999). As in the swift parrots, successful reproduction in the females appears to be linked to an annually unreliable food source: the heavy crops of fruit and seed, known as mastings, which occur in the New Zealand forests at intervals of up to six years (Higgins 1999). However, attempts to stimulate reproduction by supplementary feeding have produced equivocal results (Clout and Merton 1998).

The responses to experimental administration of exogenous GnRH, known as a GnRH challenge, provide further evidence for the onset and duration of testicular regression and photorefractoriness in swift parrots. A GnRH challenge tests the responsiveness of the HPG axis by stimulating LH release from the pituitary and reproductive steroids (T and E2) release from the gonads (Dawson et al. 1986; Lacombe 1990; Schoech et al. 1996).

In swift parrots, the response to GnRH challenge in September, at the beginning of the breeding period, is variable, with two birds showing a decrease in plasma T after GnRH administration and the other four birds responding with a significant increase in plasma T concentration. This variability may reflect individual

variation in endogenous rhythms and responses to environmental cues. The two birds showing a decrease in plasma T after GnRH challenge may have already begun to secrete high endogenous levels of GnRH. The remaining male swift parrots responded to GnRH with the expected increase in plasma T concentration, suggesting that although the testes were able to respond, endogenous GnRH production and secretion were not yet at peak levels.

October to November is the peak period of male reproductive activity as assessed by testis size and activity, and plasma T concentration. The lack of response to exogenous GnRH seen in the captive male swift parrots during the breeding period (October to November) may simply reflect maximal concentrations of endogenous GnRH at this time. Therefore, environmental and endogenous cues will already be stimulating the synthesis and release of endogenous GnRH from the hypothalamus (Dawson et al. 1986). However, unlike the swift parrots, in domestic ganders, *Anser domesticus*, there was an increase in plasma T concentration in response to exogenous GnRH during the peak period of breeding activity (spring) (Hirchenhauser et al. 2000). This difference in response may be related to the type of gonadotropin used. The study in ganders (Hirchenhauser et al. 2000) used a superactive GnRH analogue (Ovurelin®) which may have a greater effect on hormone production than the cGnRH used to challenge swift parrots.

At the end of the breeding season, during December and January, when circulating plasma concentrations of plasma T were already low, the testes were regressing, and moult has begun, the lack of response to exogenous GnRH is probably indicative of absolute photorefractoriness. Swift parrots are probably similar to the extensively studied white crowned sparrows, *Zonotrichia leucophrys gambelli*, in which photorefractoriness is induced in late summer and associated with post-nuptial moult (Farner et al. 1983). The responses of plasma T concentrations of male swift parrots to exogenous GnRH are similar to that seen in domestic ganders, *Anser domesticus* (Hirchenhauser et al. 2000), in that no response to GnRH was seen in the expected photorefractory period (summer).

In swift parrots, the duration of absolute refractoriness appears to be short, as a significant response to GnRH is seen as early as February. This suggests that the gonadal recrudescence is physiologically possible much earlier than it actually

occurs. An increase in plasma T concentration in response to GnRH challenge was seen in domestic ganders (Hirchenhauser et al. 2000) at the time when sexual reactivation was expected (autumn). The possibility that photoperiod is a major synchronising factor to the endogenous reproductive rhythms of swift parrots is supported by these findings and further studies are indicated. The future examination of plasma LH concentrations in plasma samples taken in this study, both basal levels and in response to exogenous GnRH, will help clarify the reproductive cycles of swift parrots.

Based on the results of this study, I suggest the following model for reproduction in swift parrots. Gonadal development begins after the birds' arrival in the Tasmanian breeding grounds in late August and September. Reproductive activity and gonadal steroidogenic activity peak in October and November. Gonadal regression, probably associated with the development of absolute photorefractoriness, begins in late December and January, and is associated with the commencement of the moult: there is no evidence of moult-breeding overlap in swift parrots. Photorefractoriness dissipates quickly, but the migration of the birds to the mainland, and the behavioural changes associated with migration (see Chapter 10) prevent any autumnal recrudescence of reproductive activity. The gonads remain potentially responsive to development in the winter period. The mechanisms that inhibit reproduction in this period are uncertain but possibilities include photoperiod and lack of suitable resources. Migration south in August and September into potentially abundant foraging resources stimulates the recrudescence of the gonads and completes the annual cycle.

The implication of this model is that reproduction in swift parrots is restricted temporally by the migratory rhythms and by the onset of moult. It is likely that the most critical period for the initiation of reproduction in swift parrots is from August to October in the Tasmanian breeding grounds. The availability of foraging resources in this period is likely to directly influence the birds' physiological decision on whether or not to breed, and this decision is likely to be driven by the needs of the female birds. Swift parrots rely heavily on the foraging resources provided by the Tasmanian blue gum during this period. Further investigation into the role of the

flowering of the Tasmanian blue gum as a proximate factor affecting reproduction in swift parrots is warranted.

Chapter 3. Egg incubation and chick growth parameters

INTRODUCTION

This chapter describes my investigations into the period of reproduction that encompasses the incubation of the eggs and the rearing of chicks. These reproductive events are energetically costly (Klasing 1999), and the constraints associated with this period may influence parental decisions on clutch size (Monaghan and Nager 1999; Reid et al. 2000), or whether or not to breed (Orell et al. 1994). The costs of these decisions may be immediate in terms of offspring and parent survival (Orell et al. 1994; Monaghan and Nager 1999), or may carry over to influence reproductive success in subsequent years (Orell et al. 1994; Monaghan and Nager 1999; Reid et al. 2000). Reproductive success may be affected by either nutritional or behavioural constraints during these periods of reproduction (synchronising and integrating factors, Chapter 1) or by disruption of reproduction once it has already commenced (modifying factors, Chapter 1). Understanding the constraints during these periods of reproduction is also of practical importance to managers of captive stocks, providing a scientific basis for artificial incubation and hand-rearing of chicks as adjunctive breeding tools to increase reproductive output.

The preferred nesting site of swift parrots in the wild is in a hollow in the trunk, branch or spout of a eucalypt tree, usually *Eucalyptus obliqua, Eucalyptus pulchella* or *Eucalyptus globulus* (Brown 1989; Brereton 1996a; b). The mean height of preferred hollows has been estimated at 15 m above ground level (range 6 – 35 m, n = 61) (Brereton 1996b) and 13 m (range 6 - 28 m, n = 40) (Brown 1989). The hollows have a mean depth of 41 cm (range 30 – 60 cm, n = 18) (Brown 1989). The parrots will irregularly re-use hollows between years, but only when a reliable food source is nearby (Brereton 1996b). Captive birds will use a variety of artificial nest-boxes but those with natural spouts are preferred (Laubscher 1999).

Incubation behaviour of the parent birds has been studied in the field (Brown 1989; Brereton 1996) and there is anecdotal information from observations of captive birds (Forshaw 1981, Hutchins and Lovell 1985). In captivity and the wild, both parents are involved in searching for a suitable nest-hollow, but, like most of the

Platycercinae (Higgins 1999), only the female incubates the eggs. The male parent is solely responsible for the provision of food to the female throughout the incubation period and during the first two weeks of brooding (chick rearing). In the field, feeding occurs every three to five hours (Brown 1989), but in captivity, the interval between feeds has been recorded as being approximately hourly (Hutchins and Lovell 1985).

Our knowledge of reproductive parameters for this species is limited. No information on incubation and growth of chicks of wild swift parrots has been recorded. Similarly, despite swift parrots being well established in aviculture, there is little detailed information of the incubation of the eggs and chick growth in captivity. Incubation length has only been recorded in captivity and is reported variously as ~25 days (Hutchins and Lovell 1985), 29 and 30 days (Lendon 1973). Clutch size varies between 3 and 5 (Forshaw 1981). Between 1927 and 1931, 88 eggs were collected for museums and private collectors from twenty clutches of either 4 or 5 eggs on Bruny Island (Hindwood and Sharland 1964). The young are hatched with a sparse covering of down but the changes in feathering between this stage and fledging have not been recorded (Higgins 1999). Age at fledging has been recorded as 6 to 8 weeks in captivity (Shephard 1989; Hutchins and Lovell 1985).

The aims of this chapter are:

1. to describe parenting behaviour throughout the incubation and chick-raising period in captive swift parrots;
2. to document incubation length and clutch size in captive swift parrots;
3. to describe the staging of embryos of swift parrots and;
4. to describe the growth rate of nestling swift parrots in captivity in terms of mass, skeletal structures and feathering stages.

MATERIALS AND METHODS

1. Incubation behaviour

The behaviour of two pairs of captive parent birds was observed for 30 minutes daily from oviposition until just after fledging. Observations were carried

out between 8 am to 10 am each day. The roles of each parent in incubation and the frequency of feeding were observed. These subjective observations formed a pilot study in preparation for similar observations of wild birds. Unfortunately, the poor reproductive performance of the wild population during the three years of this study, and the extreme difficulty of close observations of wild nesting behaviour precluded detailed observations in the wild.

2. Egg incubation

I inspected nestboxes daily throughout the breeding seasons and individually marked eggs with a graphite pencil as they were laid. Eggs were checked twice daily as hatching approached. The fertile eggs were used to assess the following characteristics of natural incubation in captive swift parrots. I defined the **incubation length** as the interval from oviposition (the first appearance of an egg ± 24 hrs) until the completion of hatching (the emergence of a chick completely from the egg). The **external pip-to-hatch interval** is the period from the appearance of the first puncture of the external shell caused by the chick, until the emergence of the chick from the egg (± 12 hrs). I weighed eggs at least once in three periods during the incubation period; these periods were defined as the first trimester of incubation (days 1 to 6 after oviposition), the second trimester (days 7 to 12 after oviposition) and the third trimester (days 13 to hatching). The **percentage loss of total egg mass** during incubation for each egg was calculated as follows (Joyner 1994).

$$\textbf{Loss of total egg mass (\%)} = \frac{[\text{average daily mass loss (g)} * \text{total incubation period (days)}]}{\text{laid mass (g)}} * 100$$

Eggs were transilluminated (candled) daily using a 100-Watt cold light source and a fibre-optic cable, the procedure being carried out under a heavy blanket to reduce ambient illumination. Identifiable features of the developing embryos and associated blood vessels were recorded and photographed.

3. Embryo staging

A nestbox near a feeding station in Spreyton was noted to be abandoned by wild swift parrots. Within 48 hours of abandonment, I collected three fertile swift parrot eggs, opened the eggs and noted morphological features of the embryos. The developmental stages of the embryos were identified from these features based on the standard techniques developed for domestic poultry (Freeman and Vince 1974). Further comparisons of developmental stage were made with published descriptions of the embryonic stages of *Nymphicus hollandicus*, the cockatiel, which is the only parrot species for which this information has been published (Abbott et al. 1991; Joyner 1994). The incubation period of cockatiels is 19-21 days (Higgins 1999). Therefore, I estimated the ages of the swift parrot embryos assuming a rate of development equivalent to that of cockatiels.

4. Chick growth rates

I measured mass (± 0.1 g), skeletal growth (± 0.2 mm) and feather development (± 0.2 mm) in five swift parrot chicks daily, from the time of hatch until fledging. The sixth chick died from unknown causes shortly after hatching. These measurements were carried out between the hours of 8 am to 10 am each day. Mass was determined by direct weighing on a Mettler balance; all other measurements were taken using vernier callipers. I provide a detailed description of the skeletal characteristics (Figure 1) measured below, based on the terminology used by the Australian Bird and Bat Banding Service (Lowe 1984).

Head length was defined as the distance from the nape of the neck to the distal curve of the upper beak; this was measured with the beak closed. **Bill depth** was defined as the distance from the proximal extent of the nares to the distal tip of the upper beak. **Carpo-metacarpal length** was measured as the distance from the carpal joint to the tip of the second phalanx with the wing in a flexed position. The **tarso-metatarsal length** was measured as the distance from the plantar aspect of the tibio-tarsal distal condyles to the distal tip of the tarso-metatarsus with the leg in a flexed position. The **body length** was measured as the distance from the crown of the head to the tip of the pygostyle with the neck in full extension and the body flattened.

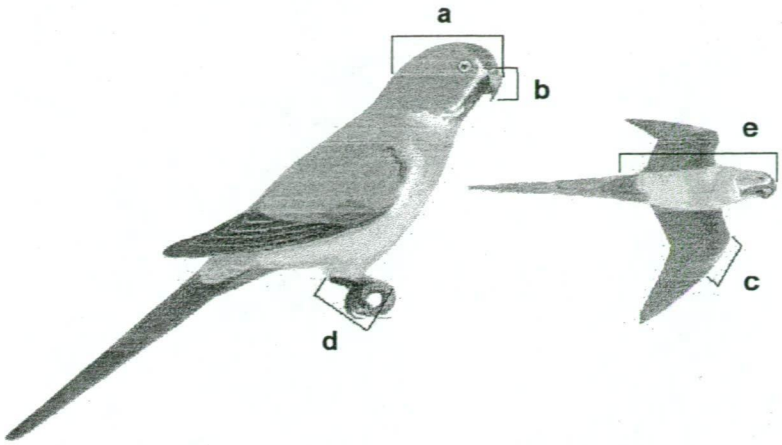


Figure 1. Skeletal characteristics measured on swift parrot nestlings (adult bird is illustrated). a = head length, b = bill depth, c = carpo-metacarpal length, d = tarsometatarsal length, e = body length.

The feather characteristics measured and described were as follows. **Primary remiges (wing feather) length** was measured as the distance from the feather's emergence from its follicle to its distal tip on the ninth primary feather. **Primary retrices (tail feather) length** was measured as the distance from the feather's emergence from its follicle to its distal tip on one of the two central retrices. **Contour feather development** was qualitatively assessed by the structure and pattern of emergence of the contour (body) feathers.

Other morphological features such as the body position and changes to the eyes, ears and legs were assessed subjectively.

Statistics

All objective measurements are presented as mean value ± one standard error unless otherwise noted. A multi-variate repeated measures analysis of variance [(M)ANOVA], using the Pillai's trace to assess significance, was used to assess changes in egg weight over the period of incubation. *Post hoc* t-tests were used to determine the nature of any differences found (See Chapter 2 for details). Growth curve equations were calculated by applying the sigmoidal equation below (Bucher 1983).

$$\text{Growth variable}_t = a / (1 + e^{-k(t-i)})$$

where Growth variable$_t$ = the growth variable at time t (days), a = the asymptote of the growth curve, k = the growth rate constant, and i = the inflection point of the growth curve. The parameters a, k and i were estimated using non-linear regression techniques using Systat 7.0 for Windows. Bucher (1983) used this formula to describe the increase in nestling mass of four species of small parrots (*Agapornis personata* and *Agapornis roseicollis*, African lovebirds; *Bulborhynchus lineola*, barred parakeet; and *Enicognathus ferruginous*, Austral conure), and it is, therefore, considered appropriate for the swift parrots.

RESULTS

1. Incubation behaviour

The female swift parrots spent increasing periods in the nestbox for seven days prior to laying the first egg. Only the female bird carried out incubation, relying on the male to provide food to her, although the food was available *ad libitum* in feeding bowls only two metres from the nestbox. The interval between feeding bouts in the first and second trimester of incubation was 25.6 ± 4.48 minutes (n = 10 observations). The female birds spent the first two weeks of brooding in the nestbox, only emerging to be fed by the male. The females spent no more than five minutes outside the nestbox for each feeding period. During brooding, both parents entered the nestbox, presumably to feed the chicks. After fledging, the male birds took greater responsibility for feeding the chicks until weaning.

Early in the incubation period one female, on a clutch of four eggs, showed the development of a brood patch, ie. an area of no feathers, thickened skin and increased vascularity on the ventral thorax (Figure 2). This is not usually a feature of parrot species and was not noted in the other hen, which incubated only a single egg.

2. Egg incubation

During the study, the females of two pairs of swift parrots in the research aviaries laid clutches of eggs. All eggs were oval shaped, smooth, lustreless and

white (Figure 3). One clutch consisted of two fertile eggs; however, one egg was broken early in incubation. The eggs in this clutch were laid 48 hours apart. The other clutch consisted of five fertile eggs. The eggs in this clutch were laid at the following intervals after the previous egg: 24 hrs; 48 hrs; 72 hrs; 24 hrs. The incubation length of swift parrot eggs in captivity was 19 ± 0.45 days (n = 6; range 18 - 20). The mean external pip-to hatch interval was 40 ± 16 hrs (n = 6). There was a significant loss of mean egg mass over the three periods of incubation ($F_{(2, 4)}$ = 13.013, P = 0.018) (Figure 4). The mean weight of the eggs in the first trimester of incubation was 6.0 ± 0.12 g (n = 6) and had dropped significantly ($t_{(5)}$ = 4.757, P = 0.005) to 5.5 ± 0.19 g (n = 6) in the second trimester. The egg mass decreased significantly ($t_{(5)}$ = 3.276, P = 0.022) from the second trimester to 5.2 ± 0.25 g (n = 6) in the third trimester. The mean percentage loss of initial egg mass during incubation was 22.1 ± 3.28 % (n = 6).

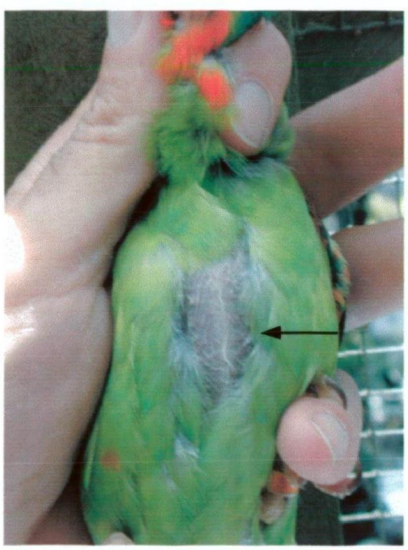


Figure 2. A female swift parrot showing the formation of a brood patch (arrow) during incubation.

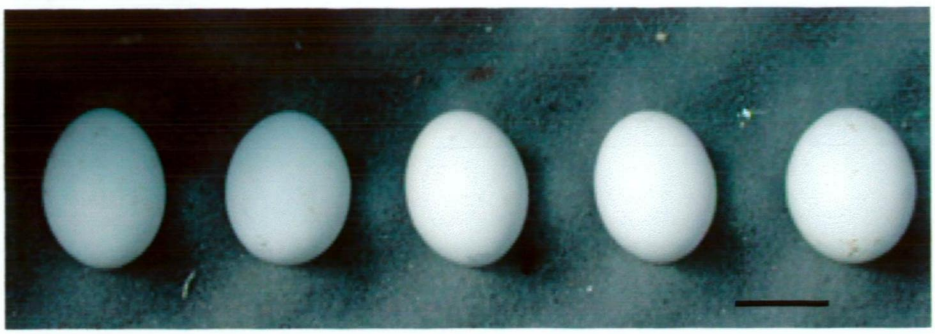


Figure 3. A single clutch of five swift parrot eggs from the research aviaries, December 2000. Scale bar represents 20 mm.

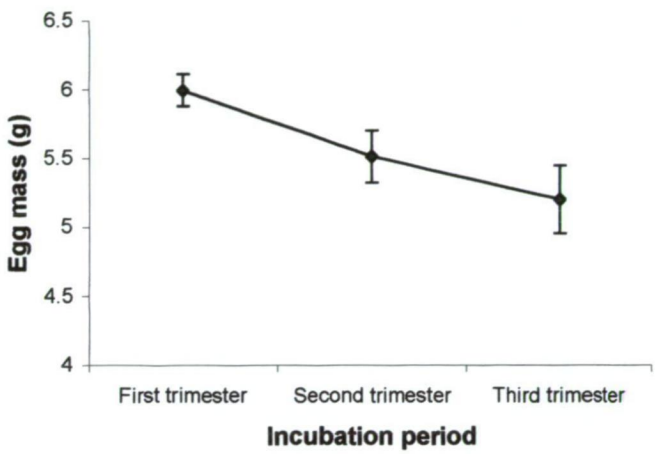
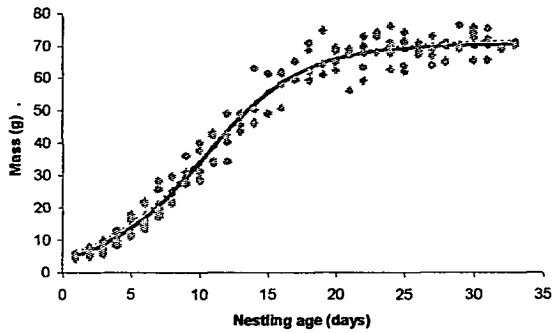


Figure 4. The mean mass (g) of swift parrot eggs (n = 6) throughout incubation in captivity. First trimester was from days one to six following oviposition, second trimester was from days seven to twelve and the third trimester was from days thirteen to hatching. Error bars represent one standard error. There was a significant decrease in egg mass ($P = 0.018$) over the period of incubation. All values were significantly different from each other ($P < 0.05$).

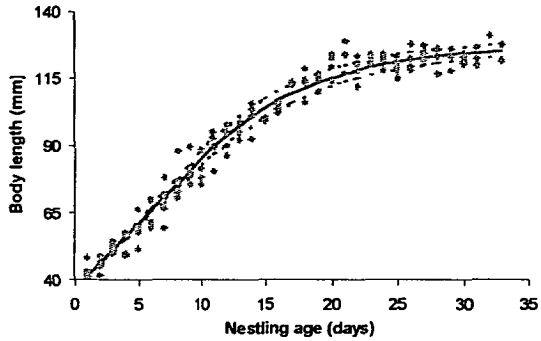
The progression of development of the swift parrot embryos and their associated structures within the eggs is presented in Table 1 and illustrated in Figures 5 to 7. Where images have been digitally enhanced to reveal more details of the embryo, this is noted in the figure legend. No image of pipping in swift parrots was available; however, the process is identical in the eggs of *Glossopsitta concinna*, the musk lorikeet, so a musk lorikeet egg at pipping is shown to illustrate this stage of incubation (Figure7ii).

Table 3. Parameter values for the growth curves of mass and skeletal characteristics of swift parrot nestlings generated from non-linear regressions of growth variables and nestling age (days) post-hatching. Upper and lower values are the 95 % confidence intervals. a is the asymptote of the growth curve, k is the growth rate, and i is the intercept of the curve.

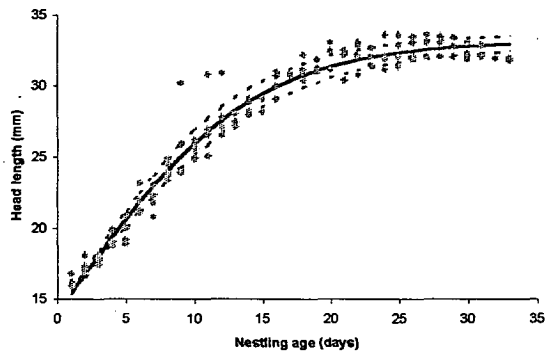
	Parameters from growth curves (Growth variable$_t$ = a / (1+ $e^{-k(t-i)}$)							
	a			k			i	r^2
Growth variable	lower	mean	upper	lower	mean	upper	mean	
Mass	69.51	70.59	71.68	0.29	0.27	0.25	10.25	0.972
Body length	125.06	126.76	128.47	0.15	0.16	0.17	5.50	0.974
Head length	32.77	33.21	33.64	0.15	0.16	0.17	1.85	0.966
Bill depth	13.46	13.63	13.81	0.15	0.16	0.18	0.40	0.951
Carpal length	36.86	37.52	38.19	0.15	0.17	0.18	8.65	0.956
Tarsal length	17.69	17.90	18.11	0.26	0.28	0.31	3.20	0.949



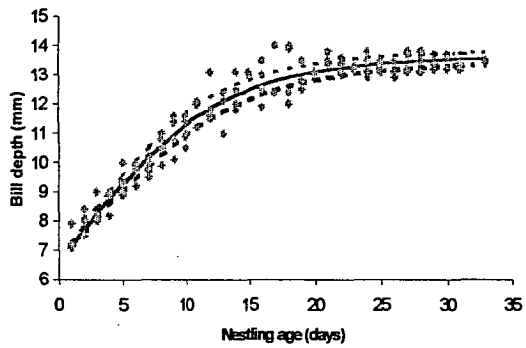
i) $Mass_t$ (g) = 70.594 / (1 + $e^{-0.272(t-10.250)}$)



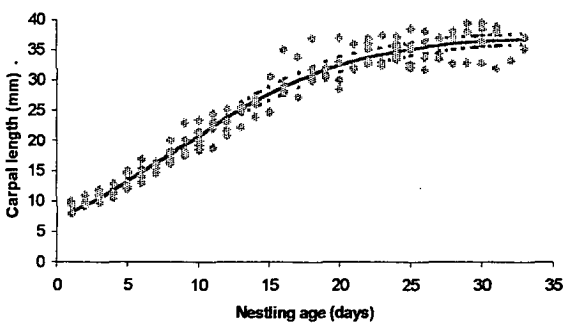
ii) Body $length_t$ = 126.764 / (1 + $e^{-0.159(t-5.500)}$)



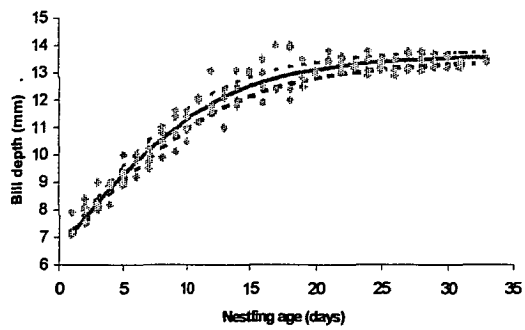
iii) Head $length_t$ = 33.206 / (1 + $e^{-0.157(t-1.850)}$)



iv) Bill $depth_t$ = 13.630 / (1 + $e^{-0.164(t-0.400)}$)

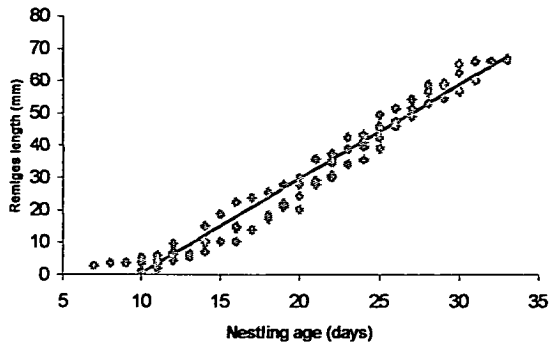


v) Carpal $length_t$ = 37.523 / (1 + $e^{-0.165(t-8.65)}$)

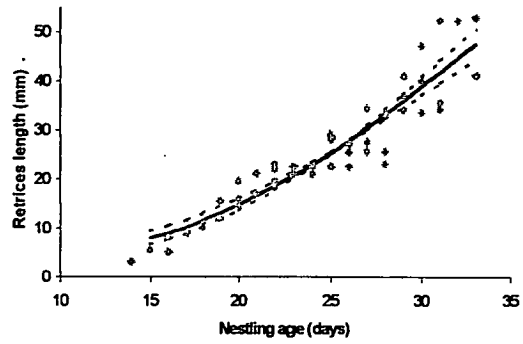


vi) Tarsal $length_t$ = 17.902 / (1 + $e^{-0.281(t-3.20)}$)

Figure 8. Relationships between swift parrot nestling age (t) in days post-hatching and the following growth variables: i) mass (g); ii) body length (mm); iii) head length (mm); iv) bill depth (mm); v) carpal (carpo-metacarpal) length (mm); and vi) tarsal (tarso-metatarsal) length (mm). Grey diamonds are the observed measurements. The solid lines represent the predicted growth curve and the dotted lines represent the upper and lower 95 % confidence intervals for the predicted growth curves.



i) Remiges length$_t$ = 2.912*t - 28.655



ii) Retrices length$_t$ = 80.56 / (1 + $e^{-0.143(t-30.5)}$)

Figure 9. Relationships between swift parrot nestling age (t) in days post-hatching and feather growth variables: i) remiges (primary wing feather) length (mm); and ii) retrices (tail feather) length (mm). Grey diamonds are the observed measurements. The solid lines represent the predicted growth curve and the dotted lines represent the upper and lower 95 % confidence intervals for the predicted growth curves.

The changes in the appearance and actions of nestling swift parrots from hatch to fledging are described in Table 4 and Figure 10 (i-vi). All swift parrot nestlings proceeded uniformly through the stages described, regardless of hatching order within the clutch (n= 4) or whether raised as a sole chick (n = 1).

The growth curves of the swift parrot nestlings follow similar patterns to those of other parrot species (Bucher 1983). The growth period is characterised by two main stages. The initial phase is mainly one of skeletal growth. All of the skeletal characteristics showed maximal rates of growth until approximately day seventeen. The only feather development that occurred during this period was the appearance of grey body down. The second period of growth, from approximately day fourteen until fledging, is dominated by feather development with the emergence of the primary remiges, retrices and juvenile plumage contour feathers.

Table 4. Appearance of major feather tracts, other key structures and landmark events during the nestling phase of growth of swift parrot chicks (n = 5).

Age of chick (days)	Structural development	Figures
1	Long, white, stringy down over body Unable to hold head upright	10 i, ii
3-5	Primary wing feather follicles visible Able to hold head up, ear canals open	-
4-6	Contour feather follicles visible	10 iii
6-8	Loss of egg tooth Pigmented scales on plantar pads appear Eyes open	-
8-10	Soft grey body down emerging from follicles Down on nape forms round white spot Primary wing feathers emerging from follicles	10 iv
11-15	Primary wing feathers emerging from sheath Primary tail feathers emerging from follicles Juvenile plumage contours emerging from sheaths over body and legs	10 iv
16-20	Facial feathers emerge from follicles Juvenile plumage wing coverts emerging from sheaths	10 v
20-25	Cere feathers emerging Juvenile plumage neck contour feathers emerging from sheaths	-
25-30	Cere feathers fully emerged Loses last of white hatching down Tail feathers fraying and breaking at end	-
30-40	Fledging	-

Figure 10. The appearance of swift parrot chicks at: i) one and two days after hatching; ii) two days old. Note the egg tooth (small arrow) and closed eyelids; iii) 6 days of age. Note the dark feather follicles present over the body (black arrow) and along the wings; iv) (A) 8 days of age, (B) 9 days of age, (C) 13 days of age and (D) 15 days of age. Note the prominent white downy spot on the nape of all chicks (black arrows) and the emergence of the juvenile plumage (contour feathers) over the body and wing (grey arrow); and v) 16 days old showing emergence of facial feathers. Scale bars represent 1 cm.

DISCUSSION

Some aspects of the reproductive strategy used by swift parrots, including incubation parameters and the raising of chicks, are likely to represent phylogenetic traits. In particular, female-only incubation, the incubation length, embryo development, and the nestling growth curves observed in the swift parrots are typical of most parrots. However, there are some differences from other members of the Platycercinae, such as clutch size, and time to fledging, that may be related to their unusual life history.

In Table 5, some of the parameters of incubation for swift parrots are compared to other Tasmanian parrots. The species represented include a nomadic, nectarivorous lorikeet (*Glossopsitta concinna*, the musk lorikeet); two resident, granivorous rosellas (*Platycercus caledonicus*, the green rosella and *Platycercus eximius*, the eastern rosella); and finally a migratory, granivorous grass-parrot (*Neophema chrysogaster*, the orange-bellied parrot). It should be noted that the information from Higgins (1999) is a compilation of avicultural records of varying dependability and no sample sizes were recorded.

Table 5. Mean clutch size, length of incubation and time to fledging in parrot species breeding in Tasmania. * = data from Higgins (1999).

Species	***Lathamus discolor*** **(this study)**	*Lathamus discolor**	*Glossopsitta concinna**	*Platycercus caledonicus**	*Platycercus eximius**	*Neophema chrysogaster**
Mean clutch size	**3.5**	4.45	2	6	5.6	4.5
Incubation length (days)	**19**	25	22	20	19.5	21
Time to fledging (days)	**30 - 40**	45 - 55	45 - 50	45	30 - 40	28 - 35

Incubation length does not vary markedly between these species, suggesting that this feature of reproduction is unlikely to be adaptive. However, the difference between incubation length observed in swift parrots in this study and that reported by

Higgins (1999), suggest the possibility of some flexibility in incubation length in this species, at least in captivity. In other avian species, even short absences of the incubating parent and subsequent cooling of the eggs results in delayed embryo maturation and hatching (Delany et al. 1999; Tazawa and Whittow 2000). In parrots, the strategy of female-only incubation means the female parent is only off the eggs for short periods during the transfer of food from the male bird.

During incubation, the male swift parrot fed the female at short (~ 25 mins), regular intervals. In wild orange-bellied parrots, feeding bouts during incubation were at a mean interval of 2.9 hours (Higgins 1999). The results from the captive swift parrots do raise the possibility that the feeding intervals recorded in the field, of every three to five hours during incubation (Brown 1989), should be re-assessed. However, the frequency of feeding bouts for the incubating female, and then the growing offspring, may be related to the proximity and abundance of food resources (Brereton 1996a). Alternatively, the male swift parrot may have been stimulated to more constant feeding by the proximity of the nest-box that occurs under captive conditions. If so, significant variation between results from the field and birds in captivity would be expected. This period may, therefore, be a critical bottleneck for resources in the swift parrot life history. The interval between feeds may limit the clutch size that can be efficiently incubated (Monaghan and Nager 1997) and reduce the chicks' growth rates (Krebs and Magrath 2000). Future field studies should aim to correlate the frequency of feeding bouts, the abundance of local food resources and the success of reproduction during this period. Food supplementation experiments were used in the Florida scrub-jay, *Aphelocoma coerulescens*, to confirm that the nutritive needs of the female were limiting the initiation of laying and clutch size (Schoech 1996). Similar experiments on wild swift parrots may aid in elucidating the critical points of food-limitation in swift parrots. Captive studies could also be used to assess the effect of limiting feeding bouts during incubation and chick rearing.

The variation in mean clutch size seen within the Psittacidae may reflect adaptations to diet and life history. The resident, granivorous rosellas have larger clutches. The average clutch size of six Australian species of rosellas (*Platycercus caledonicus, Platycercus eximius, Platycercus elegans, Platycercus adscitus, Platycercus venustus* and *Platycercus icterotis*) is 5.2 eggs per clutch (Higgins 1999).

The large clutch size may represent either the ability to provide a higher quality diet for raising offspring or a lack of time constraints allowing a longer interval to fledging and a slower growth rate of chicks. In contrast to the rosellas, the nomadic, nectarivorous musk lorikeet has a small clutch size but a similar time to fledging for the chicks. The average clutch size of six Australian lorikeet species (*G. concinna, Trichoglossus haematodus, Trichoglossus chlorolepidotus, Psitteuteles versicolor, Glossopsitta pusilla* and *Glossopsitta porphyrocephala*) is 3.0 eggs per clutch (Higgins 1999). This raises the possibility that the predominantly nectarivorous diet may constrain clutch size. However, the clutch size, incubation period and time to fledging of the orange-bellied parrots, *Neophema chrysogaster*, is similar to that of swift parrots. This similarity suggests that the time constraints imposed by migration may also limit the number of young that can be raised.

An alternative to increasing clutch size to improve fecundity is the ability of birds to produce multiple broods in a single year. No confirmed cases of double brooding in wild swift parrots have been documented. The appearance of offspring late in January has lead to the suspicion that double brooding occurs (Brereton 1996a); however, these late offspring could simply be due to the late initiation of breeding, or failed first clutches. Swift parrots can produce multiple clutches in captivity (Febey K, pers. comm; Cook B, pers. comm.) For example, one pair of birds in captivity in Hobart has reliably raised two clutches a year for the past three years, and in the summer of 2001 laid a third clutch for the year (Cook B, pers. comm.). However, this level of fecundity is unlikely to be achieved in wild birds, where food is unreliable, and lower in protein content than most captive diets fed (Chapters 8 and 9), and where the birds are temporally and energetically constrained by the demands of migration (Chapter 10).

The normal avian egg loses ~ 20-22 % of its initial mass as water loss during incubation (Ar 1991). This weight loss is mainly due to evaporation; the eggshell and membranes do not permit water movement into the egg (Ar 1991). The metabolic water production of the embryo and, more importantly, the relative humidity of the incubation environment (Ar 1991) therefore affect the rate of egg weight loss during incubation. Understanding the normal egg weight loss for the swift parrot eggs during incubation allows egg weights to be used as an indicator of embryo health

during both natural and artificial incubation (Joyner 1994). Further, during artificial incubation, monitoring the egg weight loss allows manipulations of the relative humidity of the incubator to ensure conditions remain ideal for embryonic growth (Joyner 1994). The information presented here on the weight loss of the swift parrot eggs is typical of parrot species (Joyner 1994).

The candling stages of the eggs of swift parrots during incubation suggests that the eggs follow a similar developmental pattern to *N. hollandicus*, the cockatiel (Abbott et al. 1991; Joyner 1994) and *Amazona amazonica*, the orange-winged Amazon parrot (Delany et al. 1999). The three embryos that were obtained for staging also showed features typical of an identical progression of embryogenesis in swift parrot eggs to cockatiels (Abbott et al. 1991; Joyner 1994). Similarly, the growth curves of the swift parrot nestlings are similar to those of other parrots studied (Bucher 1983). The adaptive significance of the altricial state of parrots at hatching and their comparatively slow growth rate when compared to other avian species is unknown (Bucher 1983).

This study has documented the separation of growth in the nestlings into two largely separate stages of first skeletal growth and then feather growth. I suggest three possible explanations for this separation. Firstly, it minimises the period that feathers are exposed to the potentially damaging conditions within the nest-hollow. In support of this hypothesis, note that the primary remiges (tail feathers) emerge late in the nestling period and are not fully emerged at fledging. Even so, abrasion of the distal tip of the remiges is common and many recently fledged juvenile parrots have broken tail tips. Early in the nestling phase, the chicks develop a grey body down but then halt further feather development until after skeletal growth is largely accomplished. The grey body down is presumably necessary for thermo-regulation. The female parent remains predominantly within the nest-hollow for the first two weeks post-hatching. This period sees the development of the down and may relate to the chicks' thermoregulatory independence. The presence of a white spot of down on the nape of the neck is characteristic of the Platycercinae (Forbes 1879; Homberger 1980) but its function remains unknown. It may help to locate the chicks' head within the darkness of the nest-hollow.

Secondly, if the primary feathers were to emerge at the same time as the growth of the long bones, the weight of these feathers may result in the malformation of the developing bone. This syndrome (known as "Angel Wing") occurs in juvenile waterfowl that are overfed resulting in excessively rapid growth (Olsen 1994). The delaying of the main period of feather growth until after the skeleton is relatively mature may have evolved to circumvent this problem. Finally, the high energetic requirements of skeletal and feather growth (Klasing 1999) may prohibit them from occurring together.

In the nests of *P. elegans*, the crimson rosella, parents equally distribute food between nestlings, ensuring similar rates of growth, regardless of hatching order. This is in contrast to most passerines in which chicks hatch asynchronously, and the parents simply feed the most competitive chick (Krebs and Magrath 2000). The comparable growth of swift parrot chicks from a clutch of four suggests that the parents are distributing food evenly throughout the clutch, but further studies are necessary to confirm this early impression.

In conclusion, dietary and time constraints may be acting on the incubation and chick-rearing period of reproduction in swift parrots. The high nutritional requirements of both parents and offspring in these periods mean that the quality, abundance and proximity of food resources may influence clutch size, and time to fledging. The temporal demands of migration may be acting to limit the time available for preparation for breeding, raising young and multiple brooding.

PART B

PHYSIOLOGICAL CONSTRAINTS ON REPRODUCTION AND THEIR IMPLICATIONS FOR CONSERVATION

Chapter 4.

The Nutritional, Morphologic, and Physiologic Bases of Nectarivory in Australian Birds

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Abstract: This review provides an overview of the nutritional constituents of natural foods used by avian nectarivores in Australia, and details some of the adaptations correlated with nectarivory in birds. Nectar provides abundant amounts of energy for those birds able to harvest it. However it contains very low levels of amino acids, vitamins and trace minerals, all of which are necessary for avian maintenance, growth and reproduction. Pollen, manna, honeydew, lerp, insects, and fruits are alternative resources used by different species to meet their nutritional requirements. Nectarivorous birds have developed a variety of adaptations. The morphological adaptations include changes in body size, plumage, beak and tongue structure and in the alimentary organs. The physiology of nectarivory is still poorly understood but there are indications that adaptations may include lowered metabolic rates, lowered protein requirements and changes to digestive and renal physiology. Considerable work is needed to illuminate the specific nutritional requirements of nectarivores for maintenance, growth, and reproduction.

Keywords: nectar, pollen, lorikeets, honeyeaters, alimentary ecomorphology, nutritional constituents

Introduction

Nectarivorous birds are those that rely for food predominantly on the products of flowering trees and shrubs (Simpson and Day 1984). Australian avian nectarivores include honeyeaters of the family Meliphagidae (Ford and Paton 1985), order Passeriformes (Table 1) and lorikeets and swift parrots (*Lathamus discolor*) of the family Psittacidae, order Psittaciformes (Christidis and Boles 1994).

Table 1. Main genera of Australian honeyeaters (Paton 1986).

Genus	Common Name	Beak length (mm)	Weight (g)	Species (No.)
Meliphaga (incl. *Lichenstomus*)	Typical honeyeaters	12-16	17-35	22
Melithreptus	Dark-headed honeyeaters	12-15	12-20	6
Manorina	Miners	15-20	35-50	4
Phylidonyris	Yellow winged honeyeaters	16-22	14-20	5
Acanthorhynchus	Spinebills	20-25	10-14	2
Myzomela	Myzomelas	16	8	4
Anthochaera (incl. *Acanthagenys*)	Wattlebirds	20-24	45-150	4

There is no clear division between the dietary items of nectarivores and those of insectivores and frugivores in Australia. Instead, all species use a variety of food sources, and the labels denote the predominant food source. This is subject to considerable inter- and intraspecific variation dependent upon the availability of food resources and the level of interspecific competition (House 1997).

Considerable work is needed to illuminate the specific nutritional requirements of nectarivores for maintenance, growth, and reproduction. There are a number of reviews published on the ecology and resource partitioning of nectarivorous birds (Carpenter 1978; Feinsinger and Colwell 1978; Paton 1986a;

1986b; Pyke et al. 1996) and the pollination of plants by birds (Keast 1968; Paton and Ford 1977; Vogel 1983; House 1997). There is one early and limited review of the physiologic and morphologic correlates of nectarivory (Brown et al. 1978), and there is also an emerging body of work on sugar preferences, particularly of hummingbirds (Lopezcalleja et al. 1997).

The aim of this paper is to review the nutritional constituents of natural foods consumed by avian nectarivores in Australia and to detail some of the adaptations associated with nectarivory in birds.

Nutritional Constituents of Natural Foods of Nectarivorous Birds

Nectar is a sugar-rich, liquid food source that provides abundant amounts of energy for birds that are able to harvest it (Keast 1968; Vogel 1983; House 1997). However, nectar contains very low levels of amino acids, vitamins, and trace minerals (Lüttge 1976; Vogel 1983), all of which are necessary for avian maintenance, growth, and reproduction (Roudybush 1986; Brue 1994). Therefore, birds classified as nectarivores need to forage for other food resources. Pollen, manna, honeydew, lerp, insects, and fruits are alternative resources used by different species to supplement nectar and meet their nutritional requirements (Paton and Ford 1977; Paton 1980; Richardson and Wooller 1990; Oliver 1998). Manna is a sugary exudate from damaged eucalypt leaves or woods; honeydew is the sugary excretion of nymphal stages of aphids, coccids, and psyllids; and lerp is a waxy material secreted as a protective scale by insects belonging to the family Psyllidae (see below).

Composition of nectar

Nectar is one reward that plants provide to attract pollinators (Vogel 1983). Each flower of *Eucalyptus* and *Callistemon* can produce up to 5 ml of nectar during its 10- to 30-day lifespan (House 1997). Plants have evolved to reach a balance between providing enough nectar to sustain their favored pollinators and limiting the amount that pollinators can harvest to ensure that they use a sufficient number of flowers, thus ensuring cross-pollination (Keast 1968; House 1997).

Nectar is composed of a variety of sugars that make up close to 100% of its dry weight (Lüttge 1976). The predominant sugars include sucrose, glucose, fructose, and, rarely, xylose (Lotz and Nicolson 1996; Nicolson and Vanwyk 1998; Jackson et al. 1998a; Jackson et al.1998b). These sugars are frequently in the form of oligosaccharides composed of glucose and fructose units (Lüttge 1976). Sucrose, fructose, and glucose are digested efficiently, and nectarivorous birds usually show little preference for any sugar (Del Rio and Karasov 1990; Lotz and Nicolson 1996; Downs 1997; Nicolson and Vanwyk 1998; Jackson et al. 1998a; Jackson et al.1998b). Xylose, which is found in Proteaceae, is avoided by some nectarivores and is inefficiently digested (Nicolson and Vanwyk 1998; Jackson et al. 1998a).

Sugar concentration in eucalypt nectar was equivalent to 7.6% sucrose in one study (Bond and Brown 1979); however, other reports indicate that the nectar of plants relying on bird pollinators tend to have a sugar concentration of 13–40% (Vogel 1983). Nectars produced primarily for insect pollinators tend to be more concentrated (for example, 30–74% sugar in nectar for honeybees) (Vogel 1983; Lüttge 1976).

Substances other than sugars are found only in very low amounts in nectar. The respective amounts of other nutrients in nectar of banana flowers (*Musa sapientum*) are as follows (Lüttge 1976) (in percentage dry matter [% DM]) amino acids, 0.0005–0.019%; K^+, 0.012–1.2%; Na^+, 0.0043–0.15%; Ca^{2+}, 0.0004–0.039%; Mg^{2+}, trace–0.034%; and PO^{3-4}, 0.0019–0.198%. Di- and tricarboxylic acids and vitamins are found in trace amounts only. The gross energy value of nectar has been calculated as 16.7 kJ/g (Lüttge 1976).

The level of crude protein in most flower nectars is extremely low; an analysis of banana flower nectar is shown in Table 2 (Lüttge 1976). Captive lorikeets maintained solely on artificial nectar with low protein levels showed weight loss and reduced activity, while lorikeets fed the artificial nectar supplemented with protein did not lose weight (Cannon 1979). Unfortunately, the level of protein supplementation and the amino acid composition of the diet fed in this study were not recorded (Cannon 1979).

Table 2. Nutrient content of the nectar of banana flowers (Lüttge 1976).

	Average quantitative estimation % DM[a]
Glucose	24.5
Fructose	24.5
Sucrose	49
Crude Protein	1.85
Inorganic phosphate	0.098
Phosphate in organic compounds	0.039

[a] DM indicates dry matter.

Composition of pollen

The primary function of pollen is to serve as the vehicle and conductor of male plant gametes, but it is also one of the primary attractants that plants use to reward pollinators (Vogel 1983). Active pollen harvesting by Australian birds has been recorded only in rainbow lorikeets (*Trichoglossus haematodus*) (Richardson and Wooller 1990), purple-crowned lorikeets (*Glossopsitta porphyrocephala*) (Churchill and Christensen 1970) and swift parrots (*Lathamus discolor*) (Gartrell et al. 2000a; Chapter 5).

Pollen protoplasm is composed of highly digestible protein, contains a diverse amino acid profile, and is, therefore, a good food source for nectarivorous birds (van Tets and Hulbert 1999). Pollen is a food source for the queen and nurse bees that produce royal and worker jelly (Vogel 1983). Two studies report digestion of pollen as it moves through the gastrointestinal tract of birds (Churchill and Christensen 1970; Brice et al. 1989); however, Brice et al. (1989) found that pollen is minimally digested in adult hummingbirds and lorikeets. These investigators used pollen that had been bee-collected and stored frozen, and others have speculated that these techniques may have influenced the digestion of pollen from the exine shell (Richardson and Wooller 1990), but further studies are required to explain this

apparent contradictory result. It is thought that the pollen grain is digested in birds through the pores in the exine coat via direct enzymatic action, but the actual biochemical mechanism is unknown (Richardson and Wooller 1986).

There are a large number and variety of compounds in pollen, and the cell constituents vary with age, hydration, viability, and taxonomic order. The major constituents of pollen and their relative concentrations are listed below (Vogel 1983). The gross energy value of pollen has been calculated as 11.3 kJ/g (Churchill and Christensen 1970).

1. *Mineral content (2.5–6.5% DM):* Major elements are nitrogen, potassium, phosphorus, calcium, sulfur, sodium, and magnesium. Iron and boron occur in trace amounts.

2. *Crude protein (16–30% DM):* Free amino acids are considered to be present in copious amounts (Table 3).

3. *Vitamins:* Ascorbic acid (vitamin C), tocopherol (vitamin E), and B vitamins are found in pollen (percentage weights not given). α-Carotene and β-carotene (precursors of Vitamin A) are responsible for the yellow color of most pollens.

4. *Carbohydrates (4–10% DM):* This figure includes starch and soluble sugars (fructose, glucose, and sucrose) but not the indigestible cell walls. The amount of caloric energy gained from pollen is insignificant when compared with nectar.

5. *Lipids (1–20% DM):* Triglycerides and phospholipids predominate in pollen. Fatty acids include palmitic, stearic, oleic, linoleic, and linolenic acid. The long-chain lipids of the cell wall are indigestible and are not included in the above figure.

6. *Other Constituents:* These include sterols, steroids, triterpenes, and flavonoid glycosides that are all suspected to influence pollen color and taste. Toxic substances (especially alkaloids) occur in some pollens but not in those plants using pollen as a reward.

The amino acid content of eucalypt and *Banksia* pollen, compared with that of mealworms (*Tenebrio molitor*) and the vitellogenin of the domestic chicken egg, as well as the recommended nutrient requirements for psittacine and passerine birds (Anon. 1998), are shown in Table 3.

Table 3. Amino acid composition of dietary protein sources for birds (% dry matter basis) and the percentage of the total amino acid requirements for psittacine and passerine birds (van Tets and Hulbert 1999; White 1991; Anon. 1998).

Amino acid	Eucalyptus pollen	*Banksia ericofolia* pollen	*Tenebrio molitor* haemolymph	*Gallus gallus* vitellogenin	National Research Council recommendations	
					Psittacine species	Passerine species
Methionine[a]	1.7	1.6	0.1	2.5	2.5	2.9
Lysine[a]	5.5	7.6	4.4	6.7	5.4	6.3
Arginine[a]	5.9	9.5	2.3	5.9	5.4	6.3
Histidine[a]	1.9	3.1	5.9	2.5	-	-
Iso-leucine[a]	3.3	3.5	2.4	5.5	-	-
Leucine[a]	6.6	7.2	1.6	7.8	-	-
Phenyl-alanine[a]	3.0	4.3	1.3	2.8	-	-
Threonine[a]	4.2	4.5	0.4	4.9	3.3	3.8
Tryptophan[a]	NM[b]	2.8	0.6	0.1	-	-
Valine[a]	4.7	4.1	5.7	6.7	-	-
Alanine	8.6	5.0	2.8	7.2	-	-
Aspartic acid	11.6	9.9	3.0	4.4	-	-
Cystine	4.5	1.1	NM[b]	1.9	4.2[c]	4.8[c]
Glutamic acid	10.9	12.1	17.3	5.5	-	-
Glycine	9.8	4.3	1.4	4.7	-	-
Proline	10.6	8.3	43.9	4.8	-	-
Serine	6.3	4.9	4.7	13.9	-	-
Tyrosine	1.0	6.6	2.3	3.0	-	-

Percentage of each amino acid in the total amino acid composition of eucalypt pollen, *Banksia ericofolia* pollen, the mealworm, *Tenebrio molitor* and vitellogenin of the domestic chicken, *Gallus gallus*

[a] Essential amino acid.

[b] NM indicates not measured.

[c] Combined value for methionine and cystine used by National Research Council recommendations.

It should be noted that the recommended nutrient requirements used here are based on extrapolations from the National Research Council requirements for poultry and the "research and formulation experience" of the Nutrition Expert Panel (Anon. 1998).

The vitellogenin of egg yolk is used for comparison because it is considered one of the highest quality forms of protein: it is highly digestible and has an amino acid profile considered to be optimal for avian embryonic growth (White 1991; Downs 1997). It can be seen that pollen from both genera of plants fulfil most of the recommended nutrient profiles for amino acid composition (except for methionine) and have an amino acid composition that is very similar to vitellogenin.

A nectarivorous marsupial, the Eastern pygmy possum (*Cercartetus nanus*), maintained a positive nitrogen balance and its dietary maintenance nitrogen requirements were exceptionally low when maintained on an eucalypt pollen diet (2.6 mg N/day compared with 9.5 mg N/day on mealworms), thus providing further evidence for the high quality of pollen protein (van Tets and Hulbert 1999).

Composition of manna, honeydew, and lerp

Manna is a sugary exudate from damaged eucalypt leaves or woods (Ford and Paton 1985) that appears as white nodules of interlaced acicular crystals (Basden 1965). Manna occurs at sites of damage caused by phytophagous insects, and its constituents differ markedly from leaf sap (Paton 1980; Basden 1965). Manna from eucalypts is composed of about 60% sugars, of which raffinose makes up the majority (65–80%), with the rest composed of melibiose, sucrose, glucose, fructose, and stachyose (Basden 1965). Other constituents include water (16%), pectin and uronic acids (20%), and some minerals (Basden 1965). Protein is present as less than 0.2% of wet weight (Paton 1980).

Honeydew is the sugary excretion of nymphal stages of aphids, coccids, and psyllids. It contains sugars (33%), protein (2.6%), esters and acids (5%), and minerals (11.4%). Minerals in honeydew include sodium (26%), potassium (12%), calcium (7%), magnesium (10%), silicon (3%), phosphorus (13%), and sulfur (22%) (Basden 1965). The sugars are mainly small polysaccharides with some glucose, fructose, and sucrose (Basden 1967a; 1967b).

Lerp is the waxy material secreted as a protective scale by insects belonging to the family Psyllidae (Basden 1966;1967a; 1967b). Lerp is composed of polymers of glucose (90%) and water (10%) and is entirely devoid of amino acids (Basden 1966;1967a; 1967b).

Manna, honeydew, and lerp are often found in abundance on Australian eucalypts (Basden 1966; Paton 1980) and have been noted as a common food source for a variety of honeyeaters (Ford and Paton 1985; Richardson and Wooller 1990; Oliver 1998) and nectarivorous parrots (Brown 1989; Brereton 1996a; Higgins 1999). However, given the high carbohydrate and low protein content of these foods, it is likely that they are used only as alternatives to nectar (Paton 1980).

Composition of psyllids

Psyllids are the family of insects that produce lerp (Basden 1965; 1966), and they are sometimes consumed along with their sugary exudate (Paton 1980). The dry weight of the psyllid can be up to 30 times less than that of the lerp, and the insect may be selectively left behind (in up to 77% of instances) by some honeyeaters (Paton 1980). It is therefore likely that they have little role in the nutrition of most honeyeaters that eat lerp (Paton 1980). There is no published information on the nutritional content of psyllids.

Composition of other insects

Many authors suggest that insects are the main source of protein for nectarivores (Paton and Ford 1977; Ford and Paton 1985; Richardson and Wooller 1986; Oliver 1998). There is some evidence that insects make up the largest proportion of dietary items fed by honeyeaters to their nestlings (Miller 1994; Oliver 1998), possibly reflecting the high protein requirements of growing chicks.

However, there are few studies investigating either protein content or other nutritional value of commonly taken insects. Australian nectarivores have been recorded eating insects, including those of the orders Ephemeroptera (mayflies), Orthoptera (grasshoppers), Hemiptera (cicadas and psyllids), Neuroptera (lacewings), Coleoptera (lycid beetles), Diptera (robber flies), Lepidoptera (moths),

and Hymenoptera (wasps) (Paton and Ford 1977; Oliver 1998; Higgins 1999). There is limited information on the nutrient composition of these Australian invertebrates.

A partial analysis of the nutrient composition of mealworm larvae, crickets (*Acheta domesticus*), waxworms (*Galleria mellonella)*, fruitflies (*Drosophila melanogaster*), and earthworms (*Lumbricus terrestris*) has been reported, as summarized in Table 4 (Barker et al. 1998). Larval invertebrates had a higher fat content (>30% DM) than adult species. Total nitrogen ranged from ~5% to 10%, of which 3–10% of this total was chemically bound. Levels of chitin, which is indigestible for birds, averaged 15.3% in all species except earthworms (51%). Vitamin E levels were adequate for poultry requirements, but vitamin A levels were either very low or nonexistent. All insects except crickets contained very low levels of calcium as well as unbalanced calcium-to-phosphorus ratios. Trace minerals were considered to be adequate for avian requirements on the basis of poultry studies (Barker et al. 1998).

If these insect species are considered typical of most insects in their nutritional composition, it is likely that nectarivorous birds obtain a good supply of protein from insects. The hemolymph provides a range of the essential amino acids necessary for avian protein synthesis (Brue 1994). The amino acid profile of the hemolymph of mealworms is shown in Table 3. Note that this figure does not include the protein available in the muscle bundles of mealworms (Barker et al. 1998).

However, not all birds are capable of digesting all insects (le Mar 1993). The strength and structure of the gizzard is important for birds to be able to shatter the realtively indigestible insect exoskeletons before digestion of the contents (Richardson and Wooller 1986). However, this fracturing is also dependent on the physical properties of the insect carapace and body structure. (le Mar 1993). The hard carapace of mealworm adults and the soft body of *Heliothis* moth larvae both require a similarly large amount of force and work to fracture (le Mar 1993). Some authors state that all Australian honeyeaters take insects as a source of protein (Richardson and Wooller 1986; House 1997), but further work is required to determine if insects are the major source of amino acids for nectarivores.

Table 4. Nutrient composition of various invertebrates (Barker et al. 1998).

Species (n)	Water (%)	% Dry Matter					IU/kg (mean (± SD)	
		Crude fat	Total N	ADF-N	NDF	Ash	Vitamin A	Vitamin E
Meal worms (6)	62.9	31.1	8.3	0.5	14.5	4.3	811 (±324)	30 (±3)
Adult Crickets (6)	73.2	22.8	10.3	0.7	19.1	5.1	811 (±849)	81 (±41)
Juvenile Crickets (8)	66.8	9.8	8.8	0.6	16.4	9.1	471 (±585)	71 (±42)
Waxworm (6)	61.9	51.4	6.6	0.4	12.1	3.3	150 (±160)	509 (±232)
Fruitfly (4)	67.1	17.9	9.0	1.0	16.2	5.2	Not detected	23 (±13)
Earthworms (6)	74.5	12.6	5.2	0.2	51.2	45.7	2400 (±279)	70 (±12)

ADF-N indicates acid detergent fiber nitrogen as a measure of chemically bound nitrogen; N, nitrogen; ND, not detected; NDF, neutral detergent fiber as a measure of chitin; and SD, standard deviation.

Composition of fruits

Domestic fruits are typically low in protein, rich in simple carbohydrates, and high in bulk (Levey and Karasov 1989) and are hence considered to be a nutrient-dilute food (Levey and Karasov 1994). Most primarily frugivorous species will lose weight when fed exclusively on fruits (Levey and Karasov 1989; Izhaki and Safriel 1989) unless the diet is supplemented. Some investigators speculate that it is not simple protein deficiency that causes weight loss but, instead, interference in nitrogen metabolism by secondary compounds in ripe fruits (Izhaki and Safriel 1989). They suggest that it may be a subtle adaptation by some plants to increase seed dispersal by discouraging birds from becoming solely reliant on their fruit (Izhaki and Safriel 1989).

Australian nectarivores eat a wide variety of native fruits, including the figs of *Moraceae*, mistletoe fruits, and the fruit of the camphor laurel (*Cinnamomum camphora*) (Higgins 1999), but there is limited information on the composition of these fruits. Birds have rapidly adapted to the availability of introduced fruits and are often pests in orchards, feeding on grapes, citrus fruits, pears, and apples (Higgins 1999). The composition of fruits varies widely (Izhaki and Safriel 1989; Del Rio and Karasov 1990), but most fruits contain high levels of simple sugars that provide easily digestible energy. Bird-dispersed fruits have sugar concentrations ranging from 6–22% of solutes and protein levels ranging from 1.5–4.5% DM (Witmer 1998).

The average composition of some fruits is described in Table 5. Native and introduced fruits are considered to be excellent sources of Vitamin A and C (Brandmiller and Holt 1998).

Table 5. Nutritional composition[a] of some fruits (Levey and Karasov 1989).

Fruit	Water, %	Seeds, %DM	NDS, %	Nitrogen, %DM	Protein, %DM	GE, kJ/g	Ash, %DM
Dogwood (*Cornus racemosa)*	76	54	81.2	1.22	7.6	21.5	3.8
Wild Grape (*Vitis sp.*)	81	44	68.1	1.02	6.4	17.6	3.8
Viburnum (*Vibernum dentatum*)	74	52	91.5	0.68	4.2	22.2	3.7
Banana (*Musa sapientum)*	85	0	85	2.22	13.9	17.3	4.1

[a] All fruits are mature and cultivated. Nutritional information is for pulp and skin only and excludes seeds. DM indicates dry matter; NDS, % dry matter soluble in neutral detergent (that is, potentially digestible); GE, gross energy.

Avian Adaptations to Nectarivory

Nectarivorous birds have developed a variety of adaptations allowing them to partition resources in abundant supply (for example, nectar and insects) (Paton and Ford 1977). Morphologic adaptations include changes in body size, plumage, beak and tongue structure, and the alimentary organs. The physiology of nectarivory is still in its infancy (Del Rio and Karasov 1990), but there are indications that physiologic adaptations may include lowered metabolic rates, lowered protein requirements, and changes to digestive and renal physiology.

Body size

The most pervasive adaptation seen in nectarivores is reduced body size (Brown et al. 1978). This results in relative increased energetic costs (per gram) of maintenance, temperature regulation, foraging, and reproduction (Brown et al. 1978). The abundant energy supplied by nectar allows birds to overcome these higher costs, and, therefore, different species can exploit a range of flower resources with reduced interspecific competition (Brown et al. 1978).

The smallest nectarivores are the hummingbirds of the American continents, which have the ability to enter nocturnal hypothermic torpor. The average body mass of bee hummingbirds is 2 g, allowing them to use flowers that cannot sustain larger nectarivores (Brown et al. 1978). At the other extreme, the largest nectarivores, the Australian red wattlebird (*Anthochaera carunculata*; average body mass of ~120 g) and yellow wattlebird (*A. paradoxa*; ~130 g) are able to aggressively defend trees with the richest nectar resources (Ford and Paton 1985; Kennedy 1998). However, both wattlebird species supplement their diet with large amounts of insect prey (Ford and Paton 1985; le Mar 1993). This may simply be to satisfy their protein requirements (Paton and Ford 1977), or they may have exceeded the upper limit of energy expenditure that can be supported by nectar alone (Brown et al. 1978).

The largest nectarivore of the order Psittaciformes in Australia is the rainbow lorikeet (~130 g) (Higgins 1999) and the smallest is the little lorikeet (*Glossopsitta pusilla*, ~40g) (Higgins 1999). Given this size range, it is likely that this order is bound by the same size constraints as passerine nectarivores.

Metabolic rate

The ability to reduce metabolic rate by hypothermic torpor has been reported only for small birds with a limited capacity for energy storage, in particular the hummingbirds (Brown et al. 1978). It appears that torpor is used only when food is scarce and birds are having trouble maintaining positive energy balance (Brown et al. 1978). Torpor tends to be nocturnal because at night-time birds are unable to forage, the temperatures are lowest, and the risk of predation is least (Brown et al. 1978). Nocturnal torpor has not been reported for any Australian nectarivorous bird (Brown et al. 1978).

The field metabolic rates for New Holland honeyeaters (*Phylidonyris novaehollandiae*), eastern spinebills (*Acanthorhynchus tenuirostris*), and a crescent honeyeater (*Phylidonyris pyrrhoptera*) were much lower than would be predicted allometrically for a similar-sized hummingbird (Paton 1982; Weathers et al. 1996). This may reflect the low-cost foraging technique used by honeyeaters whereby they consume nectar while perched (Weathers et al. 1996).

Protein requirements

New Holland honeyeaters (House 1997), Costa's hummingbird (*Calypte costae*) (Brown et al. 1978), and the frugivorous cedar waxwing (*Bombycilla cedrorum*) (Witmer 1998) are all considered to have much lower maintenance protein requirements (~1.5% dietary protein) than granivorous birds (~8%) (Witmer 1998). The reason for the lower protein requirement is unknown, but it may reflect a physiologic adaptation to a carbohydrate-rich diet (Witmer 1998).

Plumage

The plumage of lorikeets and the swift parrot tends to be tighter and glossier than that of granivorous parrots (Holyoak 1973). This is thought to be an adaptation to prevent feather soiling by nectar (Holyoak 1973). Australian honeyeaters do not share this adaptation, but the bare facial skin (Ford and Paton 1985) of some species such as friarbirds (*Philemon* genus) and blue-faced honeyeaters (*Entomyzon cyanotis)* may serve a similar function.

Beak structure

The beaks of hummingbirds, sunbirds, and honeycreepers of other continents are long and have varying degrees of curvature, features that result from coevolution with flower morphology (Carpenter 1978). In Australian honeyeaters, the beaks of the more specialized birds (for example, *Myzomela* and *Acanthorhynchus* species) are also long and slightly curved (House 1997). Those birds with shorter beaks are thought to be more insectivorous (Ford and Paton 1985). Nectarivorous Australian parrots have slender beaks that are much weaker (structurally) than those of similar sized granivorous parrots, reflecting their reduced dependence on hard foodstuffs (Holyoak 1973).

Tongue structure

In most nectarivorous birds, the tongue is modified to aid in the harvesting of nectar and, in some cases, pollen (Simpson and Day 1984; Ford and Paton 1985; Higgins 1999). Australian honeyeaters have extensible brush tongues, in contrast to the tubular tongues of hummingbirds, that allow them to use alternative food sources such as manna and honeydew (House 1997). Australian lorikeets and the swift parrot have a specialized brush tip on their extensible and muscular tongues (Güntert and Ziswiler 1972; Holyoak 1973; Smith 1975). The brush tip consists of a cluster of thread-like papillae that increase the surface area of the tongue and may produce a capillary effect, allowing the rapid harvesting of nectar (Churchill and Christensen 1970).

Alimentary modifications

Proventriculus: In nectarivorous parrots, the proventriculus has compound glands arranged in longitudinal rows with gland-free spaces to allow for the distension of the glandular stomach (Ziswiler and Farner 1972). This may be an adaptation to pollen digestion (Güntert and Ziswiler 1972).

Gizzard: Honeyeaters that are primarily nectarivorous have smaller, less muscular gizzards than insectivorous passerines of similar size (Richardson and Wooller 1986). The physical properties of the type of insect prey determine the development of gizzard musculature in insectivores (le Mar 1993). However,

nectarivorous passerines that consume a substantial amount of fruit have gizzards similar in size to those of insectivores (Richardson and Wooller 1986).

Nectarivorous psittacines also have varying degrees of reduction in gizzard muscle, with the most extreme reduction seen in lorikeets of the genus *Glossopsitta* in which the gizzard is barely recognizable (Güntert and Ziswiler 1972; Richardson and Wooller 1990; Gartrell et al. 2000a; Chapter 5). The proventricular and pyloric openings of the gizzard in lorikeets and honeyeaters both lie in the median plane, which is thought to allow rapid passage of ingesta (Richardson and Wooller 1990). The rainbow lorikeet and the swift parrot both have a relatively muscular gizzard, which may reflect an increased use of insects (Richardson and Wooller 1990; Gartrell et al. 2000a; Chapter 5).

The koilin layer of the gizzard is relatively thin and lacking in striae in those birds that feed on soft foods (Ziswiler and Farner 1972). The koilin striae play a vital role in the disruption of insect exoskeletons before digestion (le Mar 1993); for example, the red wattlebird has a large gizzard with prominent koilin striae (le Mar 1993).

Intestine: Both insectivores and nectarivores tend to have shorter intestines than those of similarly sized herbivorous or granivorous birds (Richardson and Wooller 1986). It has been postulated that a longer gastrointestinal tract allows an organism to increase retention time and thereby increase digestive efficiency (Geluso and Hayes 1999). It is likely, therefore, that the shorter intestines of nectarivorous and insectivorous birds are a response to their highly digestible diet (Richardson and Wooller 1986).

Nectars are hypertonic relative to avian plasma (Skadhauge 1981), and nectarivores must consume fresh water on a regular basis to prevent dehydration. Hummingbirds are thought to have intestinal membranes that are relatively impermeable to the passive uptake of glucose but instead rely on high rates of carrier-mediated glucose uptake, which may prevent osmotic dehydration (Del Rio and Karasov 1990).

Large ceca are the site of digestion by microbial fermentation of cellulose, but when they are present as vestigial remnants they tend to have large amounts of lymphoid tissue (Lumeij 1994a). The ceca are absent or vestigial in hummingbirds,

small in insectivorous passerines, and well developed in herbivorous or omnivorous passerines (Ziswiler and Farner 1972).

Renal modifications

Nectarivorous birds ingest large fluid loads and produce copious dilute urine (Goldstein and Bradshaw 1998a). Tubular reabsorption of water in nectarivorous birds on concentrated diets is at very high rates (>90%), with little change in glomerular filtration rate (Goldstein and Bradshaw 1998a). There are substantial variations in water and sodium fluxes within the family Meliphagidae that relate to body size and, more importantly, to diet (Goldstein and Bradshaw 1998b).

Honeyeaters differ from other passerines in the microscopic architecture of the kidneys (Casotti et al. 1993; Casotti and Richardson 1993). The glomeruli are similar, but the proximal tubule cells contain wide intercellular spaces filled with basolateral cell membrane interdigitations (Casotti et al. 1993). Medullary nephron tubules are arranged in a sequential manner, and the ultrastructural morphology of the limbs of Henle differs from that of granivorous passerines (Casotti et al. 1993). The thin limb of Henle consists of only one epithelial cell type with wide intercellular spaces. The thick limb of Henle consists of two epithelial cell types, each having narrow intercellular spaces but varying degrees of cell membrane infoldings. Nephron characteristics are thought to enable honeyeaters to resorb a large proportion of solutes and water from the glomerular filtrate (Casotti et al. 1993).

There are differences in the histologic anatomy of the kidneys between wet zone and arid zone honeyeaters (Casotti and Richardson 1993). Wet zone honeyeaters have a higher percentage and absolute volume of renal cortex and, in the medulla, a higher percentage and absolute luminal surface area of collecting ducts. Arid zone honeyeaters have a higher percentage and absolute volume of renal medulla, which has a higher percentage and absolute surface area of capillaries than that of wet zone honeyeaters, allowing more efficient water conservation (Casotti and Richardson 1993).

Conclusion

The specific nutritional requirements of nectarivores for maintenance, growth, and reproduction are as yet not known. Dietary information for most psittacine nectarivores is mostly anecdotal and incomplete, and the physiologic mechanisms and controls of nectarivorous digestion and fluid balance remain largely unknown.

Understanding the nutritional basis of the natural diet of a bird is a starting point in investigating the nutritional requirements of its species. Over-reliance on this technique in designing captive diets can be deleterious (Brue 1994), but knowledge of natural foods is necessary if we are to understand the morphologic specializations that evolve in response to natural diet (Levey and Karasov 1994). Until our knowledge of the nutritional requirements of nectarivores improves, we will be forced to rely on commercial diets developed from avicultural lore and trial and error feeding practices.

Chapter 5.

Morphological adaptations to nectarivory of the alimentary tract of the Swift Parrot *Lathamus discolor*.

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Summary: The morphology and microscopic architecture of the alimentary tract of the Swift Parrot was compared to that of the Green Rosella and Musk Lorikeet. Gut contents were evaluated grossly and by light microscopic examination. There were significant differences between the Swift Parrot and the Green Rosella in the scaled measurements of the length of the distal oesophagus and proventriculus and in the width of the crop, duodenum, intestine and cloaca. There were significant differences in the scaled measurements of the length of the gizzard and the width of the oesophagus, gizzard and duodenum between the Swift Parrot and the Musk Lorikeet. The results support previous findings that the Swift Parrot has retained some of the alimentary features of its granivorous ancestors. However, our findings suggest that the Swift Parrot may have adapted to nectarivory, not only with the development of a brush tongue, but also in changes to the crop, proventriculus and duodenum. The larger, more muscular gizzard and longer intestine may allow the Swift Parrot to use a diversity of dietary items when nectar and pollen are not readily available.

Introduction

The Swift Parrot *Lathamus discolor* has attracted the attention of taxonomists since the late nineteenth century but there was no consensus in attempts to classify it morphologically. In some studies, the Swift Parrot was positioned taxonomically with the Loriinae (lories and lorikeets) due to similarities in tongue morphology and the tight, glossy plumage (Holyoak 1973; Smith 1975), but other authors have disputed this classification. The Swift Parrot also shows anatomical similarities with members of the Platycercinae subfamily (Australian broad tailed parrots), such as the rosellas (Forbes 1879; Homberger 1980) and recent DNA hybridisation studies (Christidis et al. 1991) have indeed placed the Swift Parrot in this subfamily. Morphological similarities between Swift Parrots and the lorikeets may be concluded to be due to evolutionary convergence (Christidis et al. 1991).

Güntert & Ziswiler (1972) compared the alimentary structures of three Swift Parrots to those of other nectar feeding parrots. They found the only adaptation to

nectarivory was the development of a brush tongue, with the gizzard remaining relatively muscular, and the intestine longer, than that of the Loriinae and *Loriculus* species dissected. They concluded that the Swift Parrot was a less advanced form of nectarivore than these other parrots. The Swift Parrot is thought to have adapted to nectarivory from granivorous ancestors living in the southeastern eucalypt forests of Australia filling a niche available in the absence of lorikeets. The lorikeets now present in these forests are thought to be recent arrivals, having spread south from New Guinea (Christidis et al. 1991).

In ecological terms, the Swift Parrot has adapted to nectarivory by becoming nomadic, following the flowering of eucalypts around Tasmania during the spring and summer and then migrating to mainland Australia. There it winters in the southeastern mainland forests (Brown 1989; Brereton 1996a). On the mainland they have been recorded feeding on mainly eucalypt nectar, pollen, lerp and psyllids. They will also opportunistically take seed, fruit, honeydew and poplar catkins (Hindwood & Sharland 1964; Kennedy 1998; Brereton unpubl. obs.). Breeding has only been recorded in Tasmania; during the breeding season, the Swift Parrot relies predominantly on the nectar and pollen of the Tasmanian blue gum *Eucalyptus globulus* (Hindwood & Sharland 1964; Brereton 1996a).

While alimentary tract morphology is generally regarded as adapted to the predominant diet of a species, work on passerines has shown that alimentary structures are remarkably adaptive to differing diets, even within an individual. Differences in the stomach morphology of Australian passerines and lorikeets appear to be functional rather than reflecting phylogenetic relationships (Richardson & Wooller 1986; 1990). Small passerines with a diet of nectar and small insects have a smaller, less muscular gizzard and a shorter intestine than dedicated insectivores of comparable body size (Richardson & Wooller 1986). In captive starlings and poultry, it has been possible to alter the thickness of the ventriculus musculature and intestinal length in as little as seventeen days by modifying the diet (Duke 1986; Biviano et al. 1993). In the study by Güntert & Ziswiler (1972), only three specimens of Swift Parrot were examined. As this

study was carried out in Europe, it is likely that the birds were from aviary collections. Standard avicultural practice is to maintain Swift Parrots in captivity on a mixed seed and nectar diet (Hutchins & Lovell 1985), which may have altered the dimensions of the alimentary tract.

The aim of the present study was to re-examine the alimentary features of Swift Parrots in the light of this understanding of alimentary flexibility. We also wished to make more detailed comparisons with granivorous and nectarivorous parrots.

Methods and Materials

The Swift Parrot population in Tasmania suffers significant mortality each year due to impact with windows, fences and automobiles (Brown 1989; Brereton 1996a). Tasmanian Parks and Wildlife Service collected the Swift Parrot carcasses (n = 25) used in this study over the years 1995 to 1998. For comparison, Green Rosellas *Platycercus caledonicus* (n = 4) and Musk Lorikeets *Glossopsitta concinna* (n = 4) were used; these birds were mortalities resulting from cat attacks or motor vehicle collisions.

The Green Rosella was used because it is a closely related granivorous psittacine and is thought to be typical of the granivorous ancestors of the Swift Parrot (Güntert & Ziswiler 1972). The Musk Lorikeet was used as an example of a trichoglossid nectarivore more distantly related to the Swift Parrot (Christidis et al. 1991). Further comparisons were made with published data on other wild caught trichoglossid lorikeets (Richardson & Wooller 1990). Only birds from wild populations were used to eliminate the possibility of a captive diet modifying alimentary features. The oral cavity, beak and tongue were not considered in this study as these have been extensively examined in the taxonomic literature (Güntert & Ziswiler 1972; Holyoak 1973; Smith 1975).

Twelve Swift Parrots collected prior to September 1997 were frozen intact at -5°C; they were subsequently thawed and dissected. These birds were used to establish gross morphological measurements but cellular disruption due to freezing made histological analysis from these birds impossible. From September 1997, thirteen birds

were dissected fresh, within twelve hours *post mortem* and histological preparations were of much higher quality.

Gut contents were evaluated grossly and by light microscopic examination (magnification 100x and 400x) of gut-fluid smears. These smears enabled identification of pollens to genus level and revealed the chitinous remains of insects, which were not further identified. Weights and morphological measurements were taken prior to sampling of the alimentary tract. Pectoral muscle condition was scored from 1 (poor) to 4 (excellent) as in Harrison & Ritchie (1994). For the purposes of measurement, the alimentary tract was divided into the sections proximal oesophagus, crop, distal oesophagus, proventriculus, gizzard, duodenum, intestine and cloaca as in Ziswiler & Farner (1972) and Duke (1986). All measurements were made using vernier calipers by a single investigator (BG). To overcome the effect of the expansibility of the crop and oesophagus, these organs were emptied, and measurements were taken from the flattened, but not stretched, viscera.

To further characterise any differences, the microscopic architecture of the proventriculus of the Swift Parrot (n = 13), the Green Rosella (n = 2) and the Musk Lorikeet (n = 1) was examined. The tissue samples were fixed in a 10% formaldehyde solution equivalent to ten times their mass, embedded in paraffin wax and cross-sectioned (3μ) in a rotary microtome. Sections were stained with haematoxylin and eosin and then examined microscopically. The tissue layers were defined as in Randall & Reece (1996).

Statistical methods

To make interspecific comparisons of alimentary morphology, it was necessary to scale for the size differences between the species. Body weight in this study showed a strong correlation to pectoral muscle condition, making it unreliable as a scaling factor for size. Tarsometatarsal (tarsal) length was used as an indicator of overall body size (Cubo & Casinos 1994) and was used to scale the alimentary measures because all three species examined are of similar skeletal configuration. Additional tarsal measurements

from the field capture of all three species were used to supplement the *post mortem* material. The tarsal lengths (mean ± one s.e.) for Swift Parrots (16.2 ± 0.20 mm, $n = 54$), Musk Lorikeets (16.9 ± 0.28 mm, $n = 7$) and Green Rosellas (25.2 ± 0.37 mm, $n = 5$) were used to determine scaling factors of 1.56 for Green Rosellas and 1.04 for Musk Lorikeets. A two-tailed *t*-test assuming equal variances was applied to the scaled data for Swift Parrots, Green Rosellas and Musk Lorikeets to test for significant differences ($P \leq 0.05$).

Results

Gross and histological measurements of the alimentary tract

Comparisons of the Swift Parrot data with the scaled values for the other species showed significant differences in a number of measurements (Table 1). The distal oesophagus ($t = 2.785$, $P = 0.011$) and proventriculus ($t = 2.234$, $P = 0.035$) were longer in the Swift Parrot than in the Green Rosella and the crop ($t = 2.385$, $P = 0.026$), duodenum ($t = 4.388$, $P = 0.0002$), intestine ($t = 2.652$, $P = 0.014$) and cloaca ($t = 2.213$, $P = 0.039$) were wider in the Swift Parrot than in the Green Rosella. The gizzard was longer ($t = 4.927$, $P < 0.0001$) and the oesophagus ($t = 2.208$, $P = 0.037$), gizzard ($t = 2.135$, $P = 0.044$) and duodenum ($t = 2.489$, $P = 0.021$) were wider in the Swift Parrot than in the Musk Lorikeet. The microscopic architecture of the proventriculus was similar except for the arrangement of the compound glands in the lamina propria. In the Swift Parrot and the Musk Lorikeet, the glands were arranged as overlapping bundles in the lamina propria. In the Green Rosella, the compound glands were only present as a single layer (Figure 1).

Alimentary contents

The content of the alimentary tract was identified in twelve Swift Parrots whose onset of death was rapid. The alimentary tract contained insect remains in seven birds (58% of birds examined), pollen in four birds (33%), lerp and psyllids in one bird (8%), and plant matter in one bird (8%).

Table 1. Comparison of alimentary measurements of Swift Parrots to scaled values for Green Rosellas and Musk Lorikeets. *n.s. = no significant difference (P>0.05).* **Intestine excludes duodenum.*

Species	Swift Parrots	Green Rosellas		Musk Lorikeets	
N	25	4		4	
Scaling factor		/1.56		/1.04	
		Scaled value	Significance	Scaled value	Significance
Proximal oesophagus					
Length (mm)	27.7	27.1	*n.s.*	31.3	*n.s.*
Width (mm)	2.3	2.1	*n.s.*	1.3	*P = 0.011*
Crop					
Length (mm)	28.8	23.1	*n.s.*	22.5	*n.s.*
Width (mm)	17.1	12.9	*P = 0.026*	16.0	*n.s.*
Distal oesophagus					
Length (mm)	23.1	14.3	*P = 0.010*	23.9	*n.s.*
Width (mm)	2.2	1.5	*n.s.*	1.3	*P = 0.037.*
Proventriculus					
Length (mm)	24.0	19.1	*P = 0.035*	27.2	*n.s.*
Width (mm)	5.2	4.7	*n.s.*	5.0	*n.s.*
Gizzard					
Length (mm)	13.8	12.4	*n.s.*	8.3	*P < 0.0001*
Width (mm)	8.5	8.7	*n.s.*	6.0	*P = 0.044*
Duodenum					
Length (mm)	73.4	64.7	*n.s.*	69.0	*n.s.*
Width (mm)	4.6	2.8	*P = 0.0002*	3.5	*P = 0.021*
Intestine*					
Length (mm)	329.5	359.2	*n.s.*	291.8	*n.s.*
Width (mm)	2.2	1.3	*P = 0.014*	1.7	*n.s.*
Cloaca					
Length (mm)	13.5	11.5	*n.s.*		
Width (mm)	6.1	4.0	*P = 0.039*		

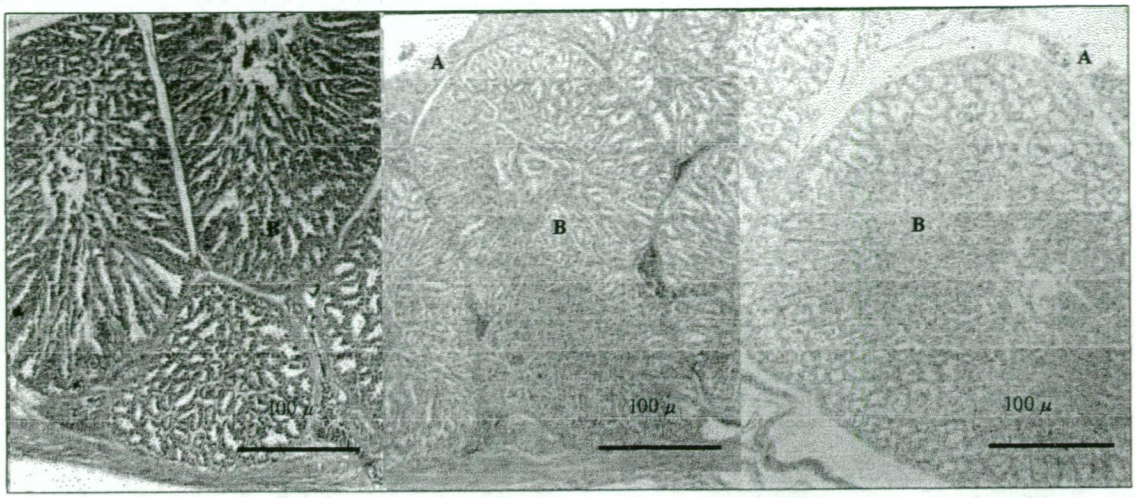

Figure 1. Histomicrographs of Swift Parrot (left), Musk Lorikeet (middle) and Green Rosella (right) proventricular lamina propria showing differences in glandular structure. A = Proventricular mucosa, B = Compound glands of the lamina propria of the proventriculus.

Discussion

Some features of the alimentary tract (as they relate to the scaled values) are an indicator of the Swift Parrots' adaptation to nectarivory in comparison to the Green Rosella and the Musk Lorikeet. These features will be discussed in their functional order.

Oesophagus

The oesophagus of birds is solely a food conduit (Ziswiler & Farner 1972; Duke 1986). It is difficult to assign a functional significance to the shorter distal oesophagus of the Green Rosella but it may simply relate to relative neck length. Similarly, the narrow oesophagus of the Musk Lorikeet may not have any functional significance.

Crop

In most parrots, the crop serves as a reservoir for food prior to digestion, allowing the birds to harvest food at a faster rate than it can be digested (Ziswiler &

Farner 1972; Duke 1986). In the Swift Parrot, the crop is wider than in the Green Rosella; this may be an adaptation to allow the Swift Parrot to collect large volumes of nectar. Field observations show that many birds will harvest nectar and pollen until the crop is visibly distended; non-breeding birds will spend time preening while digestion takes place. Breeding males need to ferry large volumes of nectar to nest hollows during the incubating period to feed their mates. Preliminary observations of captive birds indicate that incubating females are fed every twenty minutes when food is readily available (Chapter 3).

Proventriculus

The proventriculus is the glandular stomach of birds and the compound glands of the lamina propria are responsible for the production of hydrochloric acid and pepsin (Ziswiler & Farner 1972). In the Swift Parrot, the proventriculus is longer than that of the Green Rosella and the arrangement of the glands of the lamina propria more closely resembles that of the Musk Lorikeet, although this observation is based upon only very few rosella and lorikeet specimens (Figure 1). Further studies are needed to determine if this apparent change in histological structure represents greater acid production capacity. In birds, crop-emptying time appears to be set by the time taken for acidification of stomach contents; thus, a greater acid production capacity may result in faster crop emptying (Richardson & Wooller 1990). Crop emptying seems to limit the frequency of feeding bouts in hummingbirds (Diamond et al. 1986) and a similar mechanism may be possible in the Swift Parrot and lorikeets. Further, a greater degree of acidification may also aid in the digestion of pollen by opening pollen germination pores; experimental studies are needed to test this hypothesis.

Gizzard

The gizzard or ventriculus is considered to be the site of mechanical digestion and its size has been related to the presence of hard dietary items, such as seeds (Joseph 1986) and hard bodied insects (Richardson & Wooller 1986). The gizzard dimensions of

the Swift Parrot were not significantly different to those of the Green Rosella, supporting Güntert & Ziswiler (1972) but were larger than those of the Musk Lorikeet. If we compare these results to the study on Australian lorikeets by Richardson & Wooller (1990), we find similar trends (Table 2). The Western Rosellas *Platycercus icterotis* have similar gizzard dimensions but shorter intestines than the Swift Parrots; a similar pattern to the Green Rosellas. The Purple-Crowned Lorikeet *Glossopsitta porphyrocephala* and the Rainbow Lorikeet *Trichoglossus haematodus* are similar to the Musk Lorikeet in having a smaller gizzard, when scaled for size, than the Swift Parrot, but a relatively shorter intestine. The retention of the larger gizzard may allow the Swift Parrot to opportunistically use a variety of harder foods such as seeds and grasses; such foods have been observed in the guts of specimens collected on the mainland of Australia (Hindwood & Sharland 1964).

Table 2. Comparison of the gross alimentary measurements of this study with the published data of Richardson and Wooller (1990). All measurements are means. * = data from the present study. Intestine length includes duodenum.

Species	N	Body weight (g)	Gizzard length (mm)	Gizzard width (mm)	Intestine length (mm)
Swift Parrot*	25	63	13.8	8.5	402.4
Platycercinae					
Western Rosella	2	74	12.5	12.5	350
Green Rosella*	4	148	19.3	13.5	661.3
Trichoglossinae					
Purple-Crowned Lorikeet	6	48	7.4	5.9	245.2
Rainbow Lorikeet	3	151	11.8	9.8	248
Musk Lorikeet*	4	56	8.6	6.3	375.3

Duodenum and intestine

The Swift Parrot duodenum was significantly wider than that of the Green Rosella and Musk Lorikeet, suggesting that this adaptation may not be a product of

nectarivory but may allow greater digestion of insects and pollen. The Swift Parrot also retains the relatively long intestine of most granivorous parrots. The length of the intestine is necessary for the digestion of complex carbohydrates and fats from a seed or insect based diet (Ziswiler & Farner 1972; Duke 1986; Richardson & Wooller 1990; Del Rio & Karasov 1990). We speculate that this allows the Swift Parrot to use a wider range of food sources than previously expected outside the breeding season, possibly as an adaptation to the Swift Parrots' migratory habits. This may account for the species' ability to use fruit and seeds opportunistically, and to survive in captivity on a seed diet.

Alimentary contents

The Swift Parrot feeds mainly on eucalypt nectar and pollen in both the breeding and non-breeding seasons (Brown 1989; Brereton 1996a; Kennedy 1998). From our specimens, collected during the breeding season, there were no instances of seed or fruit usage. Lerp and psyllids were found in a single bird. Insect remains were present in a large proportion of the guts examined suggesting that insects may be an important source of protein during the breeding season. The presence of eucalypt pollen grains in high concentrations was also a frequent feature, suggesting direct pollen harvesting. Field observations of the Swift Parrot suggest they use the tip of their muscular tongue to compress the anthers of *Eucalyptus globulus* flowers against their hard palate and, therefore, are probably harvesting pollen. This is a different feeding technique to that used by the birds when harvesting nectar: here the tongue is extended into the floral cup, while the maxillary beak is resting on the rim of the floral receptacle. This is a similar feeding action to that which has been recorded in Purple-Crowned Lorikeets (Hopper & Burbidge 1979).

Conclusions

Our investigation of the alimentary features of the Swift Parrot support the assertion that the species has retained some features from its granivorous ancestors: specifically the muscular gizzard and the long intestine (Güntert & Ziswiler 1972).

However, our findings suggest that the Swift Parrot may have adapted to nectarivory, not only with the development of a brush tongue, but also in changes to the crop, proventriculus and duodenum.

If the assumptions about the flexibility of the avian alimentary tract in response to dietary changes are correct, then it could be reasonably expected that the ancestors of the Swift Parrot would have evolved alimentary features similar to those of the Loriinae as they adapted to nectarivory. However the results of this and previous studies (Güntert & Ziswiler 1972) tend not to support this hypothesis. If we assume that the Swift Parrot is primarily nectarivorous, then the larger, more muscular gizzard and longer intestine may allow the Swift Parrot to use a diversity of dietary items when nectar and pollen are not readily available.

Chapter 6.

Eucalyptus Pollen Grain Emptying by two Australian Nectarivorous Psittacines

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Abstract: The relative importance of pollen as a source of protein to vertebrates is controversial. In nectarivorous psittacine birds, field studies support its importance, but an experimental study in a nectarivorous parrot showed that less than 7% of pollen grains were emptied. We investigated pollen grain emptying by two nectarivorous Australian parrots, the Swift Parrot *Lathamus discolor* and the Musk Lorikeet *Glossopsitta concinna*. We used a controlled experiment, and examined pollen located at different levels through the alimentary tract of wild *L. discolor*. There was significant emptying of pollen grains ($\bar{x}$ = 45.4 % ± 1.91 s.e.) by all birds in the experimental trials. There was also a progressive increase in the percentage of pollen grains emptied at different sites along the alimentary tract in wild birds (crop $\bar{x}$ = 24.2 % ± 4.44 s.e., proventriculus $\bar{x}$ = 34.0 % ± 7.29 s.e., duodenum $\bar{x}$ = 54.3 % ± 5.42 s.e. and distal intestine $\bar{x}$ = 64.2 % ± 4.68 s.e.). The percentage of pollen grains emptied by captive *L. discolor* in the experimental trial ($\bar{x}$ = 44.1 % ± 2.77 s.e.) was not significantly different from that found in wild *L. discolor* ($\bar{x}$ = 40.3 % ± 4.25 s.e.) Both species of nectarivorous parrot were able to rapidly ingest large quantities of *Eucalyptus* pollen and appeared to empty the pollen grains efficiently. *Eucalyptus* pollen appears to be an important source of protein for these birds.

Pollen is both the vehicle and conductor of male plant gametes, and a floral attractant used to reward pollinators (Vogel 1983). Pollen represents a significant source of protein; the interior protein of the pollen grain, known as the protoplast, has a diverse amino acid profile (van Tets and Hulbert 1999). The protoplast, however, is surrounded by an indigestible cell wall, known as the exine shell (Stanley and Linskens 1974). Bees use pollen as a food source for the queen and for nurse bees that produce royal and worker jelly (Vogel 1983). In Australian mammals, a number of field studies of faecal contents have identified evidence of pollen grain emptying in species from a range of marsupial families (Turner 1982; 1984a;b; van Tets and Whelan 1997). Experimental studies of *Banksia* pollen grain emptying in two Australian nectarivorous marsupials, the eastern pygmy possum *Cercartetus nanus* and the honey possum *Tarsipes rostratus*, confirmed the ability of the animals to empty pollen grains (Turner 1984a). These studies suggested they would be able to satisfy their protein requirements with a low number of *Banksia* inflorescences if pollen grain emptying represents digestion of the protoplast (Turner 1984a). *C. nanus* maintained a positive nitrogen balance on a *Eucalyptus* pollen diet (van Tets and Hulbert 1999) and, in comparison to its requirements when on a mealworm diet, its dietary maintenance nitrogen requirements were exceptionally low. This provides further evidence for the high quality of pollen protein and suggests that pollen grain emptying may accurately reflect protoplast digestion (van Tets and Hulbert 1999).

The Australian avifauna includes two groups of birds that are specialised nectar feeders; the passerine honeyeaters of the Family Meliphagidae (Ford and Paton 1985) and the lorikeets and the Swift Parrot *Lathamus discolor* of the Family Psitticidae (Christidis and Boles 1994). The aim of the present study was to investigate the emptying of pollen grains by two nectarivorous Australian parrots: the Swift Parrot and the Musk Lorikeet, *Glossopsitta concinna.* The Swift Parrot is an endangered species that is believed to have evolved from granivorous ancestors in the southeastern eucalypt forests of Australia, filling a niche made available by the absence of lorikeets (Christidis

et al. 1991). The Musk Lorikeet is a more distantly related trichoglossid nectarivore that is thought to have arrived in these forests relatively recently, spreading south from New Guinea (Christidis et al. 1991). Trichoglossid lorikeets are considered to be highly specialised nectarivores (Güntert and Ziswiler 1972). We investigated the ability of these species to empty pollen grains using a controlled experiment, and by examination of pollen located at different levels along the alimentary tract.

Although most parrots are specialised granivores, the nectarivorous psittacines have morphological adaptations that aid in the harvesting and digestion of nectar (Güntert and Ziswiler 1972; Gartrell et al. 2000a; Chapter 5). In particular, all species have a specialised brush tip to their tongue, which is thought to facilitate nectar uptake by capillary action (Churchill and Christensen 1970). The brush tongue has also been implicated in the harvesting of pollen (Hopper and Burbidge 1979, Gartrell et al. 2000a; Chapter 5). Such anatomical adaptations suggest that these birds are specialised for feeding on pollen and nectar.

Nectar is extremely low in amino acids (Lüttge 1976), but the maintenance protein requirement, on a mg kg^{-1} basis, of nectarivores is not remarkably low compared to that of granivorous birds (Klasing 1998). Therefore, nectarivores must obtain their protein from other sources and both insects and pollen have been suggested as the main sources of protein for most nectarivores (Ford and Paton 1985; Klasing 1998). However, pollen's relative importance as a source of protein to nectarivorous psittacine birds is controversial. Evidence supporting its importance has come from field studies recording pollen harvesting and digestion in Rainbow Lorikeets *Trichoglossus haematodus* (Wooller et al. 1988), Purple-crowned Lorikeets *Glossopsitta porphyrocephala* (Churchill and Christensen 1970; Wooller et al. 1988) and Swift Parrots (Gartrell et al. 2000a; Chapter 5). In contrast with the field evidence, an experimental study of *Eucalyptus* pollen grain emptying in Rainbow Lorikeets showed less than 7% pollen grains emptied in adults and 24% in nestling birds (Brice et al. 1989). This study concluded that because of the low efficiency of pollen grain emptying, pollen could not furnish a significant source of energy or protein to these birds (Brice et al. 1989).

Richardson and Wooller (1990) suggested that the storage of pollen used by Brice et al. (1989) might explain the low rates of pollen emptying observed in the latter study. We used both fresh and frozen pollen in this study to determine if the storage of pollen affects emptying.

The mechanism of pollen grain emptying in birds is poorly understood. Pollen grains may be emptied through pores in the exine coat. This emptying is thought to be via direct enzymatic action or osmotic pressure but the actual mechanism is unknown. The presence of an empty exine shell is often considered evidence of digestion (Brice et al. 1989; Herrera and del Rio 1998). A difficulty in establishing rates of pollen grain emptying from pollen grain morphology in field studies is the variability in the numbers of empty pollen grains that occur naturally in *Eucalyptus* flowers (Wooller et al. 1988). To assess the extent of pollen grain emptying accurately, a measure of the number of empty pollens in flowers prior to ingestion must be made.

Our specific aims were to determine if: (1) there is significant emptying of *Eucalyptus* pollen grains by these birds; (2) there are differences in the percentages of pollen grains emptied between adult and juvenile Swift Parrots. Juvenile birds were included because of the increased rates of pollen grain emptying seen previously in nestling rainbow lorikeets and hummingbirds (Brice et al. 1989); (3) there are differences in the proportion of pollen digested between fresh and frozen pollen.

Materials and methods

Experimental trials

Two species of birds were used: adult Swift Parrots (n = 4) and Musk Lorikeets (n = 4). A third group made up of juvenile Swift Parrots (n = 4), less than twelve months old, was included to determine if the increased pollen grain emptying seen in fledglings (Brice et al. 1989) carried through into the first year of life. All birds were acquired either from registered aviculturists or as injured wild birds (under Tasmanian Parks and Wildlife Service permit FA 99109 and University of Tasmania animal ethics permit 98060). All birds had been in captivity for longer than six months and were being

maintained on artificial nectar and either an extruded pellet (Swift Parrots; Roudybush® maintenance parrot diet) or dry lorikeet powder (Musk Lorikeets; Avione® Lorikeets rearing and conditioning food) diet throughout the experimental period. The manufacturers of the diets reported that no maintenance dietary items contained any pollen and examination of the birds' faeces prior to the experiments failed to detect pollen grains.

A pilot study examined faeces from four Swift Parrots and four Musk Lorikeets at 30-min intervals following presentation of flowers. The first period in which pollen grains were present in faeces was four hours after presentation in both species. However, average gut-passage time for pollen grains could not be calculated as birds were not given a bolus of pollen (Herrera and del Rio 1998).

Four treatments were used: fresh or frozen *Eucalyptus globulus* flowers; fresh or frozen *E. ovata* flowers. Flowers were picked by hand from eucalypt trees in the Hobart region. Fresh flowers were collected on each day of treatment. Frozen flowers were stored in plastic bags for one to four weeks at -20°C and removed from storage approximately 15-min prior to each trial. Flowers were presented intact and offered *ad libitum* to each bird in an individual cage for four hours. No other food was available to the birds during this period.

Each bird was exposed to all treatments using a cross-over design. The sequence of treatments was assigned using a digram balanced Latin square with an extra period, which enabled analysis of the data for carryover, sequence and period effects with full efficiency (Ratkowsky et al. 1993). Each bird had at least a 48h rest period between treatments during which it was fed its maintenance diet. A small quantity of faeces smeared onto a glass slide and examined by light microscopy prior to each trial confirmed that no residual pollen grains from previous treatments remained in the bird's gut.

Quantities of pollen grains from flowers from each treatment group were obtained prior to every trial and examined by light microscopy. Fresh faeces were collected at four hours after presentation of the flowers and smeared onto microscope

slides. Slides were immediately stained with a Modified Giminez stain (Campbell 1994), which stains the empty exine shells light green and the full and partially digested pollen grains dark purple. These slides were examined under light microscopy at 100 x magnification and one operator (BG) counted empty and full pollen grains. Grains that contained approximately half or more of their protoplasm were considered full and those with less than half their contents were considered empty. The number of pollen grains counted was either all pollen grains present on the slide or 1000. This technically simple method of estimation of the percentage of pollen grains emptied has been shown to provide a conservative estimate of the ability of flower feeding animals to extract nitrogen from pollen (van Tets and Hulbert 1999).

Alimentary tract surveys

Eleven Swift Parrots that had been killed in collisions with windows or motor vehicles were collected over 1997-1999 by the Tasmanian Parks and Wildlife Service and then forwarded to the University of Tasmania under Tasmania DPIWE permit numbers FA 98107, FA 98108, FA 98122. Carcasses were either refrigerated or frozen when collected. Refrigerated specimens were dissected within 24h of collection. Frozen specimens were stored at –20°C and dissected later. Samples were taken from the alimentary tract at the crop, proventriculus, duodenum, and distal intestine. A thin smear of these samples was made onto microscope slides. Smears were stained and the number of full and empty pollen grains was counted using the protocol above. We used the percentage of empty pollen grains in the crop as an estimate of the percentage of empty pollen grains ingested. The percentages of empty pollen grains in the proventriculus, duodenum, and lower intestine were adjusted to give an estimate of pollen grains emptied in each of these alimentary organs by subtracting the crop value. Estimates of pollen grain emptying in wild birds were compared to the results of the experimental trials.

Statistical analysis

For the experimental trials, a factorial analysis of variance (ANOVA) using SAS (Version 6.12, SAS Institute Inc., Cary, NC) was employed to analyse for treatment, interaction and carryover effects (Ratkowsky et al. 1993). A two-tailed t-test was used to compare the means of the pooled data between empty pollen grains on pre-trial flowers and in faeces, using Systat® (Version 7.0, SPSS Inc). All values are given as means ± one standard error.

A univariate repeated measures ANOVA was used to evaluate the effect of level of alimentary tract on pollen grain emptying using Systat®. This analysis was used because different regions of the gut within several individuals were examined. This test was carried out on both the raw data and the data adjusted by using crop values to estimate the percentage of empty pollen grains ingested.

The percentage of empty pollen grains in the *Eucalyptus* flowers in the experimental trials was compared to the percentage of empty pollen grains in the crop of wild Swift Parrots by a two-tailed t-test using Systat®.

The percentage of pollen grains emptied by Swift Parrots in the experimental trials was compared to the percentage of pollen grains emptied in the distal intestine of the wild Swift Parrots by a two-tailed t-test using Systat®.

Results

Experimental trials

Both Swift Parrots and Musk Lorikeets were able to ingest large amounts of pollen from the flowers in the short period of the experimental trial. All used the tip of their muscular tongue to compress the anthers of the flowers against their hard palate, in a similar manner to that used by Purple-crowned Lorikeets (Hopper and Burbidge 1979).

There was significant pollen grain emptying (45.4% ± 1.91) by all birds that ingested pollen in the experimental trials ($t = -21.06$, $df = 58$, $P < 0.001$). There were no detectable effects of carryover, sequence, or interaction when the results were analysed by factorial ANOVA (Table 1).

There was no significant difference in the percentage of empty pollen grains from the pre-trial flowers among treatment groups (Figures 1 and 2) and there was no significant effect of treatment group (ie. neither species of *Eucalyptus* nor fresh or frozen flowers) on pollen grain emptying (Figures 1 and 2). Furthermore, both species emptied pollen grains with the same efficiency (Table 1 and Figure 1).

Table 1. Analysis of variance (ANOVA) values for (1) the experimental trial of pollen digestion in Swift Parrots and Musk Lorikeets and (2) repeated measures ANOVA results for the survey of percentage of empty pollens in different locations in the alimentary tracts of wild Swift Parrots.

Variable	df	F	P
1. Experimental trials			
Carryover	3,37	1.37	0.27
Sequence	4,37	0.23	0.92
Interaction	6,48	1.03	0.42
Percentage of empty pollens in pre-trial treatment group	3,55	0.56	0.64
Pollen digestion by:			
1. Treatment groups	3,48	2.17	0.10
2. Bird groups	2,48	0.68	0.51
2. Alimentary tract survey			
*Empty pollen grains	3,24	32.49	0.000
*Pollen grains emptied	2,18	26.82	0.000

* = significant difference ($P<0.01$)

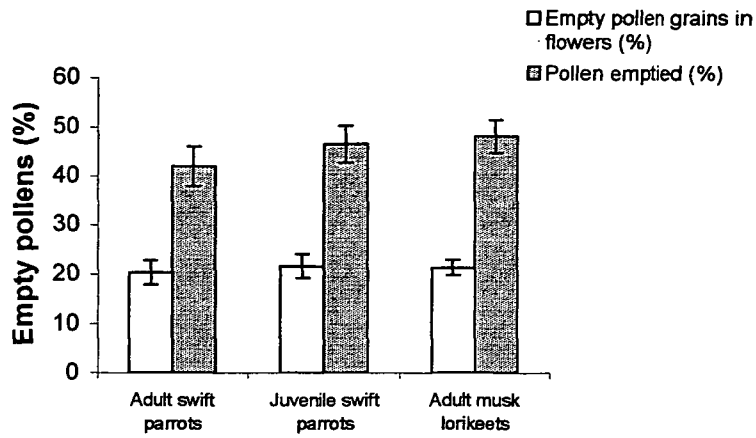

Figure 1. Percentage of empty *Eucalyptus* pollen grains in flowers ingested and percentage of pollen grains emptied by adult (n = 20 trials) and juvenile Swift Parrots (n = 19 trials) and adult Musk Lorikeets (n = 20 trials). Percentage of pollen grains emptied by the birds is calculated by subtracting the percentage of empty pollen grains in flowers from the percentage of empty pollen grains in faeces after four hours. Error bars represent one standard error for the total percentage of empty pollen grains.

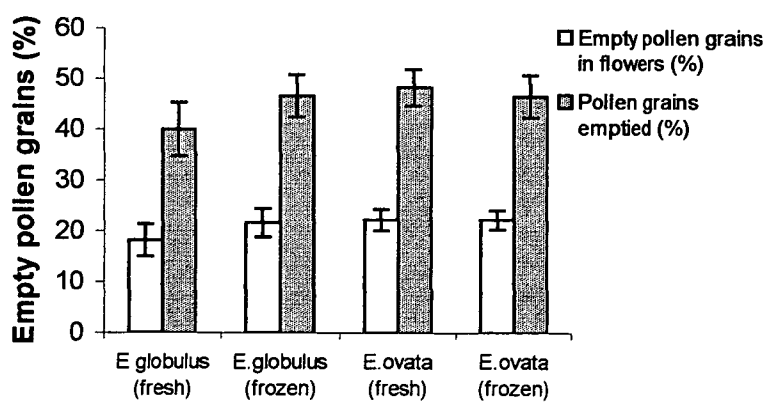

Figure 2. Percentage of empty pollen ingested by birds in flowers and pollen emptied by Swift Parrots and Musk Lorikeets, of *E. globulus* and *E. ovata*, fresh and frozen (n = 20 trials). Error bars represent one standard error for the total percentage of empty pollen grains.

Alimentary tract surveys

Only *Eucalyptus* pollen grains were found in large numbers in the wild-killed Swift Parrots. Small numbers of *Banksia* pollen grains were seen but not counted. There were significant differences in the percentage of empty pollen grains present at different sites in the alimentary tract (Table 1 and Figure 3). The percentage of empty pollen grains in the crop was not significantly different from that in the proventriculus but was significantly smaller than the percentage of empty pollen grains in the duodenum and the distal intestine (Table 1 and Figure 3). The percentage of empty pollen grains in the proventriculus was significantly different only from that in the distal intestine. The percentage of empty pollen grains in the duodenum was not significantly different from that in the distal intestine.

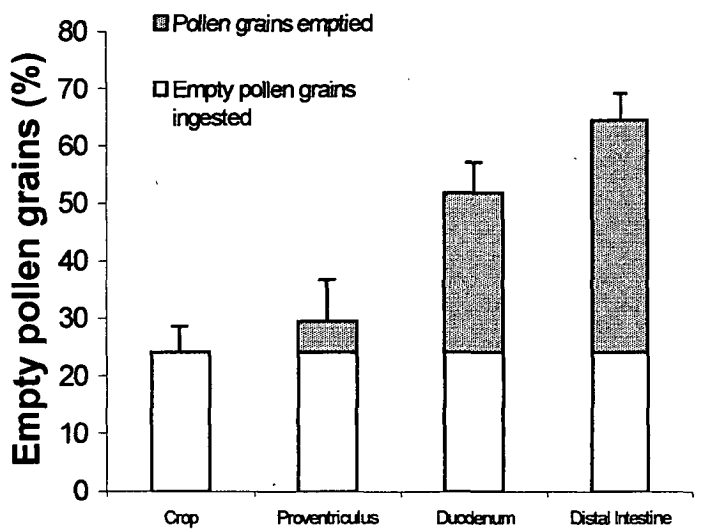

Figure 3. Percentage of empty pollen grains in the alimentary tract of wild-killed Swift Parrots (n = 11), and pollen grains emptied by the birds, calculated by subtracting the percentage of empty pollen grains in the crop as an estimate of empty pollen grains ingested. Error bars represent one standard error for the total percentage of empty pollen grains.

The percentage of empty pollen grains in the crop of wild-killed birds (24.2 % ± 4.44) was not significantly different from the percentage of empty pollen grains in the experimental trial flowers (21.1 % ± 1.26) ($t = 0.640$, $df = 9$, $P = 0.538$). This indicates that the crop percentage of empty pollen grains is typical of those ingested recently. When the alimentary tract data were then adjusted by subtracting the percentage of empty pollen grains in the crop from the values from other regions, there was a significant difference in the percentage of pollen grains emptied at different regions in the alimentary tracts (Table 1 and Figure 3). There appears to be progressive emptying of pollen grains as ingested material moves through the alimentary tract.

The percentage of pollen grains emptied by live adult Swift Parrots in experimental trials (41.9 % ± 4.03) was not significantly different ($t = -0.407$, $df = 8$, $P = 0.695$) from that found in the distal intestine of the wild killed Swift Parrots (40.3 % ± 3.50).

Discussion

The percentage of empty pollen grains found in the pre-trial flowers collected for the experimental trial was similar to that seen in field studies (Wooller et al. 1988), that is, a significant proportion (21.1 % ± 1.26) of the pollen grains ingested by the birds were already empty. Thus, the percentage of empty pollen grains in ingested flowers must be estimated if the pollen grains found in faecal or gut contents are to be interpreted. In our experimental trials, we were able to accurately measure this variable in each sample of flowers; however, studies of wild birds must necessarily contain a degree of inaccuracy because birds may feed on a large number of flowers in a short period and flowers have a variable proportion of empty pollen grains. It is possible to collect a large number of pollen grains from the plumage of captured nectarivorous birds; however, it has been previously shown that pollen grains carried on the feathers are not a reliable indicator of pollen recently ingested as it may represent flowers previously visited over several days (Wooller et al. 1988). We had access to dead specimens, and so were able to use crop pollen grains to estimate pre-digestion

percentages of empty pollen grains when assessing pollen grain emptying in wild Swift Parrots. Our method is based on the assumptions that pollen grain emptying does not begin until the proventriculus and that the pollen grains in the crop are typical of those that were ingested recently. This assertion is strengthened by the finding that the percentage of empty pollen grains in the crop of wild Swift Parrots did not differ significantly from the percentage of empty pollen grains in *Eucalyptus* flowers in the experimental trials.

All three groups of birds were able to empty a significant fraction of the pollen grains ingested. It has been suggested that mechanical abrasion of the germination pores may be needed to allow digestion (Richardson et al. 1986). In birds, the main site of mechanical abrasion is the gizzard. There are significant morphological differences in the structure of the gizzard between Swift Parrots and Musk Lorikeets. Swift parrots have a muscular gizzard while Musk Lorikeets possess a vestigial gizzard that contains no grit (Gartrell et al. 2000a; Chapter 5). If mechanical digestion of pollen grains is important, it might have been expected that Swift Parrots would show a higher percentage of pollen grain emptying, especially between the proventriculus and the duodenum. Our results do not support this hypothesis. Exposure to the acid contents of the proventriculus may lead to protoplast ejection. If *Banksia* pollen is exposed to lactic acid *in vitro,* some protoplast ejection will occur (Stanley and Linskens 1974; Turner 1984a). Other suggested mechanisms include bursting of the pollen grain due to osmotic gradients, or germination of the pollen grain within the alimentary tract (Stanley and Linskens 1974; Turner 1984a); however, cracked or germinated pollen grains were not noted in this study.

Further studies are required to determine the mechanism of pollen grain emptying. Given that a small percentage of the pollen grains were emptied in the proventriculus, it is possible that the acidification of pollen grains is important for the extrusion of their contents. However, the majority of pollen grain emptying in these birds occurred in the duodenum and intestine.

The juvenile birds were included in the study because nestling birds have been previously found to show a greater emptying of pollen grains than adults (Brice et al 1989). If such a difference occurs in the nestlings of the Swift Parrot, it would appear that it is not retained in the first six months after fledging (Table 1 and Figure 3).

The lack of significant difference in the digestion of the two different *Eucalyptus* species' pollen was not surprising given the morphologically identical features of their pollen grains. Both species of eucalypt should be able to supply protein to nectarivorous psittacines foraging from them. Interestingly, breeding in the Swift Parrot has been correlated with the flowering of *E. globulus*; in years when these trees have poor flowering, Swift Parrots rely on *E. ovata* but do not breed well (Brereton 1996a).

Richardson and Wooller (1990) have suggested that the storage of pollen used by Brice et al. (1989) might explain the lower rates of pollen emptying observed in that study than has been seen in field studies. However, in the experimental trial, both fresh and frozen pollen grains were emptied with equal efficiency. Intuitively, it might be expected that freezing would actually increase the digestibility of pollen grains as internal expansion of the protoplasm might open the germination pores. The reasons for the efficiency of *Eucalyptus* pollen grain emptying in Rainbow Lorikeets (Brice et al. 1989) remain unclear.

It is possible that the Rainbow Lorikeets are different in some way from the two species examined here, but the Musk Lorikeet is closely related to the Rainbow Lorikeet (Christidis et al. 1991) and has similar feeding habits. It is also possible that the pollen from *Eucalyptus calophylla* used in the previous study differs from the two species of *Eucalyptus* pollen examined here. The pollen grains of these *Eucalyptus* species are highly conserved morphologically (McPhail et al. 1994) and we were not able to distinguish between the two species we tested and it is likely that their physical properties, including digestibility, are likewise very similar. An intriguing speculation is that pollen, which has been collected by bees, as was used by Brice et al., may be subject to some secretions from the bees that alter the digestibility of the pollen grain.

Examination of wild birds indicates that pollen grain emptying occurs in wild Swift Parrots at comparable efficiencies to that seen in the experimental trial. This level of pollen grain emptying is similar to that seen in field studies of Purple-crowned Lorikeets (Wooller et al. 1988). This rate of pollen grain emptying is relatively low compared to that found in honey possums (95-100%). This difference has been attributed to the longer gut transit time in honey possums than in psittacine birds (Richardson et al. 1986). It is possible that the nectarivorous parrots have adopted a strategy of rapid gut transit and, therefore, a greater food ingestion rate, at the cost of digestive efficiency. However, pollen still represents an important source of protein to these birds. This use of pollen may explain the differences in foraging behaviour seen between nectarivorous passerines and psittacines. There are many reports of nectarivorous passerines "hawking" for insects as a source of protein (Recher and Abbott 1970; Paton 1982; Oliver 1998) and some evidence that their dependence on insects increases during the rearing of juveniles (Millam 1994; Oliver 1998). There is evidence from gut content studies (Higgins 1999; Gartrell et al. 2000a; Chapter 5) that nectarivorous psittacines ingest significant quantities of insects but there are no records of them "hawking" for insects. Eucalypt trees are a good source of arthropods (Recher at al. 1996) and it is likely that the parrots glean insects opportunistically as they forage. It seems likely that the birds use both pollen and insects to satisfy their protein requirements.

Both species of nectarivorous parrots were able to rapidly ingest large quantities of *Eucalyptus* pollen and to empty the pollen grains efficiently. It is therefore likely that *Eucalyptus* pollen is an important dietary source of protein for these birds; however, future work should include nitrogen balance studies to confirm this hypothesis.

Chapter 7. Renal disease in captive swift parrots: an indication of low protein requirements in a nectarivorous species?

This chapter will be submitted, with minor modifications, to the Journal of Avian Medicine and Surgery for publication. Consequently, there is some repetition of methods previously reported in this thesis.

INTRODUCTION

The intensive management of parrots is complicated by the numerous toxins that may be present in the captive environment and by the birds' inquisitive and destructive nature. While some toxins produce clinical signs, necropsy appearances or characteristic tissue changes, there are a vast number of toxins that produce non-specific effects, making definitive diagnosis difficult (Everist 1974). Many toxins are not readily detectable even upon close inspection of the captive environment, and even apparently benign environments can contain multiple toxins.

Most reported cases of toxicity to captive parrots involve chemical poisoning (for example zinc and lead toxicoses), toxicity from plants is reported infrequently. Some authors have proposed that parrots have a high resistance to plant toxins (Dumonceaux and Harrison 1994; Bauck and LeBonde 1997). However, another explanation for the low frequency of reported plant toxicity is that the difficulties of confirming the diagnosis of plant-associated toxicities lead to a low reporting rate. Additionally, the potential toxicity of many plants is affected by a number of factors, including the condition and stage of growth of the plant, the quantity ingested, the susceptibility of the species of bird and the condition of the individual bird (Everist 1974). Such variation often means that even controlled feeding trials of potentially toxic plants give variable results (Everist 1974).

This chapter details the investigation of an episode of neurological disease and deaths in the captive research colony of the endangered swift parrot. The syndrome manifested as a combination of neurological and renal disorders that affected birds irregularly through the course of eleven months from October 1999 to

September 2000. Likely sources of toxins included zinc galvanising, nitrate fertilisers and the nesting material that contained shavings from the Tasmanian sassafras, *Atherosperma moschatum*. However, not all affected birds were exposed to these potential toxins. The investigation evolved as more evidence became available. I describe the sequential case history with associated diagnostic tests in this chapter and present clinical signs, necropsy results, histopathology, and toxicological analyses. The difficulties in confirming a toxicological diagnosis are highlighted. The following chapter (Chapter 8) details investigations of variations in plasma uric acid concentrations in the birds. Information about this metabolite provides insights into both the pathophysiology of renal disease and the possible role of diet as a contributing factor to the deaths described.

CASE HISTORY

General husbandry and nutrition of swift parrots at the University of Tasmania

A research colony of twenty swift parrots was established at the University of Tasmania, Hobart, with a mixture of injured wild-bred, and donated captive-bred, birds. The four adjoining aviaries were constructed of painted, but otherwise untreated, pine lumber, plywood and galvanised mesh wire. Eucalypt branches were used as perches throughout the aviary. Wooden (pine) nestboxes, containing untreated pine shavings, were installed during the southern hemisphere spring and summer (September to March) but were removed at other times (Chapter 2).

During the breeding season (September to January), the swift parrots were fed once a day on experimental diets consisting of nectar and pellets. Two levels of protein and energy were used (Table 1). Nectar powder was formulated as Lori-nectar® or Lori-start® Avesproduct B.V. (66.6% dry matter [DM]); Farex Original Blended Cereal® Heinz Wattie's Ltd (16.7% DM); and sucrose (16.7% DM). Pellets were either Roudybush® maintenance or breeder pellets. These diets are respectively referred to as the "low protein" (Lori-nectar and maintenance pellets) and the "high protein" rations (Lori-start and breeder pellets). Note that the metabolisable energy (ME) values reported in Table 1 are based on standard tables of ME for various foodstuffs that were derived using domestic chickens (T. Roudybush pers. comm.).

Metabolisable energy is defined as the gross energy of the diet consumed minus the energy in the excreta of the bird consuming the diet (Klasing 1998). Metabolisable energy of foodstuffs vary depending upon the species and age of bird consuming the diet (Angel 1993). The true ME of these experimental diets in swift parrots is unknown, and gross energy values for these foods are not readily available.

Table 1. Dietary levels of metabolisable energy and protein in the daily rations fed to swift parrots during the spring and summer of 1999 based on manufacturers' labelling. Combined values assume equal consumption of nectar and pellets. See notes regarding metabolisable energy (ME) in the text. Percentage of dry matter weight indicated by % DM.

	High protein diet		Low protein diet	
	ME (kJ/g)	Protein (% DM)	ME (kJ/g)	Protein (% DM)
Nectar	14.49	13.6	14.76	11.6
Pellets	13.23	20	14.36	11.0
Combined diet	13.86	16.8	14.56	11.3

The diets were supplemented with a small amount of fresh apple (1/4 apple for 6 birds every second day). In the non-breeding period (February to August), the low protein diet was fed to all birds. The birds were fed each morning and offered an equal volume of nectar and pellets. Nectar was preferentially consumed and was usually completely exhausted by early afternoon. A majority of the low protein pelleted ration (>90%) was consumed by the next morning, but approximately half of the high protein pellets remained each morning.

Prior to the breeding season of 1999/2000, the birds had been in the aviaries for a period of eighteen months without any deaths or illness noted and a pair of captive-bred swift parrots successfully reared a single chick in 1998. All of the birds underwent regular weighing and blood sampling during this period for hormone

analyses (Chapters 2, 10). Blood samples (0.3 ml) (less than 0.5 % of bodyweight) were taken from the right jugular vein weekly during the breeding season, and at monthly intervals during the rest of the year. The mean haematocrit of six birds at the beginning of sampling was 52 % (± 1.0 % standard error). The haematocrit initially decreased to a mean of 48 % but by the fourth week of sampling it had returned to initial levels and was subsequently maintained at this level throughout the sampling period. The mean bodyweight of the twenty adult swift parrots was 70.8 g (± 0.93 standard error) with no significant difference between the sexes.

In the research colony's second year (1999/2000), nest-boxes were installed and the breeding season diets were commenced on the 31st August 1999. Fresh nesting material of untreated pine (*Pinus radiata*) was placed in each nestbox. Nestbox disturbance was noted within two weeks of installation. On the 14th September 1999, as part of a collaborative study on the pollination of the Tasmanian blue gum, dwarf blue gum trees, *Eucalyptus globulus*, planted in potting mix, were placed in two of the four swift parrot aviaries.

Clinical signs of illness, necropsy and histopathology findings

Clinical signs were first observed in October 1999. A summary of clinical signs, necropsy and histopathology findings of all affected birds is given in Table 2. The detailed case history is described to establish the sequence of events and the progression of the clinical investigation. The time-line of events is presented in Table 3. The first evidence of a problem in the swift parrots was a significant loss in body weight of a juvenile bird (Bird A), recorded on the 4th October, 1999. This weight loss continued over the next week and on the 11th October, 1999 a mild, generalised muscle tremor became evident. No other clinical signs were noted.

On the same day in a different aviary, another swift parrot (Bird B) was noted to be acutely weak and ataxic with mild, generalised muscle tremors. This adult hen showed no weight loss. Radiographs, crop wash cytology, direct faecal wet mounts and faecal Gram stains revealed no discernible abnormalities. A preliminary diagnosis of zinc toxicosis was made, largely based on the neurological signs (Dumonceaux and Harrison 1994; Bauck and LeBonde 1997). Both birds were removed from the aviaries and supportive care was commenced, consisting of

warmth, chelation therapy (Calcium EDTA - Calsenate® 0.05 ml intra-muscular twice daily), fluid therapy (lactated Ringers solution 3mls subcutaneous twice daily) and antibiotics (lincomycin/spectinomycin – Lincospectin® 0.04ml intramuscular twice daily). All exposed aviary wire was treated with diluted acetic acid and copious water rinsing to reduce the exposure of the birds to zinc from the galvanising coat on the wire. Nylon shade-cloth was installed to prevent the birds gaining direct access to the roof aviary wire in all four aviaries.

Table 2. Summary and frequency of clinical signs, necropsy and histopathology findings in clinically affected swift parrots.

Pathological change	Number of affected birds
Total clinically affected	10
Birds that died	9
Clinical signs	
Neurological dysfunction (ataxia, generalised tremors)	5
Weight loss	4
Articular gout	3
Necropsy findings	
Gross nephropathy	6
Visceral gout	6
Gonadal regression or inflammation	4
Enteritis	2
Gross cerebral congestion	1
Ventral body oedema	1
Histopathology	
Renal tubular degeneration	8
Renal tubular and ureteral crystal accumulation	7
Glomerulonephritis	2
Cerebral congestion with perivascular haemorrhages	1
Wallerian degeneration in the spinal cord	1

Table 3. Time-line of events in the swift parrot research aviaries at the School of Zoology, University of Tasmania from June 1998 to September 2000.

Date	Description of events
1 Jun 1998	Aviary flock established with twenty swift parrots
1 Sep 1998	Nestboxes placed in all aviaries Breeding season diets commenced Weekly blood sampling and weighing commenced
24 Dec 1998	Successful breeding of a single pair of birds
1 Mar 1999	Nestboxes removed from all aviaries All birds returned to maintenance diet Frequency of blood sampling reduced to monthly
31 Aug 1999	Nestboxes placed in all aviaries Breeding season diets commenced
14 Sep 1999	Dwarf blue gums placed in two aviaries
4 Oct 1999	First clinical signs noted; weight loss in first affected bird (Bird A)
11 Oct 1999	Neurological dysfunction noted in two birds (Birds B and C)
16 Oct 1999	Death of Bird A
18 Oct 1999	Weight loss and progressive neurological disease in Bird C Partial recovery of Bird C after three weeks
18 Nov 1999	Weight loss, muscle tremors and death of another bird (Bird D) Weight loss, leg lameness and muscle tremors in fifth affected bird (Bird E)
9 Dec 1999	Death of Bird C
24 Jan 2000	Sudden death of sixth affected bird (Bird F)
1 Mar 2000	Nestboxes and dwarf blue gums removed All birds returned to maintenance diet
16 Mar 2000	Death of Bird B
21 Mar 2000	Sudden death of seventh bird affected (Bird G)
1 Sep 2000	Nestboxes placed in all aviaries Breeding season diets commenced
5-7 Sep 2000	Articular gout followed by death in the eighth and ninth affected birds (Birds H and I) Sudden death of tenth affected bird (Bird J) All birds returned to maintenance diets

Bird A was found dead in its hospital cage after four days of therapy. Necropsy findings included poor pectoral muscle condition, pericardial gout, an inflamed duodenum and the kidneys were pale and swollen with urate tophi present (Figure 1).

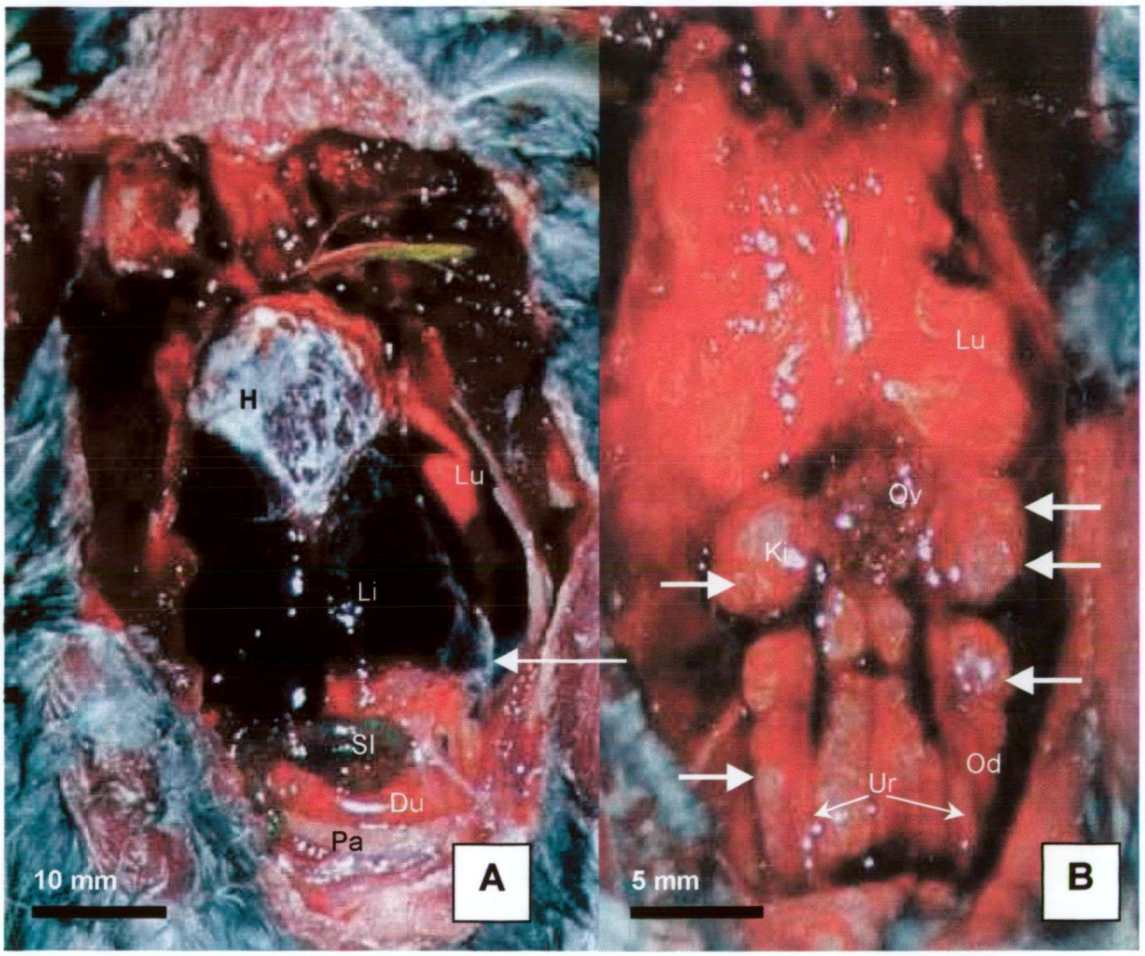

Figure 1. Necropsy changes in a swift parrot (Bird A) with renal disease. **A.** Sternum removed, body in dorsal recumbency. H = heart with pericardial precipitates of uric acid (pericardial gout). Small arrow points to uric acid precipitate on the surface of the left caudal thoracic airsac. Lu = lung; Li = liver; SI = small intestine (jejunum); Du = duodenum; Pa = pancreas. **B.** Visceral mass, heart and pericardium removed. Large arrows point to larger aggregates of uric acid precipitates within the renal parenchyma (renal gout). Ov = ovary; Ki = kidney; Od = oviduct; Ur = ureters.

The gizzard contained quartzite grit and occasional wood shavings. Histopathology revealed bacterial enteritis, ovarian congestion and haemorrhage, mild splenitis and a degenerative nephrosis of the kidneys with an accumulation of unidentified crystals in the renal tubules (Figure 2). There were no significant changes in the other organs examined, which included the brain and pancreas.

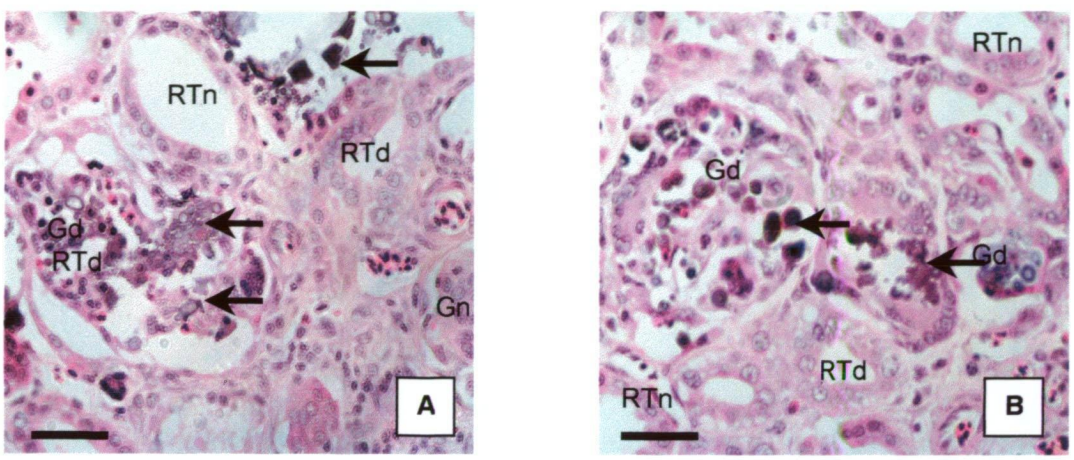

Figure 2. Histological changes in the kidneys of swift parrots with acute renal disease. **A.** Mild renal tubular nephrosis and tubular crystal accumulation present in an early acute case (Bird A). **B.** Acute glomerular crystal accumulation with minimal associated inflammation present in an early acute case (Bird A). All sections are stained with haematoxylin and eosin (H&E). Arrows point to crystal accumulations. RTn = normal renal tubule; RTd = damaged renal tubule; Gn = normal glomerulus; Gd = glomerulus with associated inflammation (glomerulonephritis). Scale bar represents 10 μm.

Bird B stabilised with the supportive treatment but the neurological signs persisted. After ten days of therapy, the chelating agent, antibiotics and fluid therapy were withdrawn. The bird remained ataxic, with minor tremors continually evident, but was otherwise stable.

On the 18th October, 1999 another adult hen (Bird C), again in a different aviary, was discovered to have lost twelve grams of body weight in one week (17 % of body weight). Clinical signs of incoordination, ataxia and mild generalised muscle

tremors developed several days later despite supportive treatment as outlined above. Radiographs, faecal wet preparations and gram-stained faecal smears revealed no abnormalities. However, after three weeks of intensive care there was a gradual improvement in body weight. Neurological signs persisted but did not worsen once the bird had been removed from the aviary.

All other birds in the captive flock were clinically normal and maintained weight. Nestbox activity continued but there was no other evidence of breeding behaviour in the swift parrots. It was hypothesised that the incident was an unusual occurrence of zinc toxicosis and no further testing was instituted.

One month later, two more swift parrots (Birds D and E) from adjoining aviaries were noted to be unwell. An adult male swift parrot (Bird D) showed an acute weight loss of eight grams in one week (~11 % of body weight); mild generalised muscle tremors were evident. A second adult male swift parrot (Bird E) acutely developed right-leg lameness and muscle tremors with no evidence of trauma. Both birds were removed from the aviaries and supportive therapy was instituted as previously outlined. Bird E recovered gradually with one week of treatment; Bird D's tremors worsened over 72 hours followed by the bird's death. Necropsy revealed congestion of the brain and pale discolouration and accumulation of urates within the renal parenchyma. Histopathology confirmed cerebral congestion with acute perivascular haemorrhages and areas of possible fibrinoid degeneration of vessels, however, there was no evidence of an inflammatory cellular reaction (Figure 3). There was also mild hepatic congestion. The renal parenchyma was congested and there were degenerative tubules, which were consistent with an acute toxicosis.

Assays of liver tissue from affected swift parrots for zinc and lead concentrations were performed. The concentration of lead in the liver was less than 0.1 μmol kg^{-1} (dry matter basis) and the concentration of liver zinc was 1.53 mmol kg^{-1} (dry matter basis), which was considered to be within normal limits for parrots (Dumonceaux and Harrison 1994; Bauck and LeBonde 1997). The preliminary diagnosis of zinc toxicosis was therefore discarded.

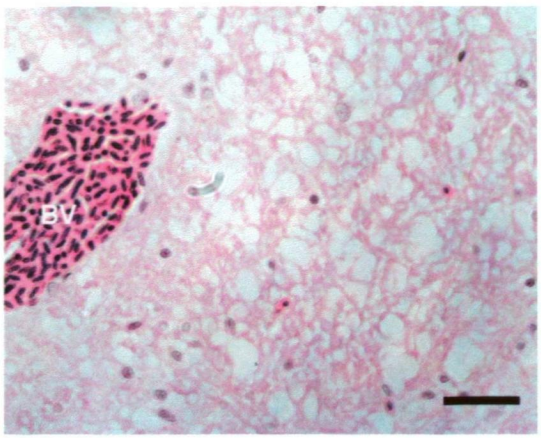

Figure 3. H&E stained section of the cerebrum of swift parrot (Bird D). 100x magnification. Note the congestion of the blood vessel (BV) without accompanying inflammation. Scale bar indicates 20 μm.

Selenium deficiency was considered as a differential diagnosis for the neurological disease (Wilson 1994), conceivably due to the incorrect formulation of one of the dietary components. The concentration of selenium in the liver was assayed, by Analytical Services Tasmania, from a composite of liver tissue from three swift parrots with neurological signs prior to death. The result was a concentration of 30.1 μmol/kg, which was considered to be in the high range of normal for poultry (L. Gabor pers. comm.) but no reference values for psittacine birds are available. Therefore, the concentrations of liver selenium were assayed from the carcases of wild parrots (swift parrots, green rosellas, *Platycercus caledonicus*, and musk lorikeets, *Glossopsitta concinna*), that had been collected for a previous study. The results are shown in Table 4. Given these results, the selenium concentration in the livers of the affected birds was not considered clinically significant.

Table 4. Selenium concentrations (dry matter basis) in the hepatic tissue of captive swift parrots and wild psittacine birds from Tasmania, Australia. All samples are composites of liver from three adult individuals. Wild birds were killed by window or motor vehicle collision.

Species	**Selenium** (μmol/kg)
Captive swift parrots, *Lathamus discolor*	30.1
Wild swift parrots, *L. discolor*	21.0
Wild green rosellas, *Platycercus caledonicus*	16.5
Wild musk lorikeets, *Glossopsitta concinna*	27.0

One of the female swift parrots with residual neurological signs (Bird C) was found dead in the aviaries on the 9th December 1999, six weeks after the onset of clinical signs. Its body weight at necropsy was 46.9 g (the previous week the weight had been 62 g). The bird had severe loss of the pectoral muscle mass. There was no food evident in the alimentary tract, and the intestinal contents were haemorrhagic. This was assumed to be a terminal haemorrhagic diathesis (Dorrestein 1997), as multiple smears of intestinal contents examined microscopically showed no other abnormalities. The ovary was regressed. Histopathology revealed bilaterally symmetrical subacute Wallerian degeneration in the dorsal lateral funiculi of the spinal cord, extending into the brainstem. There was no associated inflammation (Figure 4). The *otis media* were assessed to investigate the possibility of paramyxovirus infection but were found to be normal. These non-specific changes were again considered to be consistent with a toxicosis.

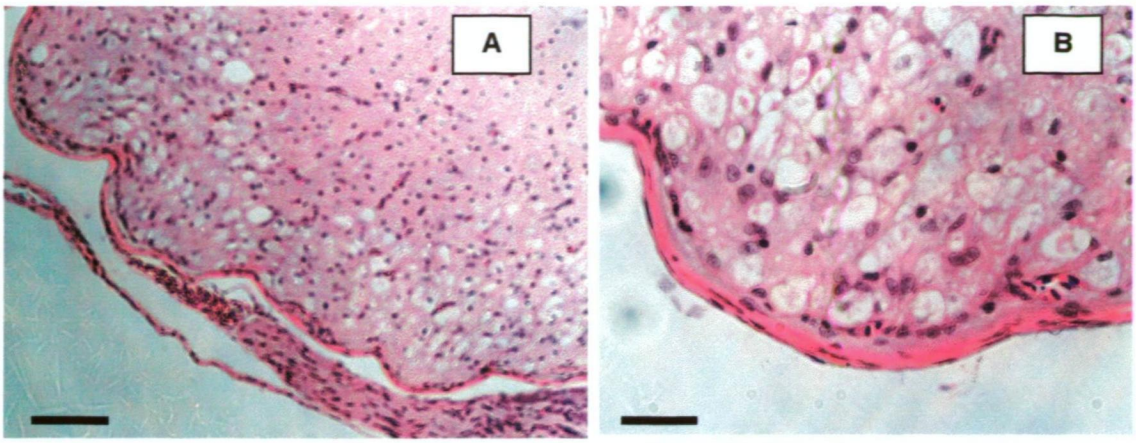

Figure 4. Haemotoxylin and eosin (H&E) stained section of dorsal lateral funiculi of the spinal cord of a swift parrot (Bird C). **A.** 100x magnification. Note the widespread nature of the myelin swelling leading to the spongy appearance. Scale bar represents 40 μm. **B.** 400x magnification. The myelin swelling shows no accompanying inflammation. Scale bar represents 10 μm.

Although the possibility of pesticide contamination of the aviaries or the diet was considered remote, an assay for organophosphates and organochlorines was carried out, by Analytical Services Tasmania, on frozen brain tissue of one of the swift parrots displaying neurological dysfunction prior to death and showing histological evidence of neuron degeneration. No detectable levels of pesticides were found (Appendix C).

On the 24th January, 2000 an adult male swift parrot (Bird F) with no previous signs of illness was found dead in the aviaries. On necropsy this bird had pericardial gout and pale kidneys with a generalised nodular change throughout the parenchyma. The testes were regressed (Figure 5) and the adrenals appeared enlarged. Histopathology revealed multiple abnormalities. In the kidneys there were: multifocal to diffuse infiltration with lymphocytes, plasma cells and heterophils; moderate to marked areas of acute necrosis of tubular epithelium associated with heterophilic casts; globular basophilic crystals which could be oxalates or uric acid; multifocal glomeruli-nephritis; and in many areas multi-nucleated giant cells surrounding the crystals. The adrenals had mild hyperplasia of the cortical cells. The

spleen exhibited: proliferation of plasma cells; aggregations of macrophages containing haemosiderin; foci of myeloid hyperplasia; no normal lymphoid follicles were present; and there were haemorrhages at the hilus. Examination of the duodenum demonstrated several foci of plasmacytic inflammation and occasional heterophils in the lamina propria. The lungs were congested and contained several foci of calcified debris surrounded by macrophages. However, the brain appeared normal. The pathology was consistent with a sub-acute to chronic nephrosis of uncertain aetiology.

Although only two of the birds affected had been in aviaries with the dwarf blue gum trees, the trees were removed and the potting mix that held the trees was investigated. Its constituents are outlined in Appendix B. The potential toxicity of the Osmocote® slow-release granules and the mineral supplement Micromax® were noted, in particular the potential for nitrate and/or nitrite poisoning.

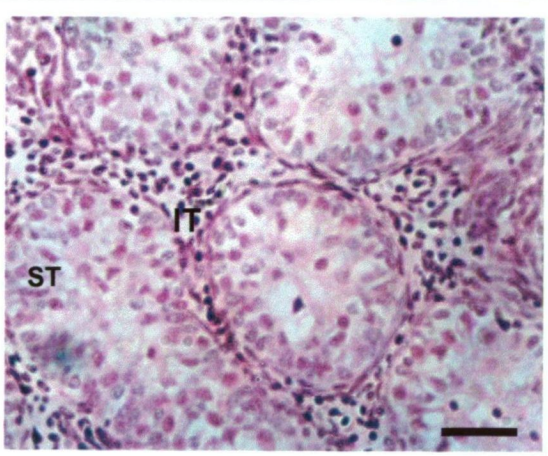

Figure 5. H&E stained section of the testes revealing early gonadal regression. 100x magnification. Note the absence of spermatogenesis in the seminiferous tubules (ST). The interstitial tissue (IT) shows a mono-cellular infiltrate. Scale bar represents 40 μm.

However, no birds were observed foraging in the potting mix, and a retrospective examination of the proventriculus and gizzard contents of all the necropsied birds failed to detect any trace of the resinous coating of the Osmocote®. It was noted, incidentally, that all these birds had grit and some had wood shavings

present in the gizzard. Eight of the birds affected had no access to the blue gum trees or the potting mix. However, upon appreciating the potential toxicity of the potting mix, the trees were removed.

The nesting material was purchased as untreated pine shavings and was used for musk lorikeets that bred successfully in an adjoining aviary and showed no signs of disease. The outbreak of disease occurred approximately one month after the introduction of the nestboxes; the swift parrots' courtship includes active nestbox inspection and the chewing of nesting material. The nesting material was analysed by a wood anatomist who reported that the wood shavings contained a mix of woods with radiata pine predominating. There were also myrtle beech *Nothofagus cunninghami*, Tasmanian sassafras *Atherosperma moschatum* and ash eucalypt *Eucalyptus regnans* (J. Ilic pers. comm.). A review of the Australian plant toxicology literature revealed the potential toxicity of the sassafras (Hurst 1942; Everist 1974). Some plants in the same family (Atherospermataceae) as the Tasmanian sassafras contain alkaloids; however, it is unknown if the Tasmanian sassafras contains these toxins. Alkaloids extracted from related trees, *Daphnandra micrantha* and *Doryphora sassafras* caused reduced responsiveness, paralysis and death when injected into frogs, cats, guinea pigs and grasshoppers (Hurst 1942). The wood shavings were removed and replaced with untreated radiata pine shavings.

On the 16th March, 2000 the swift parrot hen that had been first affected in October, 1999 (Bird B), and had remained persistently weak with mild muscle tremors, was found dead. There was evidence of a small amount of fresh haemorrhage from the vent and swelling of the tibio-tarsus and feet. On necropsy there was oedema of the ventral abdominal wall and severe nephropathy with a green discolouration of the renal parenchyma. The liver also showed a green discolouration but was of normal dimensions. There was evidence of fresh haemorrhage into the intestines. Histopathology revealed no pathological changes within the brain. In the kidney there was many cystic glomeruli, widespread renal tubular degeneration and tubular epithelial cells contained eosinophilic intracytoplasmic globules. A moderate myeloid proliferation was present as discrete foci. Many tubules contained dark globular crystals (Figure 6A).

An adult male swift parrot (Bird G) with no previous signs of illness was found dead on the 21st March, 2000. The necropsy revealed: pericardial gout; inflamed duodenal serosa; regressed testes; severe generalised renal gout and a diffuse nodular change to the renal parenchyma. Histopathology showed acute necrotising enteritis and sub-acute nephrosis. The kidney had many focal areas of tubular degeneration and tubules containing black crystals (Figure 6B). There was focal to widespread intestinal mucosal necrosis associated with abundant numbers of spore forming rods consistent with *Clostridium perfringens*. The brain appeared normal.

There were no more deaths or signs of illness over the following autumn and winter while the birds were on the low protein diet. However three birds fed the high protein ration died acutely within one week of the introduction of the breeding season diets in September 2000. Two birds (Birds H and I) had lameness and swelling with white tophi in both tarsal joints 12 hours prior to death. The third bird (Bird J) died with no evident illness. Necropsy and histopathology findings were similar for all three birds. Necropsies revealed moderate to severe visceral gout affecting the pericardium, liver and, in one case, the surface of the airsacs. Articular gout was simultaneously present in two birds. The kidneys of all three birds showed severe and generalised changes involving renomegaly, pale colouration and irregular parenchyma. Histopathology showed marked diffuse interstitial fibrosis and degeneration of the renal tubular epithelial cells. There were areas of macrophages and giant cells replacing glomerular tufts and in amongst this were many round basophilic crystals consistent with urates. No abnormalities were noted in the brain. These findings suggested that the high protein ration may be involved in the pathogenesis of the renal syndrome.

The differential diagnoses considered in the course of this investigation are summarised in Table 5.

While no diagnosis had been confirmed at the conclusion of this investigation, the most likely diagnosis was of alkaloid toxicity from Tasmanian sassafras shavings in the nesting material causing the initial neurological and renal disease. It was further possible that the high levels of protein in the diets fed to the captive birds may have exacerbated the renal damage.

The feeding trial was suspended and the remaining birds on the high protein diet were switched to the low protein ration. There have been no deaths since. Due to the high incidence of renal disease in the birds the reproductive study was abandoned for the 1999/2000 season. This made available a pool of plasma samples taken throughout this period and retrospective analysis of plasma uric acid samples was undertaken. The results of this analysis, and the further investigations it led to, are described in Chapter 8.

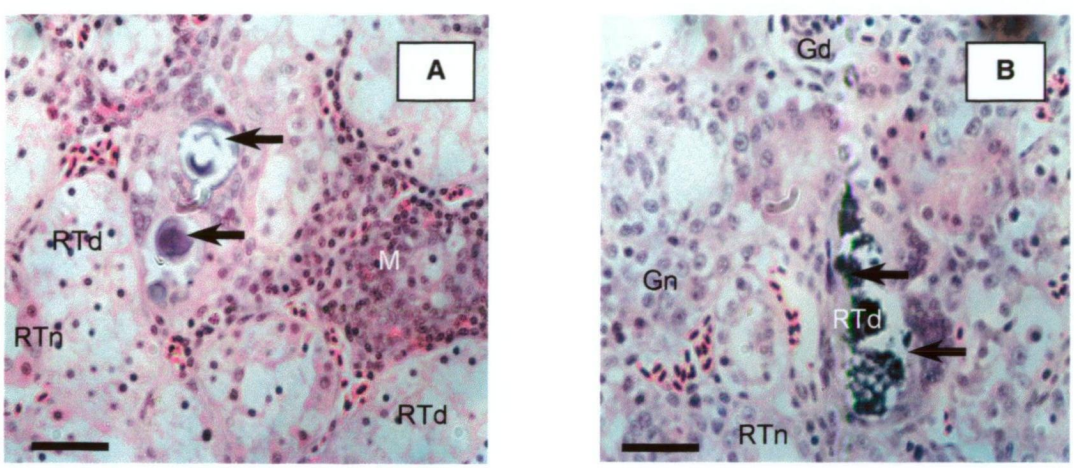

Figure 6. Histological changes in the kidneys of swift parrots with chronic renal disease. **A.** Widespread tubular degeneration, with tubular crystal accumulation resulted in destruction of the normal renal architecture in this chronically affected bird (Bird B). Myeloid proliferation (M) is present as discrete foci. **B.** Focal areas of tubular degeneration are present in this chronically affected bird (Bird G). Multinucleate giant cells containing crystalline material are present. All sections are stained with haematoxylin and eosin. Arrows point to crystal accumulations. RTn = normal renal tubule; RTd = damaged renal tubule; Gn = normal glomerulus; Gd = glomerulus with associated inflammation (glomerulonephritis). Scale bars represents 10 μm.

Table 5. The differential diagnoses considered in the course of the investigation into the deaths in captive swift parrots at the School of Zoology, University of Tasmania, Hobart. The diagnostic techniques used, the results of these investigations and an assessment of the possible role of each differential diagnosis in the disease syndrome are summarised.

Differential diagnosis	Investigative actions or responses	Result	Possible disease role
Heavy metal toxicosis (zinc or lead)	Treatment with chelating agents	No response	Nil
	Assay of liver levels of zinc and lead	Within normal limits	
Aflatoxicosis	Histopathology	No suggestive pathology	Nil
	Change batches of food	Continued deaths	
Selenium deficiency or toxicity	Assay of liver levels of selenium in swift parrots and other wild parrots	Not different from a range of free-living parrots	Nil
Pesticide contamination	Assay for pesticides in brain tissue of a parrot with neurological disorder	No pesticides detected	Nil
Nitrate/nitrite or other fertiliser based toxicity	Removal of potted trees from aviaries	Majority of affected birds in aviaries without trees. Continued deaths after removal of trees	Low possibility of causing neurological disorder and initiating renal disease in some parrots
Alkaloid toxicity	Identification of woods in nesting material	Tasmanian sassafras in nesting material	Likely (but unconfirmed) cause of neurological disorder and initiator of renal disease
	Necropsy examination of gizzards	Wood shavings present in gizzards of some birds	
Protein levels in diet	Plasma uric acid concentrations. See next chapter	Birds from both dietary groups affected. Three birds died after introduction of high protein diet	Likely contributing factor to ongoing renal damage although unlikely to be initiating cause

DISCUSSION

The initial clinical signs related to both neurological and renal dysfunction. Later, the most common pathology encountered was of renal dysfunction. This suggests that the syndrome may have been caused by an initial inciting agent, possibly alkaloid toxicity. Then the duration of the syndrome was extended by ongoing renal injury to already damaged kidneys, possibly due to the high levels of protein in the diet.

The histological changes associated with the renal tissue are non-specific and might be associated with a wide variety of nephro-toxins including, but not limited to, heavy metals, organic solvents, phenol, antibacterial agents, pesticides, and ethylene glycol (Robbins et al. 1984). Regardless of the initial cause of renal damage the overall renal pathology is that of a urate nephropathy. In humans, uric acid nephropathy is classified by histology as either acute or chronic. In acute uric acid nephropathy there is a precipitation of uric acid crystals in the tubules and collecting ducts. The crystals are amorphous and cause dilatation of the tubules and proximal obstruction (Robbins et al. 1984). This stage appears analogous to the early cases seen in the swift parrots.

In chronic uric acid nephropathy in humans, the pathology is characterised more by a chronic tubulo-interstitial nephritis due to longer periods of hyperuricaemia. There is deposition of uric acid crystals within the interstitium, tubules and collecting ducts. The crystals, in these cases, appear in the birefringent needle-like form similar to those seen in avian cases with articular gout. In human renal tissue, the urates induce a tophus surrounded by foreign body giant cells, other mononuclear cells and a fibrotic reaction (Robbins et al. 1984). This clinical picture is analogous to the swift parrots that died later in the course of this case study. This suggests that despite birds' greater ability to tolerate hyperuricaemia (Lumeij 1994b), the pathogenesis of uric acid nephropathy follows a similar pattern to that seen in human pathology.

The pathology associated with the neural tissue is also non-specific. It should be noted that while brain tissue was routinely submitted for histology, the spinal cord was only assessed in one individual, in which axonal degeneration was evident. The true incidence of spinal cord pathology within this group may be much higher than

the single case reported. The differential diagnosis for symmetrical sub-acute axonal polyneuropathies in human pathology includes: alcoholic polyneuropathy; thiamine deficiency (beriberi); arsenic; lead; alkaloid toxicosis, and "other intoxications" (Morris and Schoene 1984). For these birds there was no known access to alcohol or arsenic. Thiamine deficiency is unlikely as three of the four food sources used contain supplemental vitamins. Lead toxicosis was considered unlikely based on the results of liver tissue analysis from some of the dead birds.

Despite the intensive investigation described there is still uncertainty as to the initial cause of the neurological disease and renal damage. A number of potentially toxic substances were found in the swift parrots environment (eg. nitrate fertiliser, zinc galvanising and potentially toxic wood fragments); however not all affected birds were exposed to these substances, or diagnostic testing ruled them out of consideration.

The immediate deterioration of two swift parrots following the re-introduction of the high protein diet led to the suspicion that dietary levels of protein may be a contributing factor to the renal disease. It is not suspected that diet was the initiating cause as affected birds also came from the low protein diet group. However, if the renal tubules of the birds were damaged, as the histology suggests, this may have lead to a reduced ability to eliminate uric acid, particularly at the levels produced by the high protein diet. The result would be hyperuricaemia, continued renal damage, visceral or articular gout and death. Protocols for the clinical maintenance of birds with renal disease stress the importance of reducing the level of protein in the birds' diet to reduce the possibility of hyperuricaemia (Lumeij 1994b; Smith and Roudybush 1997). To further investigate the possible contribution of diet to the renal disease an investigation of the plasma uric acid concentrations was undertaken and this is described in the following chapter.

Chapter 8. Plasma uric acid concentrations in swift parrots: relationships with renal disease and diet

This chapter will be submitted, with minor modifications, to the Journal of Avian Medicine and Surgery for publication. Consequently, there is some repetition of methods previously reported in this thesis.

INTRODUCTION

Uric acid is the predominant end product of nitrogen (protein) metabolism in birds. Other components include ammonia, urea, creatinine, and amino acids (Skadhauge 1981; Goldstein and Skadhauge 2000). The proportion of uric acid compared to other nitrogenous components is always high but varies according to dietary state, at least in domestic fowl and the turkey vulture (Goldstein and Skadhauge 2000; Table 1). Uric acid is produced in the liver and to a lesser degree in the kidney (3-20%). Renal clearance of uric acid is primarily (>90%) via tubular secretion (Goldstein and Skadhauge 2000).

Table 1. Patterns of nitrogen excretion in avian urine (Goldstein and Skadhauge 2000). Other nitrogenous components of the urine, including creatinine, amino acids and purines, always accounted for <10% of the total nitrogen.

Species	Condition	Total Nitrogen (g/L)	Urate (% of total)	NH_4 (% of total)	Urea (% of total)
Domestic fowl (*Gallus domesticus*)	Fed	4.4	84.1	6.8	5.2
	Fasted	2.4	57.8	23.0	2.9
	Low protein	11	54.7	17.3	7.7
	High protein	13	72.1	10.8	9.7
Turkey vulture (*Cathartes aurea*)	Fed	61	87	9	4
	Fasted	13	76	17	7

The increases in post-prandial concentrations of urates in urine (Table 1) are associated with corresponding changes to plasma uric acid concentration (Goldstein and Skadhauge 2000). Post-prandial increases in plasma uric acid concentration have also been recorded in peregrine falcons, *Falco peregrinus* (Lumeij and Remple 1991), red-tailed hawks, *Buteo jamaicensis* (Lumeij and Redig 1992) and black-footed penguins, *Sphenicus demersus* (Kolmstetter and Ramsay 2000). Presumably the ability of these species to tolerate high post-prandial plasma concentrations of uric acid relates to their high protein diets. The mechanisms that prevent uric acid precipitation are unknown but elevated plasma sodium concentrations have been suggested to play a part (Lumeij 1994b). Granivorous birds have plasma uric acid concentrations that are approximately 50% lower than in carnivorous birds (Hochleithner 1994). The recommended upper normal limit of plasma uric acid concentration in cockatoos, *Cacatua spp.*, and budgerigars, *Melopsittacus undulatus*, is 500 $\mu mol.l^{-1}$(Hochleithner 1994). Plasma uric acid concentrations in nectarivorous birds have not been reported.

Diets that provide birds with dietary protein in excess of their requirements may induce elevations of plasma uric acid concentrations (Lumeij 1994b). If these elevations are greater than the kidneys' ability to clear uric acid from the plasma, then hyperuricaemia and eventually either articular or visceral gout may occur (Lumeij 1994b). Gout is defined as the precipitation of uric acid crystals from the plasma onto either joint surfaces (articular) or the surface of the internal organs (visceral) (Blood and Studdert 1990). The mechanisms that control this preferential deposition are unknown. The current hypothesis is that articular gout is a chronic process resulting from sustained hyperuricaemia and the gradual deposition of uric acid crystals in foci around the joints (Lumeij 1994b). Visceral gout is suspected to be due to a severe and rapid hyperuricaemia due to an acute obstructive uropathy (post-renal obstruction) such as the precipitation of uric acid crystals in the renal tubules, collecting ducts or the ureters (Lumeij 1994b). Other pathological causes of hyperuricaemia include catabolic states, due to the breakdown of muscle proteins, and the release of nucleic acids from cell breakdown (Hochleithner 1994). Birds with primary renal disease may also show hyperuricaemia, but only when renal function is reduced to below 30% of its original capacity (Lumeij 1994b).

In this study, I retrospectively examined the uric acid concentrations in swift parrot plasma samples collected during a period where an unidentified toxin was causing renal tubular damage (see Chapter 7). The primary aim of this investigation was to elucidate the role of diet in the pathophysiology of the renal disease, and to assess the effect of high dietary protein on renal function. Pre-prandial concentrations of plasma uric acid are the preferred samples to obtain when assessing renal function in birds (Hochleithner 1994). Unfortunately, all the plasma samples that had been collected for the reproductive biology study were post-prandial samples. Therefore, I evaluated the effects of an overnight fast and subsequent feeding on plasma uric acid concentrations in swift parrots. To further examine the effect of diet on plasma uric acid concentration I examined the plasma of wild swift parrots feeding on nectar, pollen and insects, and of swift parrots from a private aviculturist feeding on seed and commercial lorikeet nectar food.

MATERIALS AND METHODS

All blood samples were collected in heparinised syringes from the right jugular vein of the parrots. The blood was placed in plastic Eppendorf® tubes and held on ice until it could be centrifuged at 6400 rpm and the plasma removed and stored frozen at -20°C. All uric acid assays were performed on a Reflotron® Dry Chemical Analyser at the North Hobart Veterinary Hospital, Tasmania. This assay is based on the oxidation of uric acid specifically to allantoin and hydrogen peroxide. In the presence of peroxidase the hydrogen peroxide so formed oxidises the indicator producing a blue colouration. This fall in reflectance is proportional to the quantity of uric acid originally present (Merdes et al. 1985). The within-run precision values for this assay using heparinised human plasma (n = 10) ranges from 2.6 % at 120 μmol L^{-1} to 1.0 % at 790 μmol L^{-1} (Merdes et al. 1985). The lower limit of detection of the assay was <119 μmol L^{-1}. The upper limit of detection was >1190 μmol L^{-1}. If samples were found to exceed the upper limit of detection they were diluted 1:4 with lactated Ringers solution and assayed again; the results were then corrected for the dilution.

1. Retrospective analysis of plasma uric acid concentrations in captive swift parrots

The retrospective analysis compares monthly plasma samples from August 1999 to January 2000 of five swift parrots that died with histological evidence of renal tubular disease, five swift parrots that remained clinically unaffected throughout that period, and one swift parrot that was ill but recovered. I always sampled between 10 and 12 am to reduce variation from diurnal rhythms. Birds were captured from the aviary by hand-net and blood samples taken immediately; time from beginning capture to blood sampling did not exceed five minutes in any individual.

2. Comparison of pre- and post-prandial plasma concentrations of uric acid

Six swift parrots with no history of illness were used in this experiment. Four of the six had been donated to the research aviaries in June 2000 and all birds were in good health. The birds were fasted overnight and a pre-prandial blood sample was collected. The birds were then fed the low protein ration that is described in the first part of this chapter (Table 1). Post-prandial samples were collected at 2 h, at which time food was removed. A final sample was collected at 6 h after feeding. Plasma was separated and stored frozen until plasma uric acid concentration was assayed as described previously.

3. Effects of diet on plasma uric acid concentrations in swift parrots

Post-prandial plasma samples were collected from wild birds (n = 8), from captive-bred birds from a local breeder's aviary (n = 8) and from swift parrots in the research aviaries (n = 6) that were fed only the low protein ration (see Chapter 7). The wild birds were caught by mist-netting and sampled within 5 minutes of capture. All of the wild birds sampled had nectar present in their crops by palpation, and thus all samples are assumed to be post-prandial. The wild birds were feeding in Tasmanian blue gum trees, *Eucalyptus globulus,* and were assumed, based on previous gut content studies (Chapter 4, Hindwood and Sharland 1964), to have consumed a mixture of nectar and pollen plus some small insects. The average levels of energy and protein that can be digested from such a diet are unknown. If the birds

only consumed nectar they would gain ~16.7kJ/g DM of energy but less than 2% of the dry weight of nectar is protein (Lüttge 1976; Vogel 1983). The swift parrots in the local breeder's aviary were being maintained on a mixed seed and commercial nectar diet (Shep's Lorikeet Mix®). There was no history of health problems in the local breeder's aviary over the previous three years. Captive birds from both the research aviary and the breeder's aviary were sampled between the hours of 0900 to 1200 during November 2000. The wild bird samples were from November 1998 and time of sampling varied throughout the day. Plasma was separated and stored frozen until plasma uric acid concentration was assayed as described previously.

Statistics

Results are presented as means ± one standard error unless otherwise noted. All statistical analyses were carried out using Systat 7.0 for Windows®, SPSS Inc. Data from the retrospective analysis was analysed using repeated measures analysis of variance (M)ANOVA between those birds showing no clinical signs and three birds that died after January 2000. The Pillai's trace statistic was used to determine the significance of any differences because the uni-variate output of the SYSTAT programme is unsuitable due to a lack of independence of the data through time (C. Johnson, pers. comm.). Two birds that died prior to January are included in the graphical results but not in the statistical analysis due to the limitations of the (M)ANOVA when dealing with missing data points. *Post hoc* t tests were used to check for significant differences within the months assessed. (M)ANOVA and *post hoc* t tests were also used to assess the effect of diet in the clinically normal birds. The pre- and post-prandial concentrations of plasma uric acid were also assessed by (M)ANOVA. The comparison between wild birds and captive birds on differing diets was analysed using a single factor ANOVA and post-hoc pair-wise mean comparisons using the Bonferroni method.

RESULTS

1. Retrospective analysis of plasma uric acid concentrations in captive swift parrots

The monthly data for plasma uric acid samples in the swift parrots in the research aviaries are presented according to clinical outcome (Figure 1) in all the birds assessed. There is a significant difference in the monthly pattern of plasma uric acid concentration in those birds that died compared with those birds that remained clinically normal ($F_{(1,5)} = 7.358$ $P = 0.042$).

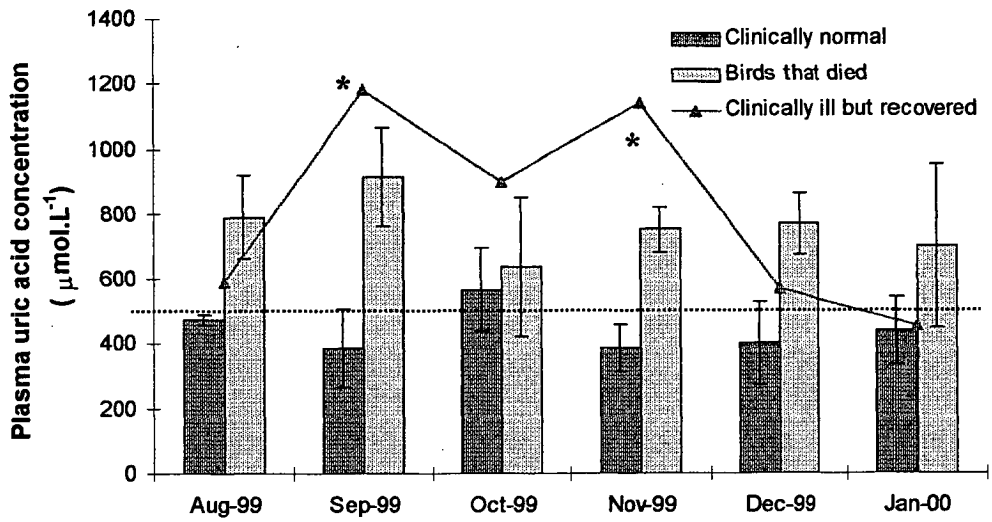

Figure 1. Plasma uric acid concentrations in swift parrots in the research aviary between August 1999 and January 2000. The samples are divided by clinical outcome: birds that died with histological evidence of severe renal tubular disease (n = 5 Aug and Sep; n = 4 Oct and Nov; n = 3 Dec and Jan); birds that were clinically normal (n = 5) and the bird that was clinically ill, with evidence of renal dysfunction, but recovered (n = 1). * indicates a significant difference ($P < 0.05$) between the birds that died and the clinically normal birds. Error bars represent one standard error. The dotted line demonstrates the upper normal limit of plasma uric acid concentration (500 µmol.l^{-1}) in cockatoos and budgerigars, *Melopsittacus undulatus* (Hochleithner 1994).

Plasma uric acid concentration is significantly higher in ill birds in September (914 ± 152 μmol.l^{-1}, $P = 0.035$), and November (750 ± 70 μmol.l^{-1}, $P = 0.040$) than in clinically normal birds (September, 387 ± 120 μmol.l^{-1}; November, 387 ± 73 μmol.l^{-1}). Differences between the two groups is not significant ($P > 0.05$) in all other months. In the bird with renal disease that survived, plasma uric acid concentrations rose to concentrations similar to those birds that died (September, 1180 μmol.l^{-1}), but recovers over a period of months to levels similar to unaffected birds (January, 453 μmol.l^{-1}).

To assess the effect of diet on plasma uric acid levels in the research birds, the group of birds that showed no clinical signs was examined with respect to diet fed during this period (Figure 2). Only birds with no clinical signs were used in order to minimise the confounding effects of renal tubular damage. It should be noted that no other objective measure of renal function (such as biopsy) was performed and that sample sizes are low. Notwithstanding these limitations, the comparison shows that the birds that were fed the high protein ration have a significantly different monthly pattern of plasma uric acid concentration than those birds that were fed the low protein ration ($F_{(1,3)} = 38.747$, $P = 0.008$). Note that at the commencement of the breeding season (August), when the birds had been fed the low protein ration over winter, the plasma uric acid concentrations are not significantly different between the two groups (High protein, 535 ± 46 μmol.l^{-1}, Low protein, 462 ± 18 μmol.l^{-1}, $P > 0.05$). However after the introduction of the ration a significant elevation ($P < 0.05$) is noticeable in the high protein group from September (961 ± 220 μmol.l^{-1}) through to November (942 ± 387 μmol.l^{-1}). The differences between the groups are not significant ($P > 0.05$) in December and January.

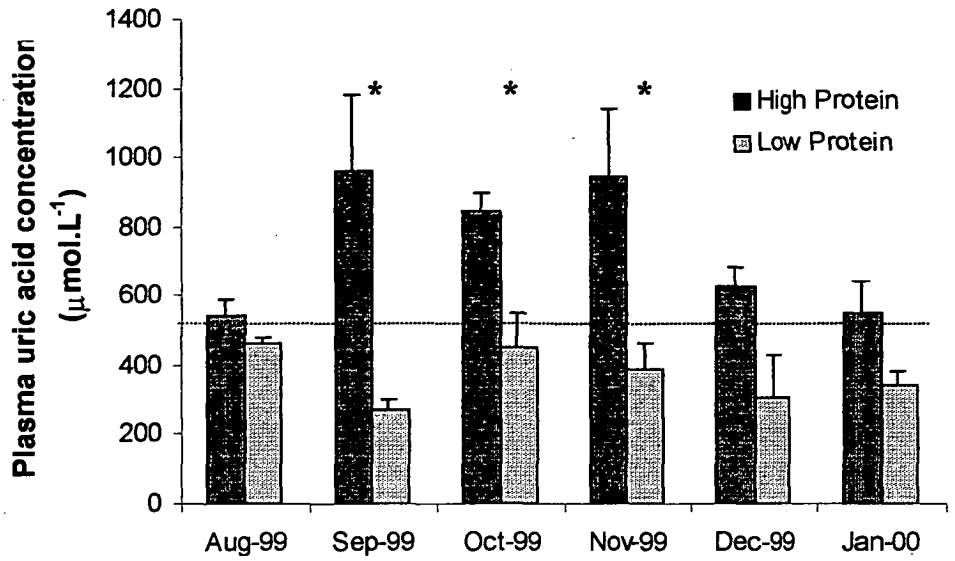

Figure 2. Plasma uric acid concentrations in clinically normal swift parrots in the research aviary between September 1999 and January 2000. The samples are divided by dietary group: high protein ration (n = 2) and low protein ration (n = 3). Error bars represent one standard error. * indicates a significant difference ($P < 0.05$) between the birds on the high protein and low protein diets. The dotted line demonstrates the upper normal limit of plasma uric acid concentration (500 μmol.l^{-1}) in cockatoos and budgerigars, *Melopsittacus undulatus* (Hochleithner 1994).

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2. Comparison of pre- and post-prandial plasma concentrations of uric acid

The results of the fasting and feeding experiment on plasma uric acid concentrations are surprising (Figure 3). The pre-prandial plasma uric acid concentration, after a 14 hour fast, is not significantly different to the two and six hour post-prandial concentrations. Statistical analysis by repeated measures ANOVA confirmed the graphical impression that there is no significant change in plasma uric acid concentration over time ($F_{(2,10)} = 2.890$ $P = 0.102$).

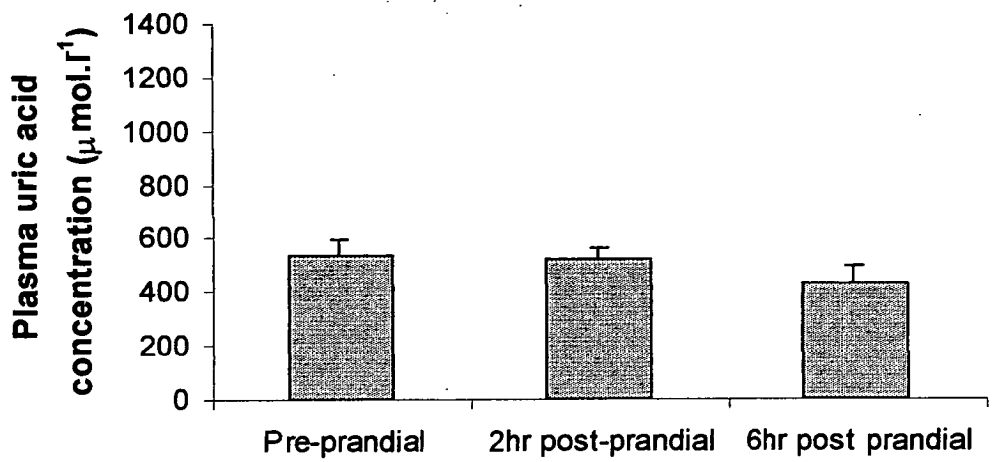

Figure 3. Effect of feeding on plasma uric acid concentrations in swift parrots (n = 6). Pre-prandial values reflect a 14 hour fast. Birds were then allowed 2 hours access to the low protein diet before the post-prandial samples were taken. Error bars represent one standard error.

3. Effects of diet on plasma uric acid concentrations in swift parrots

Post-prandial plasma uric acid concentrations of all three diet groups are significantly different from each other (Figure 4; $F_{(2,19)} = 36.928$ $P < 0.001$). The captive swift parrots fed the mixed nectar and seed diet have the highest levels of plasma uric acid (907 ± 66 μmol.l^{-1}); plasma uric acid concentrations in the wild birds were lower (179 ± 28 μmol.l^{-1}) than the concentrations of uric acid found in pre-prandial captive parrots (see Figure 4). This low value for the wild swift parrots is actually an overestimate as four of the eight birds tested had plasma uric acid concentrations below the lower limit of detection of the assay at <119 μmol.l^{-1} and were arbitrarily assigned this value. Values for the birds on the low protein diet (512 ± 93 μmol.l^{-1}) were similar to those in November 1999 (Figure 3).

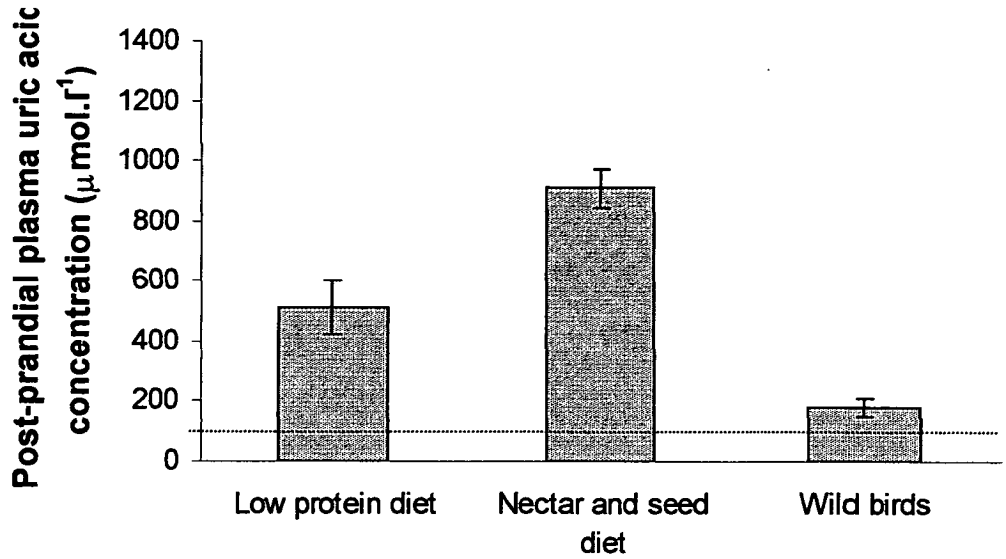

Figure 4. Post-prandial concentrations of plasma uric acid in swift parrots from captivity and the wild. The three groups represented are: birds from the research aviaries fed the low protein ration (n = 6); captive-bred birds from a local breeder fed artificial nectar and seed (n = 8); and wild birds captured and sampled when feeding on nectar and pollen from *Eucalyptus globulus* (n = 8). All groups are significantly different ($P < 0.05$) from each other. The dotted line represents the minimum detectable limit of the assay (119 $\mu mol.l^{-1}$). Error bars represent one standard error.

DISCUSSION

The plasma samples that were collected for the reproductive biology study were all post-prandial samples (~1-2 hrs post-feeding). Pre-prandial samples would have been more informative but this was a retrospective analysis. The plasma uric acid levels therefore reflect both renal tubular function and dietary protein metabolism. The high concentrations of uric acid in birds with renal tubular damage can be explained by the role of the renal tubules in the excretion of uric acid (Lumeij 1994b, Goldstein and Skadhauge 2000). The presence of high uric acid concentrations in the clinically affected swift parrots in August 1999 suggests that renal toxicity had commenced by this time. Unfortunately, no samples from these

birds are available for the months prior to August which might have allowed a better estimation of the onset of the toxicity.

The response to treatment of the single bird that was affected but survived is not apparent in the concentrations of plasma uric acid for many months afterward. While this is only a single bird, the results suggest the limitations of using uric acid as a prognostic indicator of the recovery of renal function.

The effect of the increased amount of protein in the diet is clearly evident in the birds that showed no clinical signs (Figure 2). Ideally, the effect of the high protein ration would also have been evaluated in birds that had not been potentially exposed to renal toxicity. However, this was not carried out in this endangered species due to the potential risk of nephrotoxic effects from the high protein ration itself. The birds that were retrospectively used to make this comparison have shown no clinical evidence of disease either throughout the time reported here or in the twelve months following. The hyperuricaemia seen in the birds on the high protein ration is most likely to be due to their dietary nitrogen intake exceeding their renal clearance capacity. This suggests that these levels of protein are well in excess of the birds' nitrogen requirements.

Only a limited number of studies have addressed the protein requirements of nectarivorous birds. Hummingbirds have very low protein requirements (< 3 %) when expressed as a percentage of diet (Brice and Grau 1989, 1991). A study of nitrogen excretion in New Holland honeyeaters, *Phylidonyris novaehollandiae*, calculated that the protein requirements in mg day^{-1} were 25% of that expected in a similar sized granivorous passerine (Paton 1982). In a study of the protein requirements of rainbow lorikeets *Trichoglossus haemotodus*, endogenous nitrogen losses (32 mg $N.kg^{-0.75}.day^{-1}$) were less than an eighth of the predicted values; the dietary protein requirement, expressed as a percentage of feed intake was 2.8 % (Frankel and Avram 2001). Nitrogen balance studies are needed to determine if the protein requirements of the swift parrots are similarly low.

The concentrations of uric acid that were found in the fasting and feeding trial were unexpected (Figure 3). In previous studies of plasma uric acid concentration in larger birds (eg. raptors, poultry and penguins), pre-prandial concentrations have always fallen to very low levels after an overnight fast (Lumeij and Remple 1991;

Lumeij and Redig 1992; Goldstein and Skadhauge 2000; Kolmstetter and Ramsay 2000). The recommendation based on these studies is that an overnight fast before assessing pre-prandial concentrations of uric acid gives the best assessment of renal function without the confounding effects of diet (Lumeij 1994b; Hochleithner 1994; Kolmstetter and Ramsay 2000). The swift parrots that were fasted for fourteen hours did not show a reduction in plasma uric acid levels from post-prandial concentrations. This period of fasting may be too long for small birds and they may have already begun catabolism of body protein reserves, resulting in increased levels of plasma uric acid. Food was removed two hours after feeding, and the plasma uric acid concentration had not decreased significantly four hours later. Future studies of the clearance of uric acid from the plasma of small psittacine birds are needed.

The possible effects of captive diets are highlighted in the comparisons between the concentrations of plasma uric acid in wild birds and captive birds on commonly used diets. The extremely low concentrations of plasma uric acid in the wild birds suggest that these birds are not consuming diets with excesses of protein. The high concentrations of uric acid in the plasma of the captive bred birds on the seed and nectar diet is alarming, although no history of disease was seen in these birds. When poultry are given a choice between high energy and high protein diets they can regulate both their protein and their energy intake (Denbow 1999). It can be assumed that swift parrots and other nectarivores have similar abilities. Nectar provides abundant energy but has insufficient protein to maintain body mass (Chapters 4 and 6), and so the birds must seek out alternate foods to fulfil their amino acids requirements. However, all the foods fed to captive birds in this study had high levels of both energy and protein and the birds were unable to select between protein and energy sources. If food intake regulation is predominantly driven to provide energy, then excess amounts of protein would have been consumed unavoidably. This suggests that captive feeding of nectarivorous birds may have to be reassessed. Until the protein requirements of different species are investigated it may be advisable to feed separate sources of protein and energy and allow the birds to regulate their own consumption.

CONCLUSIONS

Despite this intensive investigation (Chapter 7 and this chapter), a number of important facts remain obscure about this episode of renal disease in swift parrots. The initial cause of the renal damage remains uncertain. It is also unclear whether the elevation of plasma uric acid concentrations was a consequence of renal tubular damage or elevated dietary protein levels. However, the finding of low plasma uric acid concentrations in wild birds and high concentrations in captive birds provide support for the hypothesis that dietary protein levels were involved. As nectarivores, swift parrots may have lower protein requirements than granivorous parrots. However, confirmation of this hypothesis will require nitrogen balance studies as a direct assessment of protein metabolism.

Chapter 9. The relationship between diet and reproduction in captive swift parrots

INTRODUCTION

Environmental factors, such as food, photoperiod or the presence of nest-hollows, may stimulate reproductive development (Lack 1968; Wingfield 1983; Cockrem 1995). In particular, food may act as a proximate factor in the regulation of avian seasonal breeding (Lack 1968; Wingfield 1983; Cockrem 1995; Monaghan and Nager 1997; Hau et al. 2000a). My study (Chapter 2) showed that in male swift parrots the testes do not begin to develop until after the birds arrive in their breeding grounds and field studies provide circumstantial evidence for a relationship between successful reproduction in swift parrots and the abundant flowering of the Tasmanian blue gums, *Eucalyptus globulus* (Brown 1989; Brereton 1996a; 1996b). Experimental investigation of this relationship is limited to a single botanical study. Brown (1989) investigated the amount of nectar produced by the flowers of *E. globulus*. From cut branches in water, it was estimated the flowers of *E. globulus* produced ~ 0.15 mls of nectar per day, while measurable volumes of nectar could not be collected from the much smaller flowers of the swamp gum, *Eucalyptus ovata.* From these results, it was speculated that the volume of nectar produced by *E. globulus* may be necessary to provide sufficient energy to stimulate reproduction in swift parrots (Brown 1989). The flowers of *E. globulus* are the largest of any of the Australian eucalypts being approximately 2cm in diameter (Brown 1989). Other factors such as the standing crop of nectar, the energy value of the nectar and the visitation rates of swift parrots to flowers need to be further investigated.

Nectar does provide abundant amounts of energy for those birds able to harvest it. However, it contains very low levels of amino acids, vitamins and trace minerals (Lüttge 1976), all of which are necessary for avian maintenance, growth and reproduction (Chapter 4). My investigations, described in Chapters 5 and 6, revealed that eucalyptus pollen and insects (such as psyllids) associated with eucalypt trees are used by swift parrots during the breeding season to provide amino acids and other nutrients. Is it simply the easily harvested, abundant amounts of

energy (nectar) and protein (pollen and insects) available in the *E. globulus* trees during flowering that stimulates reproduction, or are other visual or sensory cues from the flowers involved?

Food supply has frequently been reported as a proximate control on reproduction, with experimental studies demonstrating the negative effects of nutrient deficiency and the positive effects of food abundance on reproduction in temperate zone birds (reviewed by Wingfield 1983). Tropical birds may be more responsive to changes in food abundance than temperate birds (Wikelski et al. 2000; Hau et al. 2000a; Hau 2001). There is evidence that female birds use the abundance of food resources and their own nutritional plane to time the initiation of reproduction (Wingfield 1983), although the mechanisms involved are unknown.

The rationale behind this part of my study was to assess the effects of differing levels of protein and energy on reproduction, and whether the presence of *E. globulus* flowers was necessary to initiate successful reproduction. In this chapter, I report on feeding trials in captive swift parrots, designed to elucidate some of the mechanisms that may be involved in the relationship between abundant flowering and reproductive success.

MATERIALS AND METHODS

Experimental diets

Three mixed sex groups of four to six swift parrots were established in captivity. The source and husbandry of the swift parrots used in this study are described in Chapter 2 (Materials and Methods). Three experimental diets were used during the breeding season (September to March) of 1998 and 1999. These were a high protein diet, low protein diet and low protein diet supplemented with fresh flowers of *E. globulus*. The metabolisable energy (ME) and protein content of each diet is given in Table 1. Metabolisable energy is defined as the gross energy of the diet consumed minus the energy in the excreta of the bird consuming the diet (Klasing 1998). The metabolisable energy of foodstuffs varies depending upon the species and age of bird consuming the diet (Angel 1993). Note that the ME values reported in Table 1 are based on standard tables of ME for various foodstuffs derived using domestic chickens (Roudybush T. pers. comm.). The true ME of these diets in

swift parrots is therefore unknown; however, gross energy values for these foods are not readily available. Nectar powder was formulated as Lori-nectar® or Lori-start® Avesproduct B.V. (66.6% DM); Farex Original Blended Cereal® Heinz Wattie's Ltd (16.7% DM); and sucrose (16.7% DM). Pellets were either Roudybush® maintenance or breeder pellets. The diets were supplemented with a small amount of fresh apple (1/4 apple for 6 birds every second day). One group of swift parrots was fed the low protein ration but offered access to ~ ten to twenty freshly picked flowers of *E. globulus* every day. Each group of birds was fed once a day, during the morning. The feeding trial continued through the breeding season (September to March) of 1998 and 1999. In the non-breeding period, all birds were fed on the low protein diet. In the final year of the project, due to the problems detailed in Chapters 7 and 8, all pelleted diets were replaced with a commercial lorikeet dry food (Avione® Lorikeets rearing and conditioning food, the "Lorikeet diet", see Table 1), and all birds were maintained on this diet. Information on gross or metabolisable energy was unavailable from Avione® and lists of ingredients that would have enabled calculation of energy values were similarly unavailable due to commercial concerns.

Table 1. Dietary levels of metabolisable energy (ME) and protein in the daily rations fed to swift parrots in the 1998 and 1999 breeding seasons. Values are based on manufacturers' labelling. Combined values assume equal consumption of nectar and pellets or nectar and dry powder. Metabolisable Energy for lorikeet powder not available (see text). Percentage of dry matter weight indicated by % DM.

	High protein diet		Low protein diet		"Lorikeet diet"	
	ME (kJ/g)	Protein (% DM)	ME (kJ/g)	Protein (% DM)	ME (kJ/g)	Protein (% DM)
Nectar	14.49	13.6	14.76	11.6	14.76	11.6
Pellets	13.23	20	14.36	11.0	-	-
Dry powder	-	-	-	-	*Unknown	15.0
Combined diet	13.86	16.8	14.56	11.3	*Unknown	13.3

Assessment of reproductive activity in captive birds

Two measures of reproductive activity were assessed. First, successful reproduction was defined as a pair of birds producing a live chick. However, only one adult female swift parrot was available per treatment group. Therefore, a second measure of reproductive readiness was assessed during these trials: plasma concentrations of testosterone (T) during the breeding season was determined in male birds with no evidence of renal disease (see Chapter 7). See Chapter 2 for details of the T radioimmunoassay. The pattern of plasma T over the breeding seasons was compared between dietary groups using a repeated measures (M)ANOVA using Pillai's trace to assess levels of significance.

RESULTS

Nectar and lorikeet powder were preferentially consumed, and were usually completely exhausted by early afternoon. A majority of the low protein pelleted ration (>90 %) was consumed by the next morning, but approximately half of the high protein pellets remained each morning. All birds offered *E. globulus* flowers fed immediately upon presentation of the flowers, harvesting both nectar and pollen (see Chapter 5 for descriptions of feeding actions).

Incidence of successful reproduction

In the three years of the project, there were only two successful breeding attempts in the captive swift parrots. The first breeding occurred in a pair of captive-bred swift parrots fed the low protein ration with supplemental *E. globulus* flowers. The second breeding occurred in the third year of the project in captive-bred swift parrots fed the "Lorikeet diet" of nectar and dry powder. In all of the three years of the project, there was evidence in all groups of nestbox inspection and bowl formation, which constitutes the preliminary stages of breeding (Chapter 2).

Plasma testosterone concentrations

There was no significant effect of dietary group on plasma T concentrations over the breeding season (n = 14, $df_{3, 13}$, $F = 0.966$, $P = 0.438$) (Figure 1). However, this result is complicated and obscured by low sample sizes in the high protein group (n = 1) and the blue gum and low protein group (n = 1), both of which were non-breeding birds. This is due to the combination of problems developing the hormone assays (see Chapter 2) and the deaths of many subjects (Chapters 5 and 6): only male birds showing no evidence of kidney disease have been included in these analyses. Therefore, *a posteriori* comparison was carried out after excluding these two groups. A significant interaction was found between dietary group (low protein and lorikeet diets) and month during the breeding season (n = 15, $df_{4, 10}$, $F = 4.246$, $P = 0.029$).

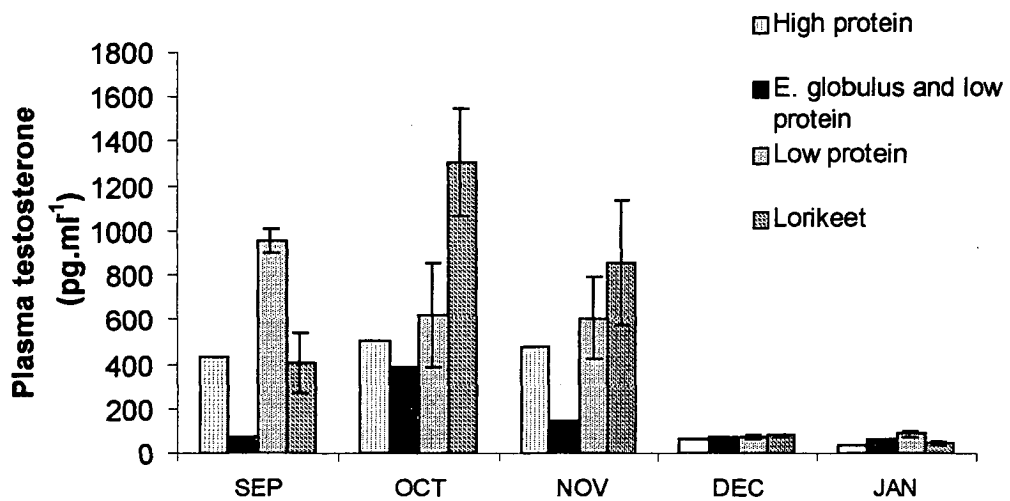

Figure 1. Monthly variations in plasma testosterone throughout the breeding season in captive swift parrots fed on different diets. Diets are: high protein nectar and pellets (n = 1); blue gum flowers plus low protein nectar and pellets (n = 1); low protein nectar and pellets (n = 3); or Lorikeet diet (n = 12). There is a significant interaction between diet and month ($P < 0.05$) for the low protein and lorikeet diet groups. Values are means ± one standard error.

DISCUSSION

The incidence of breeding success in the captive swift parrots was limited, and factors other than diet are likely to be involved in the two instances of breeding

on the low protein ration supplemented with blue gum flowers and on the lorikeet mix. Both pairs of birds were captive-bred and therefore likely to have improved chances of breeding in captivity than wild-bred birds. The limited number of female swift parrots in this study is also likely to reduce successful breeding. Forced pairing results in reduced breeding success in cockatiels (Yamamoto et al. 1989). Although each swift parrot female had a choice of mates, males were limited to competing for a single female. This may also have caused an increased need for territorial defence and reduced breeding success (Joyner 1994). Future studies should preferentially use captive-bred birds. Also, using paired birds (either exclusively force-paired or previously bonded) would eliminate some variability due to social and behavioural factors. Individually housing pairs would also allow improved monitoring of nest inspection and bowl formation (Millam et al. 1988).

There was a clear pattern of variation in plasma T in all male birds, with plasma T concentrations peaking in the spring (September to November) and decreasing significantly in December. This is in accordance with the pattern of reproductive activity described in both wild and captive male swift parrots in Chapter 2. Elevations of plasma T are associated with testes development in swift parrots (Chapter 2) and other avian species (Farner and Wingfield 1980; Wingfield 1983; Gwinner and Dittami 1990).

The significant interaction between diet and monthly plasma T concentrations in swift parrots during the breeding season indicates that diet is influencing plasma concentrations of this hormone. In male birds on the low protein diet, plasma concentrations of T were highest in September. However, the male swift parrots on the Lorikeet diet had higher concentrations of plasma T in October and November than in other months. Whether the shifting in the peak of plasma T represents an earlier readiness to breed or not requires further study; however, the result does suggest some flexibility of male swift parrot reproductive readiness. The unpaired birds on the high protein diet and the low protein diet that was supplemented with blue gum flowers also showed the elevation of plasma T in the breeding season, and its subsequent decline in December. However, the peaks in spring were much lower than in the birds on the low protein or lorikeet diet.

The interaction of diet with time on reproduction in swift parrots is in contrast to a previous study on the granivorous cockatiel, *Nymphicus hollandicus* (Millam et al. 1988). In the study of paired cockatiels, reproductive activity was assessed by nest-box inspection, bowl formation and egg laying (Millam et al. 1988). A dietary change from a maintenance seed diet to a high protein pelleted diet did not influence reproductive activity (Millam et al. 1988).

As a result of the renal disease described in Chapter 7, low sample sizes and incomplete data sets confound this study. Therefore, I am unable to draw any conclusions regarding the importance of the blue gum flowers to reproduction. The levels of protein and energy used in all the diets were probably in excess of the male swift parrots' requirements for reproduction. Male birds do not generally increase basal energy requirements by more than 1 % for testicular growth and sperm production (Klasing 1998). In contrast, female birds invest considerably more in reproduction (Klasing 1998; Visser and Lessells 2001). Female passerines increase nutrient requirements by ~45 % during reproduction (Klasing 1998) and suffer increased mortality if either incubation or egg production costs are increased (Visser and Lessells 2001). Future trials should use lower concentrations of protein to avoid the complications of renal compromise (Chapters 5 and 6) and concentrate on the responses of the female birds.

I conclude that diet did affect reproductive activity in male swift parrots, as measured by patterns of plasma T concentrations. However, the nature of the effect, and the mechanisms involved, remain obscure and further studies are needed.

Chapter 10. Migration as a temporal and physiological constraint on reproduction in swift parrots

INTRODUCTION

The migratory journeys of swift parrots dominate their life history. The swift parrots move among a variety of habitats to follow an unbroken sequence of flowering plants that provide food in the form of nectar, pollen and insects (Brown 1989; Brereton 1996a; Ford et al. 2001). The swift parrots' migratory habits may have contributed to its population decline by two possible mechanisms. First, degradation and loss of habitat at any point in the migratory journey may result in the birds compensating by ranging further to procure their foraging resources (Ford et al. 2001). This may increase the physiological costs associated with migration. Second, migration may impose both temporal and physiological constraints on reproduction, and this is the focus of this chapter.

There is a lack of information on the migratory process in swift parrots. Although the general movements of the swift parrot population have been increasingly well documented by the efforts of the Swift Parrot Recovery Team, much of these data are yet to be published (Brereton 1996a; Kennedy 1998), and there is no information on the movements of individual birds. It is not known whether the birds return to the same breeding or over-wintering areas from year to year, nor whether the migratory journeys are diurnal or nocturnal. The possible factors, both endogenous and environmental, that control the migration have not been investigated in the swift parrots, or any psittacine species.

Furthermore, migration is rarely observed in psittacines, making it difficult to extrapolate or hypothesise from work in similar species. Indeed, the only three confirmed migratory parrot species in the world breed in Tasmania (Higgins 1999). Two of these species, the swift parrot and the orange-bellied parrot, *Neophema chrysogaster*, are already endangered and the third species, the blue-winged parrot, *Neophema chrysostoma*, is considered rare (M. Holdsworth pers. comm.). The loss of habitat in the

migratory path of the regent honeyeater, *Xanthomyza phrygia*, an Australian nectarivorous passerine, is suspected to have contributed to its critically endangered status (Cooke and Munro 2000; Ford et al. 2001). This suggests that a detailed study of the constraints of migration upon reproduction should underpin any conservation effort in these endangered birds.

Avian Migration

The purpose of migratory behaviour is to reduce or avoid the impact of seasonal environmental variations (Leggett 1984). The ultimate factor driving migration is resource availability, although it does not act as a proximate factor to initiate departure or to terminate the migratory movement (Dingle 1996). Migrants may time their departure using cues that forecast resource deterioration and suppress responses to resources in transit (Dingle 1996); alternatively such timing may be due to endogenous rhythms (Berthold 1999).

It is uncertain how avian migration evolved. An early theory hypothesised that migratory behaviour evolved in resident species subjected to habitat deterioration (Baker 1978). The central idea of this theory was that each animal had a "migratory threshold", and when environmental conditions deteriorated below this threshold, animals would migrate. However, Berthold (1999) has suggested an alternative theory for the evolution of bird migration. There are five basic tenets to the theory.

1) Bird species developed migratory behaviour very early, close to their origin in space and time.
2) Migration behaviour evolved in tropical regions, long before the temperate regions were colonised, and was initially short distance and partial. Partial migration is said to occur when a significant proportion of the population remains resident rather than migrating.
3) The physiological mechanisms for migration, in particular, navigation, may have been inherited from pre-avian ancestors.

4) Partial migration was widespread as an adaptable and successful strategy. This strategy led to the stable establishment of genotypes within most avian populations for both migratory and resident behaviour and physiology.
5) Natural selection has acted and continues to act upon these populations in a range of environmental conditions, producing the whole spectrum of movements from exclusive migrants, to species that have retained the hypothesised ancestral condition of partial migration, to purely resident species.

Berthold's theory proposes that bird populations can exhibit behavioural oscillations within this spectrum of migratory behaviour when environmental conditions require it, unless the population becomes genotypically pure with respect to migration; that is, there is a loss within the population of the genotype for either migration or residency. Berthold (1999) doubts that genotypically pure populations exist. Thus, most migratory species may be expected to have some potential for plasticity in migratory behaviour.

Migration in the Family Psittacidae

Migration is an unusual strategy amongst the Psittacidae. The movements of the budgerigar, *Melopsittacus undulatus*, may be nomadic or partially migratory (Dingle 1996). However, the only three confirmed migratory psittacine species in the world breed in Tasmania (Higgins 1999). The migratory journeys of swift parrots, blue-winged parrots, and orange-bellied parrots are all classed as short distance, to-and-fro migrations (Dingle 1996) ranging between 300-1000 kms. Swift parrots and orange-bellied parrots are considered exclusive migrants; that is, they are species in which the entire population migrates (Berthold 1999; Hockey 2000). The blue-winged parrots are considered to be partial migrants, because some birds remain on the mainland of Australia to breed, and in some years, some birds over-winter in Tasmania (Higgins 1999).

Migration in swift parrots

The annual movements of the swift parrot population are summarised in Figure 1. Swift parrots migrate to Tasmania from their over-wintering grounds on the south-eastern Australian mainland between late winter and early spring. The first records of birds arriving are in early August, and the bulk of the population has arrived by mid-September (Hindwood and Sharland 1964; Brown 1989; Brereton 1996). Observations of birds arriving in Tasmania suggest that the population does not migrate together; instead, the birds appear to move south in small groups of up to ten birds (Hindwood and Sharland 1964; Brown 1989; Brereton 1996).

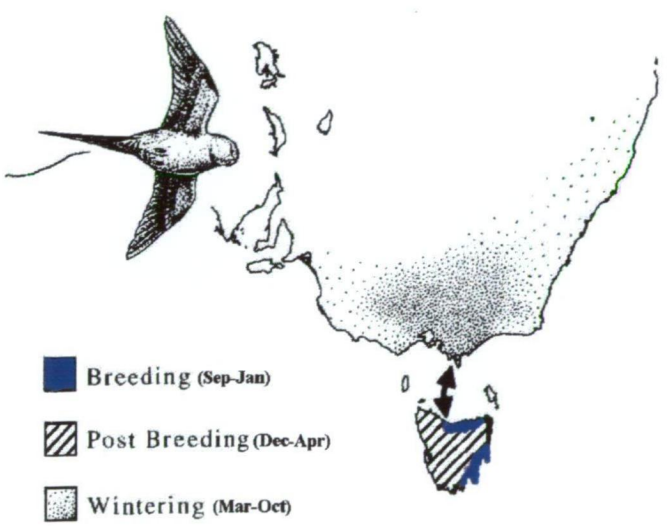

Figure 1. Distribution and movements of swift parrots throughout its annual life history. Figure modified from Dingle (1996), with permission. The double headed arrow indicates the annual to- and fro- movements of the population across Bass Strait.

During the breeding season, birds tend to congregate in two main areas of Tasmania, with the majority of the population congregating in the south-east, within 20

km of the coast (Brereton 1996a). A significant proportion of the population is reported to breed in the central-north of Tasmania (Hindwood and Sharland 1964; Brown 1989; Brereton 1996). The proportion of the population that spends the breeding season in each of these two areas varies between years. This variation has been tentatively linked to eucalypt flowering patterns (Brereton 1996).

The breeding season lasts from September through to January. From December through to April the populations begins to disperse throughout Tasmania, with movements suspected to be nomadic in response to the variable flowering of the endemic eucalypts (Hindwood and Sharland 1964; Brown 1989; Brereton 1996). The earliest reports of birds returning to the mainland are from mid-February, although most birds leave during March and April (Brown 1989; Brereton 1996). Some large flocks have been seen in the north of Tasmania during the March to April period and it is suggested that these may be pre-migratory flocks (Brown 1989). The last birds to depart leave in May; however, occasional birds have been found in very poor condition in early winter in the north of Tasmania. There are occasional reliable reports of birds in northern Tasmania in the winter (B. Munday pers. comm). These may be related to a reliable source of supplementary artificial feeding in this area (Brown and Brereton pers. comm; pers. obs.).

During the winter, the population is concentrated in Victoria and NSW, although historically the birds were once commonly seen in South Australia, and recently it has been recognised that they are regularly seen in limited areas of south-eastern Queensland (Brereton 1999). A large proportion of the population is found in the dry eucalypt forests to the north and west of Melbourne. Recent surveys of these birds show substantial variation in numbers between years, suggesting that the birds are nomadic across their over-wintering grounds (Kennedy 1998).

There are two features of the population's movements that I wish to emphasise. First, there appears to be substantial yearly variation in the swift parrot population's distribution around a framework of the annual to-and-fro movements from Tasmania to the mainland of Australia. This may be a result of the superimposition of the nomadism

associated with nectarivory onto the endogenous rhythms associated with migration. Second, there is a large temporal variation in migratory movements within the population in any year, as evidenced by concurrent observations of birds on the mainland and in Tasmania during the early and late periods of the breeding season. It is currently unknown if this temporal variation is due to annually repeated behaviour by individuals within the population, or is simply a result of the variation associated with nomadism. Similarly, it is not known if the spatial distribution of the population can be attributed to individuals returning annually to breeding and wintering sites or to the random nomadic movements of the population.

Study of migratory movements in other avian species has relied upon large scale banding recovery projects over many years or the use of radio and satellite tracking (reviewed in Berthold 1996). The small size of the swift parrots (60-70 g; Chapter 5) currently precludes satellite tracking and limits radio-tracking to short distances (~2-3 km range) and short durations (~ 6 weeks) (Brereton 1996b). Banding and recovery of such a nomadic and high arboreal species as the swift parrots has also proved problematic (Brereton 1996b; Chapter 2). However, the behaviour of caged migratory birds during migratory periods has been shown to reflect the control mechanisms of avian migration (Berthold 1996). This study, therefore, concentrates on investigating the physiological and behavioural changes associated with migration in captive swift parrots in order to assess the possible constraints of migration upon reproduction.

Physiological and behavioural changes associated with avian migration

Migration is triggered by environmental factors, particularly photoperiod, that induce both endocrine and neural changes (Berthold 1996; Dingle 1996). The bird's hormone balance shifts to stimulate changes in metabolism that support the high energetic demands of migration, while activation of central neural pathways induces migratory behaviours (Berthold 1996; Dingle 1996). A large body of experimental evidence (for reviews see Berthold 1984; 1996; Gwinner 1996) has suggested the presence of endogenous circadian and circannual rhythms that, at least, partly control

these physiological changes. Further, there is good evidence for the hereditary nature of these rhythms (for reviews see Berthold 1984; 1996; Gwinner 1996).

a. Hormonal and metabolic changes

The activation of hormonal and metabolic pathways to support and stimulate migration has been demonstrated in a wide range of migratory organisms (Dingle 1996). The control of these changes is suspected to require an interaction between endogenous rhythms and environmental conditions (Berthold 1984; 1996; Dingle 1996; Gwinner 1996). The ultimate factor driving migration is thought to be a periodic deterioration in environmental conditions (Dingle 1996; Berthold 1999). In order for migration to be successful, this deterioration must be anticipated, so that resources must be sequestered prior to migration and then mobilised during the migratory journey (Dingle 1996). Photoperiod is the most widely used environmental cue for avian migration, and the hormonal response to photoperiod via melatonin is suspected to be one of the modifying mechanisms to endogenous migratory rhythms (Dingle 1996; Gwinner 1996). Wingfield et al. (1990) state that the physiological adaptations associated with preparation for long distance migration in birds include:

1. fat deposition (lipogenesis);
2. the integration of enzyme systems for energy storage and mobilisation;
3. increased haematocrit for enhanced oxygen transport;
4. hypertrophy of flight muscles and;
5. development and synchronisation of migratory behaviour.

The hormonal control of these adaptations is only partially understood; however, hormones with multiple roles appear to support the changes associated with migration (Dingle 1996). The role of the gonadal hormones in avian migratory physiology is complex and not clearly understood. Castration of *Zonotrichia sp.* sparrows from the northern hemisphere prior to spring migration eliminated hyperphagia and pre-migratory lipogenesis and reduced, but did not eliminate, migratory restlessness (Wingfield et al.

1990). Similar experiments conducted prior to the autumn migration resulted in no effect on pre-migratory changes, suggesting that spring migration has a different hormonal basis to autumn migration (Wingfield et al. 1990; Dingle 1996). Supplementary testosterone reinstated hyperphagia and lipogenesis in castrated male and ovariectomised female *Zonotrichia sp.* sparrows (Schwabl et al. 1988), while oestradiol implants had a similar effect in ovariectomised females (Schwabl and Farner 1989).

Non-gonadal hormones may also be involved. In red-headed buntings, *Emberiza bruniceps,* circulating thyroid hormones (T3 and T4) increase prior to spring, but not autumn, migration (Dingle 1996). It is postulated that gonadal hormones, thyroid hormones and prolactin are required for a complete spring migratory response (Dingle 1996). It should be noted that the studies provide correlative, but not causative, evidence of a role for these hormones in migration. The failure of these models to account for hyperphagia and lipogenesis in both spring and autumn migration is a major flaw.

Corticosterone may have a major role in the physiological preparations of the birds for migration (Holberton 1999; but see Deviche 1995). Early studies provided equivocal evidence that interactions between corticosterone and prolactin are responsible for pre-migratory lipogenesis and migratory restlessness (Martin and Meier 1973; Meier and Wilson 1985). However, the experiments on which these assertions were based were technically flawed and have yet to be repeated successfully (Dingle 1996). Much stronger evidence for the role of corticosterone in the preparation for migration comes from several field (John 1966; Ramenofsky et al. 1995; Holberton et al. 1996; 1997) and experimental studies (Holberton 1999). These have shown a rise in the baseline plasma concentrations of corticosterone and a reduced corticosterone response to acute stress concurrent with pre-migratory development. The basis for the role of corticosterone in pre-migratory adaptations is that during the migratory period migrants show: 1) elevated baseline corticosterone, which may facilitate migratory fattening and 2) a reduced corticosterone stress response, which may protect the skeletal muscle mass against catabolism (Holberton 1999). Further, such changes have been observed in both spring and autumnal migrating birds (Holberton 1999), suggesting that corticosterone is acting

independently of gonadal and thyroid hormone systems. These models have, however, been developed using long distance migrants, and it is not known if the same hormonal changes occur in short distance migrants, such as the swift parrots.

b. Migratory behaviour

The migratory period is associated with reduced responses to stimuli such as feeding and reproduction, the development of migratory restlessness and directional orientation preferences (Dingle 1996; Gwinner 1996). Migratory restlessness, or Zugenruhe, is the increase in the activity of captive migrating birds that correlates with the period of wild migration (Gwinner 1996; Munro and Munro 1998). It has been observed in both long (Gwinner 1996) and short distance migratory birds (Munro and Munro 1998). The duration of migratory restlessness has been shown to be genetically determined in a number of species; this restlessness may be a mechanism for determining the distance flown during migration, and the temporal orientation of migration (Gwinner 1996). There have been no studies investigating the occurrence of migratory restlessness in swift parrots, nor, indeed, in any psittacine species.

The preferred directional orientation of captive migratory birds changes during the year correlating with the movements of the birds' normal migratory journey (Emlen and Emlen 1966; Berthold 1984; 1996; Gwinner 1996). This captive orientation preference represents changes in spatial orientation associated with navigating during migration (Gwinner 1996). Both nocturnally and diurnally migrating birds exhibit this behaviour (Munro and Wiltschko 1993; 1995; Munro and Munro 1998) which can be demonstrated and recorded in a funnel cage (Emlen and Emlen 1966). Understanding the mechanisms by which organisms are able to perceive their orientation in space is the subject of much current research, but will not be discussed here. There have been no previous studies assessing orientation behaviour in any of the migratory psittacine species.

Aims of this study

This study concentrates on investigating the physiological and behavioural changes associated with migration in swift parrots in order to assess the possible constraints of migration upon reproduction. The specific aims are:

1. To determine if physiological changes (eg. body weight, fat reserves, pectoral muscle condition changes) associated with migration in other species can be identified in swift parrots.

2. To determine if the plasma concentrations of corticosterone in swift parrots show seasonal variations correlated to periods of migratory activity; and to compare the annual pattern of corticosterone in swift parrots to nomadic, resident musk lorikeets, *Glossopsitta concinna.*

3. To determine, by examining orientation preferences and migratory restlessness through the year, if swift parrots have endogenous circannual rhythms of migratory behaviour.

METHODS

1. Body weight, pectoral muscle condition and fat score

Wild swift parrots were either trapped by mist-netting, or obtained after having been killed by collisions with fences, windows and motor vehicles. As the body weights of swift parrots do not differ significantly between genders (Higgins 1999; Appendix A) data from both sexes were pooled for analysis. I measured body weight with Salter scales ($g \pm 0.1$) and assessed pectoral muscle condition by subjectively comparing the pectoral muscle mass to the prominence of the sternum (Harrison and Ritchie 1994) (Table 1). Fat score was allocated by subjective assessment of deposits of subcutaneous fat in the furcula and abdomen (Table 2). In live birds, visualisation of subcutaneous fat was aided by wetting the overlying feathers with alcohol.

2. Plasma corticosterone concentrations

Sampling protocols - captive birds

I carried out blood sampling of captive swift parrots and musk lorikeets, between 10 and 12 am to reduce variation from diurnal rhythms of plasma hormone concentration. Only male birds of both species were used for assessment of plasma corticosterone concentrations. I captured birds from the aviary by hand-net and took blood samples immediately; time from beginning capture to blood sampling did not exceed five minutes in any individual. Blood was taken from the left jugular vein using heparinised syringes and the volume of blood taken from each bird did not exceed 0.4 ml. Samples were held on ice until centrifuging at 6400 rpm. Plasma was stored frozen at -20 °C until analysis.

Table 1. Scheme for the assessment of pectoral muscle condition in swift parrots by comparing the depth of the pectoral muscle mass with the level of the sternum (modified from Harrison and Ritchie 1994).

Condition score	Description
1	Poor musculature, little muscle either side of sternum
2	Moderate musculature, sternum prominent
3	Good musculature, level with sternum
4	Excellent musculature, extends beyond level of sternum

Table 2. Scheme for the assessment of fat score based on visible deposits of subcutaneous fat in the furcula and abdomen of swift parrots.

Fat score	Description
0	No fat visible in furcula and abdomen
1	Fat visible in furcula but not abdomen
2	Fat visible in both furcula and abdomen

Sampling protocols - wild birds

I captured wild male swift parrots by mist-netting combined with the use of lure birds. I monitored nets continually, and extracted and sampled birds as soon as possible after capture. However, simultaneous multiple captures, and the need to free by-catch, meant that not all birds were sampled less than five minutes after capture. Time to bleeding was recorded as: less than 5 min, 5-10 min, 10-15 min and greater than 15 min. Blood sampling and storage of samples were otherwise as for captive birds.

Radioimmunoassay

I measured plasma concentrations of corticosterone using established radioimmunoassay (RIA) techniques based on tritiated corticosterone as the radiolabel. Corticosterone was extracted from between 15 to 50 µl of plasma using one ml dichloromethane with an extraction efficiency (mean ± one standard error) of 50.0 % ± 0.72. The plasma extracts were evaporated to dryness and incubated overnight at 4 °C with 100 µl [^{3}H]-corticosterone in pH 7.6 phosphate-buffered saline, 100 µl antiserum (Endocrine Sciences B3-163, 1: 100 dilution in standard diluent) and 100 µl standard diluent (a pH 7.6 phosphate buffer containing 1.0 % bovine serum albumin). The unbound fraction was removed by centrifuging with 500 µl dextran coated charcoal (0.125%) and the radioactivity remaining in the supernatant was measured using a

Beckman LS 5801 liquid scintillation system for radioactive counting as outlined in Jones and Swain (1996). Assay results were calculated using the data capture and analysis software installed on the Beckman LS 5801 scintillation counter. The limit of detection of the assay was 25 pg (4 $ng.ml^{-1}$ for a 50 µl plasma sample) and assay values were corrected for the extraction efficiency. The intra-assay coefficient of variation was 8.5 % and the interassay coefficient of variation was 11.6 %. [^{3}H]-Corticosterone was purchased from Amersham Life Sciences (UK) and corticosterone antiserum from Endocrine Sciences. Analytical reagent grade dichloromethane was purchased from Biolab Scientific Pty, Ltd (Victoria, Australia). Scintillation fluid (Ecolite +) was puchased from ICN (Costa Mesa, CA).

3. Endogenous rhythms of migratory behaviour

There were three groups of captive swift parrots involved in these experiments. First, there were adult birds that were juveniles when they were injured and brought into captivity, and thus had never migrated. Second, there were birds that had been found in very poor condition in the north of Tasmania during the previous winter and were presumed to be over-wintering birds. Third, there were injured wild birds brought into captivity in the breeding season in the south of Tasmania as adults and were presumed to have successfully completed at least one migration. Captive musk lorikeets, a resident/nomadic species, acted as control animals in the following experiments. The captive swift parrots and musk lorikeets were held in outdoor aviaries in Hobart, Tasmania and were thus exposed to the natural photoperiod and magnetic field (42°53' S, 147°19' E).

Open sky orientation and migratory restlessness experiments

The periods examined in the open sky orientation trials (and the expected orientation of swift parrots based on life history) were: April (northerly); May (northerly); June (no preference); September (southerly); and October-November (no preference). Six adult swift parrots were used repeatedly in all periods examined. Musk

lorikeets were expected to show no directional preference in any season. For each trial, five swift parrots and one musk lorikeet were placed simultaneously into outdoor individual orientation cages (Emlen funnel cones) with mesh tops for 40 mins between 10-12 am on clear sunny days. The Emlen funnel cones were constructed after the design described in Munro and Wiltschko (1993) with the following minor modifications. The recording paper was plain paper overlain with blue film carbon (Pelikan®), and the lid of the cone was white nylon mesh (~ 1 mm gauge) allowing the birds access to solar cues and providing good ventilation. A mist net was draped over the cages as additional security against escapes. The number of trials for each period varied depending on the number of clear, sunny days available for testing and ranged from two to five. After testing, I divided the recording paper into 24 sectors (15°/ sector) using a clear plastic template as an overlay and counted the number of scratches in each sector and the total scratches per cage. These data were used to assess orientation preference and migratory restlessness respectively.

Covered orientation and migratory restlessness experiments

The results of the open sky orientation and migratory restlessness experiments indicated that a confounding factor(s) may have influenced the birds' preferences. The most likely local attractor for birds in the open sky orientation experiments was suspected to be noise from the aviaries reflecting off a brick wall. Noise from con-specifics has confounded orientation experiments in yellow-faced honeyeaters *Lichenostomus chrysops* (U. Munro pers.comm.). The design of the experiments was therefore altered and a second series of experiments carried out. The next experiments were carried out using covered cages and at a site completely removed from auditory range of the aviaries. The orientation cones were covered with a white sheet to block visual cues, while other experimental protocols were similar to that detailed above. These experiments were conducted between 8-10 am each morning, regardless of cloud cover, but not in rainy weather. The periods examined in the covered cage orientation trials (and the expected orientation of swift parrots based on life history) were:

December-January (no orientation preference), March (northerly), April (northerly) and May (northerly). Musk lorikeets were not expected to show any directional preference. The swift parrots used in these experiments were either captive-bred birds that had never migrated; wild birds who had been injured as adults and had completed a migration; or wild birds who had been injured as juveniles and had never migrated. Different birds were used for each experimental period.

Covered orientation and migratory restlessness - night experiments

In April and May, a northerly orientation preference was expected, but no orientation preference was being shown in the morning experiments. In order to assess the possibility that swift parrots may be nocturnal migrants and only manifest orientation preferences and migratory restlessness at night, further experiments the experimental design was further modified. I carried out two orientation trials using the same protocol as described previously for the covered experiments, but these trials were carried out after sunset, between 8 and 10 pm. Night experiments were carried out only for two months due to the time constraints of the project. Musk lorikeets were not included in these trials.

Statistics

Significance level for all tests was set at $\alpha < 0.05$. All analyses, except that of orientation preferences, were carried out using Systat for Windows 7.0 (1997 SPSS Inc.).

Body weight, pectoral condition and fat scores

I assessed changes in mean body weight between seasons using single factor analysis of variance (ANOVA). *Post hoc* tests were carried out using Tukey's HSD tests. Pectoral condition scores and fat scores contained too few samples in all categories for categorical analysis of monthly or seasonal differences (Ott and Mendenhall 1985).

However, sufficient samples were obtained for Chi-square analysis to detect differences in fat scores between birds sampled in summer and spring.

Plasma corticosterone concentrations

All data points were initially log transformed to satisfy the assumptions of normality and homogeneity of variance. For the captive birds, differences in plasma corticosterone concentrations between months and between species of birds were analysed by multi-variate repeated measures analysis of variance, [(M)ANOVA]. Occasional missing data points resulted from insufficient plasma remaining for corticosterone analysis after reproductive hormones had been assayed. These were assigned the mean value for birds in that period, although no more than one such value was assigned to any sample set or individual bird (Mundry 1999). This resulted in complete data sets for monthly periods during the breeding season (spring and summer). However, during autumn and winter, monthly samples had to be combined to create a full data set for each respective season. The Pillai's trace statistic was used to determine the significance of any differences between sample periods because the uni-variate output of the SYSTAT programme is unsuitable due to a lack of independence of the data through time (C. Johnson pers. comm.). However, I could not use Pillai's trace when carrying out the (M)ANOVA on monthly plasma corticosterone concentrations in musk lorikeets: a multi-variate (M)ANOVA could not be produced because the number of trials was less than the number of subjects (L. Barmuta pers. comm.); thus, the uni-variate output was used instead.

A single factor ANOVA was used to assess the concentration of plasma corticosterone in wild swift parrots over the year. The effect of time to bleeding after capture on the plasma corticosterone concentrations in wild swift parrots was analysed using single factor ANOVA and *post hoc* analysis using Bonferroni tests. For this analysis samples were pooled across months.

Orientation and migratory restlessness experiments

Orientation data is a form of circular data with a continuous (von Mises) distribution; linear statistics are therefore unsuitable (Batschelet 1981). I calculated the orientation preferences of the birds by reducing the number of scratches in each sector to a mean vector. Cages with less than 40 scratches in total were excluded from analysis as unreliable (Munro and Wiltschko 1993).

The mean vector has two components, length (r) and direction (α). Possible lengths range from 0 to 1; larger numbers indicate that the observations are clustered more closely around the mean than lower numbers. The mean direction varies from 0° to less than 360°. The mean vector was calculated for each sample using a Zbasic 3.02 (Zedcor Inc.) program supplied by Dr. U. Munro.

There is no statistical method for calculating a group mean vector that takes into account both the direction and the length of the individual vectors (H. Dingle and U. Munro pers. comm.). Current statistical methods result in vectors with a low concentration of observations around the mean (r approaching 0) being treated with the same weight as vectors with a high concentration of observations around the mean (r approaching 1). To overcome this bias, I calculated the group mean vectors after excluding all individual vectors with an $r < 0.25$. In the open sky experiments, I also calculated group mean vectors after excluding all individual vectors with an $r < 0.5$. This was not carried out for the covered experiments as it resulted in too many samples being discarded to allow any analysis.

The group mean direction of vectors was analysed by Rayleigh's test for significant directional preferences, and Watson's F test was used for assessing differences between groups (Batschelet 1981). These analyses were carried out using Oriana for Windows v1.0 (Kovach Computing Services).

Migratory restlessness was quantified by assessing the total activity of each bird in its orientation cage. I counted the total number of scratches within each orientation cage. For the open-sky experiments, six adult swift parrots were used repeatedly in all periods examined; therefore a multi-variate (M)ANOVA was performed to assess for

significant changes in the periods examined and *post hoc* t tests were used to compare the periods. *A posteriori* multi-variate (M)ANOVAs were used to assess the effect of the birds' history; that is, whether the birds were captive or wild bred and whether the birds had completed a migration, never migrated or been found over-wintering in Tasmania.

For the covered orientation experiments different birds were used in each period. Therefore, two-way factorial ANOVAs were used to assess changes by period and the effects of the birds' history. A two-way factorial ANOVA was also used to check for any effect of orientation cage position on the results. *Post hoc* tests were carried out using Tukey's HSD tests.

RESULTS

1. Physiological changes

Body weight, pectoral muscle condition and fat score

There was significant variation in body weight in wild adult swift parrots between seasons (N = 143, $F_{(3, 139)} = 2.954$, $P = 0.035$). The mean body weight (± one standard error) in spring (68.2 ± 0.62 g) was significantly higher ($P = 0.032$) than in summer (62.9 ± 2.74 g) (Figure 2). Mean pectoral muscle condition score did not appear to vary seasonally although some samples sizes were too low to allow reliable categorical analysis (Figure 3). Fat score in wild swift parrots varied seasonally (Figure 4). The mean fat score (± one standard error) of birds in spring (1.4 ± 0.07) was significantly higher ($X^2_{(2)} = 13.749$, $P = 0.001$) than in summer (0.6 ± 0.21). Sample sizes in autumn were too low for reliable categorical analysis.

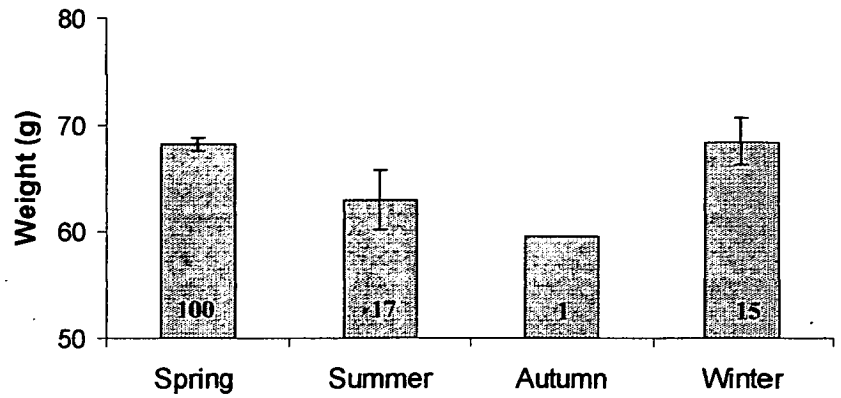

Figure 2. Seasonal variation in body weight in wild swift parrots. The figures within the columns indicate the sample sizes. Error bars represent one standard error.

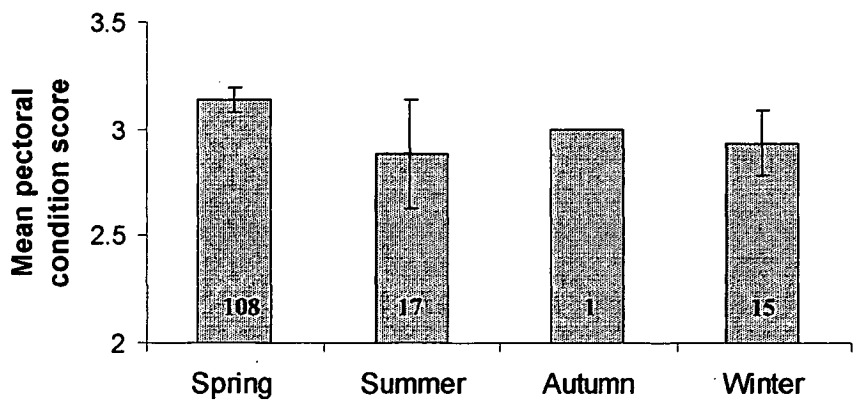

Figure 3. Seasonal changes in mean pectoral condition score in wild swift parrots. The figures within the columns indicate the sample sizes. Error bars represent one standard error.

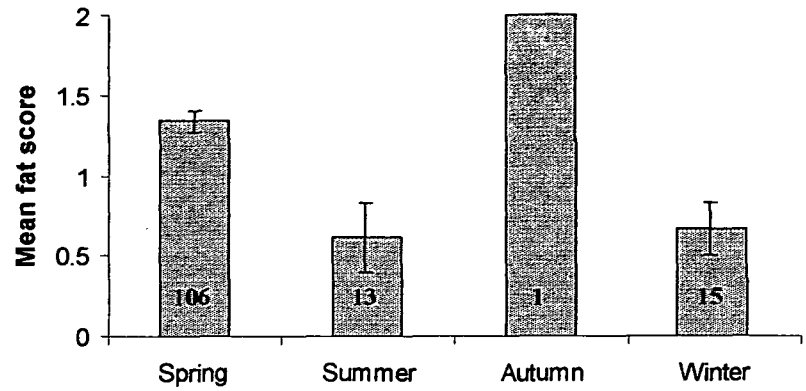

Figure 4. Seasonal changes in mean fat score in wild swift parrots. The figures within the columns indicate the sample sizes. Error bars represent one standard error.

2. Plasma corticosterone concentrations

Captive birds

There was no significant variation in plasma corticosterone concentration, in captive adult male swift parrots, when examined either by month during the year (n = 23, $F_{(7, 16)} = 0.648$, $P = 0.712$) (Figure 5), or by season (n = 23, $F_{(3, 20)} = 0.90$, $P = 0.459$) (Figure 6). Similarly, captive adult musk lorikeets showed no significant variation in plasma corticosterone concentration when examined either by month during the year (n = 5, $F_{(7, 28)} = 0.681$, $P = 0.687$) (Figure 5), or by season (n = 5, $F_{(3, 2)} = 1.571$, $P = 0.412$) (Figure 6). Plasma corticosterone concentrations did not vary significantly between swift parrots (n = 23) and musk lorikeets (n = 5) when compared either monthly during the breeding season ($F_{(7, 20)} = 0.635$, $P = 0.722$) (Figure 5), or by season ($F_{(3, 24)} = 1.404$, $P = 0.266$) (Figure 6).

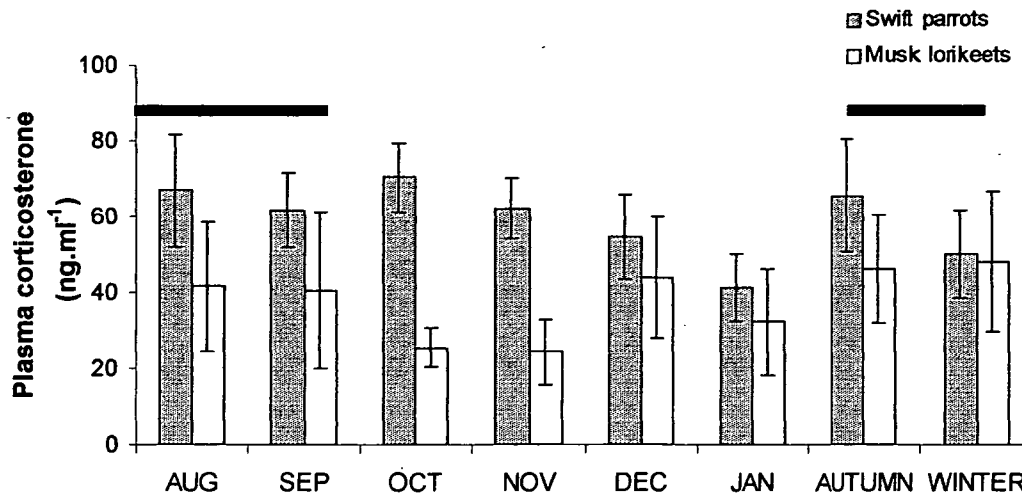

Figure 5. Plasma corticosterone concentrations in captive adult male swift parrots (n = 23) and adult musk lorikeets (n = 5) sampled repeatedly over a year. The bars indicate the migration periods. Error bars represent one standard error.

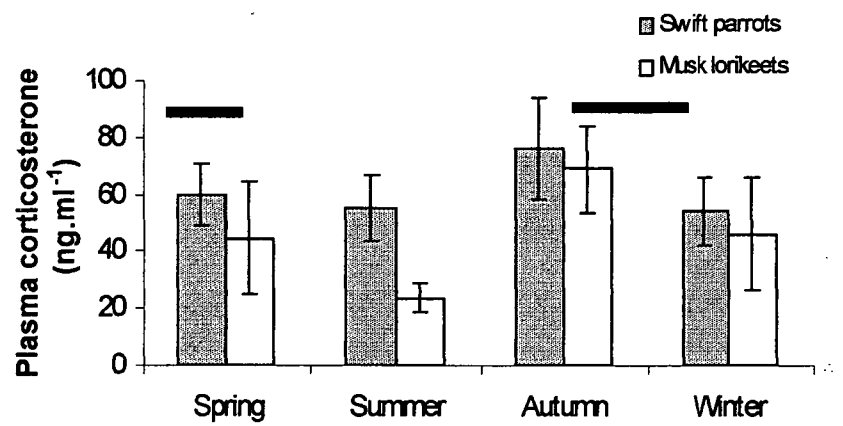

Figure 6. Seasonal changes on plasma corticosterone concentrations in captive swift parrots (n = 23) and musk lorikeets (n = 5). The bars indicate the migration periods. Error bars represent one standard error.

Wild swift parrots

The plasma corticosterone concentrations of all wild swift parrots showed a significant effect of the time to bleeding (n = 73, $F_{(3, 69)} = 15.511$, $P < 0.001$) (Figure 7). Samples taken in less than 5 minutes after capture (n = 11) had a significantly lower mean plasma corticosterone concentration (70.2 ± 10.39 ng.ml^{-1}) than all other samples ($P < 0.01$). Because of this strong effect of time after capture, any samples taken in more than 5 minutes after capture did not reflect basal concentrations of plasma corticosterone. There were insufficient blood samples taken less than 5 minutes after capture to examine the seasonal patterns of plasma corticosterone concentration in wild swift parrots.

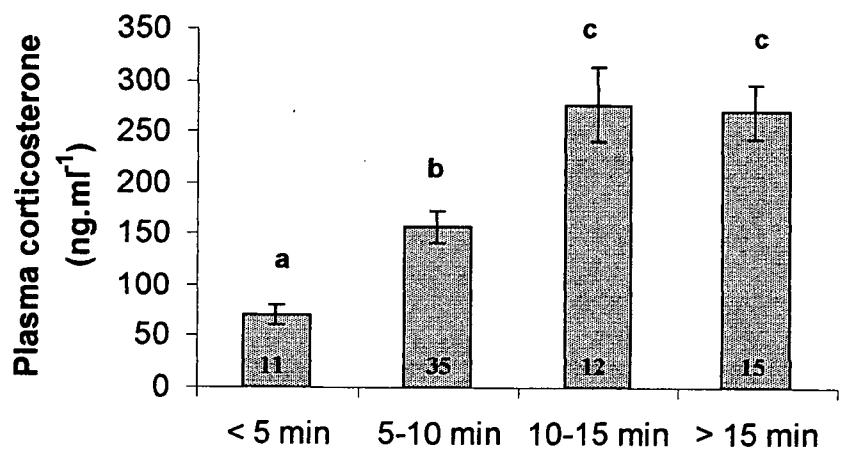

Figure 7. The relationship between time to bleeding after capture and plasma corticosterone concentrations in wild swift parrots sampled throughout the year. The figures within the columns indicate the sample sizes. Error bars represent one standard error. Columns with different letters are significantly different ($P < 0.01$).

3. Endogenous rhythms of migratory behaviour

Orientation data

Orientation in open sky cages

The results for the open sky orientation experiments, with all samples with a length of vector (r) less than 0.25 excluded (see Methods, Statistics), are summarised in Table 3 and Figure 7. The results are presented again (Table 4) with those samples with r less than 0.5 excluded. Regardless of the level of r used for exclusion, swift parrots showed a significant north to north-easterly directional preference in April ($P < 0.01$), May ($P < 0.05$), September ($P = 0.03$) and October-November ($P < 0.01$). No significant directional preference was seen in June ($P = 0.07$). Watson's F tests showed no significant variation ($P > 0.05$) between any of the periods examined.

Table 3. Orientation of swift parrots, with individual vector lengths (r) > 0.25, assessed in mesh topped Emlen funnel cones under clear skies between 10 to 12 am in Hobart, Tasmania.

	April	May	June	September	October-November
Total no. birds	14	6	18	13	19
No. of birds with r > 0.25	14	6	14	8	16
Mean direction	25.2°	33.4°	55.7°	6.4°	42.5°
Mean vector length	0.74	0.74	0.44	0.64	0.74
Rayleigh test of uniformity	< 0.01	0.03	0.07	0.03	< 0.01

Table 4. Orientation of swift parrots, with individual vector lengths (r) > 0.5, assessed in mesh topped Emlen funnel cones under clear skies between 10 to 12 am in Hobart, Tasmania.

	April	May	June	September	October-November
Total no. birds	14	6	18	13	19
No. of birds with r > 0.5	7	3	8	3	10
Mean direction	29.2°	61.0°	38.9°	327.9°	37.9°
Mean vector length	0.88	0.98	0.79	0.40	0.70
Rayleigh test of uniformity	< 0.01	0.04	< 0.01	0.66	< 0.01

Despite a low sample size, musk lorikeets (Table 5) also showed a significant north-easterly directional preference in September ($P = 0.04$). Although the musk lorikeets showed no directional preference ($P > 0.05$) in any of the other periods examined, examination of the scatter-plots (Figure 7) suggests strongly that a confounding effect was occurring.

Table 5. Orientation of musk lorikeets, with individual vector lengths (r) > 0.25, assessed in mesh topped Emlen funnel cones under clear skies between 10 to 12 am in Hobart, Tasmania.

	April-May	June	September	October-November
Total no. birds	3	5	3	4
No. of birds with r > 0.25	3	5	3	2
Mean direction	37.2°	34.0°	50.1°	58.2°
Mean vector length	0.80	0.50	0.97	0.96
Rayleigh test of uniformity	0.15	0.30	0.04	0.17

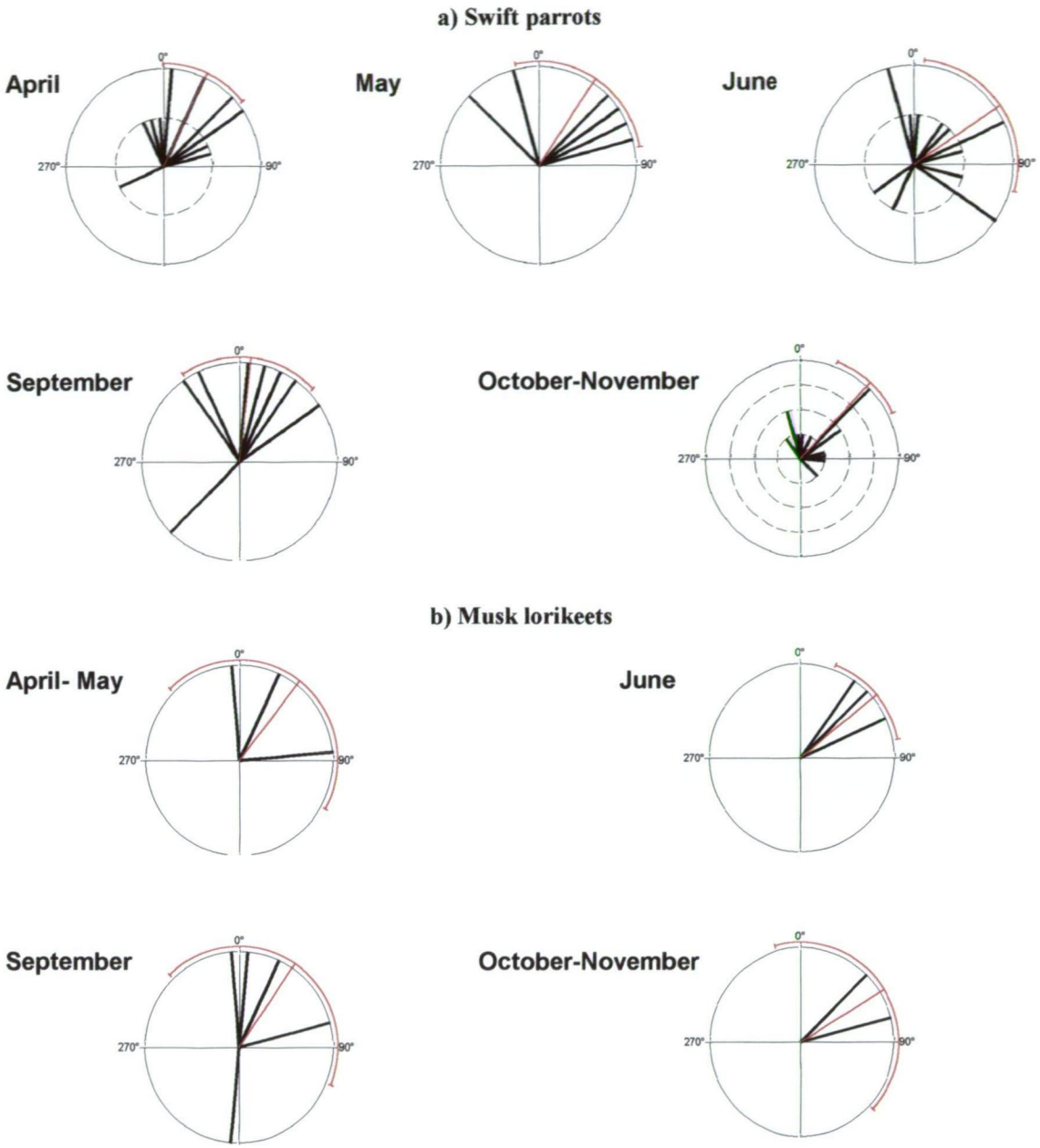

Figure 7. Orientation of a) swift parrots and b) musk lorikeets, with individual vector lengths (r) > 0.25, assessed in mesh topped Emlen funnel cages under clear skies between 10 to 12 am in Hobart, Tasmania. Black lines represent individual vector directions, red lines represent the mean direction of the vector and the red arc represents the 95% confidence interval for the mean direction. Each circle represents an n = 1 increase for the vector frequency.

Orientation in covered cages - morning experiments

The results of the daytime covered orientation experiments in swift parrots, with all samples with a length of vector (r) less than 0.25 excluded, are summarised in Table 6 and Figure 8. Swift parrots showed no significant directional preference during December-January ($P = 0.46$), April ($P = 0.10$) and May ($P = 0.74$). In March, there was a significant easterly preference (89.1°, $P = 0.02$). Musk lorikeets showed no significant orientation preference at any period during these tests ($P = 0.50$) (Table 6).

Table 6. Orientation of swift parrots, and musk lorikeets (column labelled ML), with individual vector lengths (r) > 0.25, assessed in covered Emlen funnel cones between 8 to 10 am in Hobart, Tasmania.

	December-January	March	April	May	ML
Total no. birds	7	9	19	7	7
No. of birds with r > 0.25	4	7	12	4	5
Mean direction	301.7°	89.1°	138.0°	140.4°	350.1°
Mean vector length	0.46	0.71	0.43	0.29	0.38
Rayleigh test of uniformity	0.46	0.02	0.10	0.74	0.50

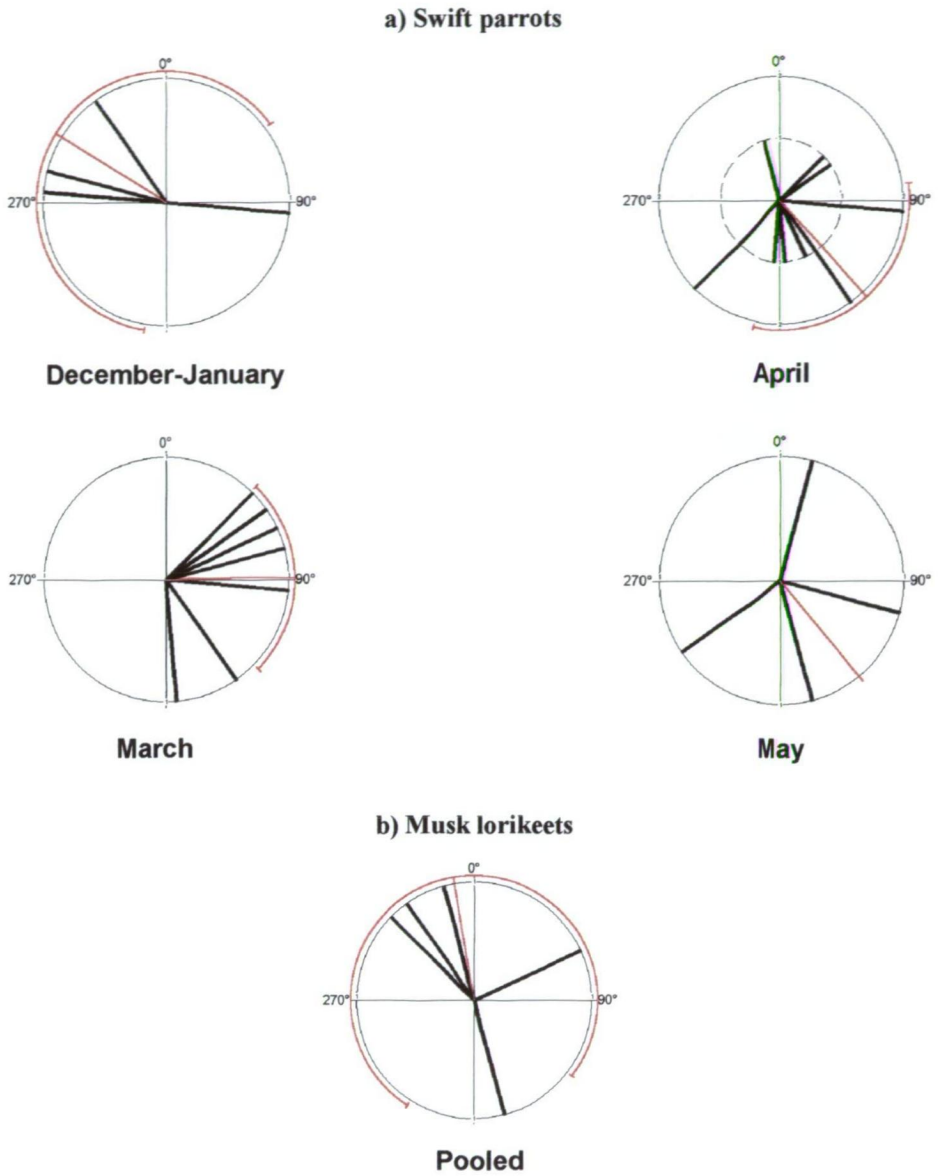

Figure 8. Orientation of a) swift parrots and b) musk lorikeets, assessed in covered Emlen funnel cages between 8 to 10 am in Hobart, Tasmania. Black lines represent individual vector directions, red lines represent the mean direction of the vector and the red arc represents the 95% confidence interval for the mean direction. Each circle represents an n = 1 increase for the vector frequency.

Orientation in covered cages - night experiments

The results for the covered orientation experiments carried out at night using swift parrots, with all samples with a length of vector (r) less than 0.25 excluded, are summarised in Table 7 and Figure 9. There was a significant northerly preference in the night trial (331.6°, $P = 0.04$) in April, although no significant directional preference ($P = 0.16$) was seen in the night trial in May. In April, swift parrots showed significantly different orientation at night to the day orientation in April ($F_{(13)} = 10.96$, $P = 0.01$), and March ($F_{(8)} = 13.27$, $P = 0.01$). Night-time orientation in May was significantly different to the day orientation in April ($F_{(15)} = 10.06$, $P = 0.01$), and March ($F_{(10)} = 5.94$, $P = 0.03$). There was no significant difference ($P = 0.15$) between day-time and night-time orientation in May.

Figure 9. Night-time orientation of swift parrots, assessed in covered Emlen funnel cages between 8 to 10 pm in Hobart, Tasmania. Black lines represent individual vector directions, red lines represent the mean direction of the vector and the red arc represents the 95% confidence interval for the mean direction. Each circle represents an n = 1 increase for the vector frequency.

Table 7. Night-time orientation of swift parrots, with individual vector lengths (r) > 0.25, assessed in covered Emlen funnel cones between 8 to 10 pm in Hobart, Tasmania.

	April	May
Total no. birds	6	6
No. of birds with r > 0.25	3	5
Mean direction	331.6°	6.4°
Mean vector length	0.99	0.60
Rayleigh test of uniformity	0.04	0.16

Migratory restlessness

Migratory restlessness in open sky cages

The activity of swift parrots within the orientation cages varied significantly between months (n = 7, $F_{(3, 4)} = 12.220$, $P = 0.018$). The birds that had over-wintered unsuccessfully had a different activity pattern to the other birds (Figure 10); therefore, these birds were excluded from further analysis. There was no significant difference (n = 5, $F_{(1, 4)} = 0.390$, $P = 0.70$) between birds that had migrated before and birds that never had the opportunity to migrate; these results are therefore pooled.

During April and May when the northerly migration occurs in the wild population, mean activity in captive birds (744.9 ± 79.33 scratches/cage) was significantly higher ($P < 0.05$) than in June (233.6 ± 44.72), September (408.6 ± 90.51), or October and November (314.3 ± 101.63). The periods when no migratory activity was expected (June, October and November) were not significantly different to the activity

within the cage in September (P = 0.059 and P = 0.584 respectively) when southerly migration occurs.

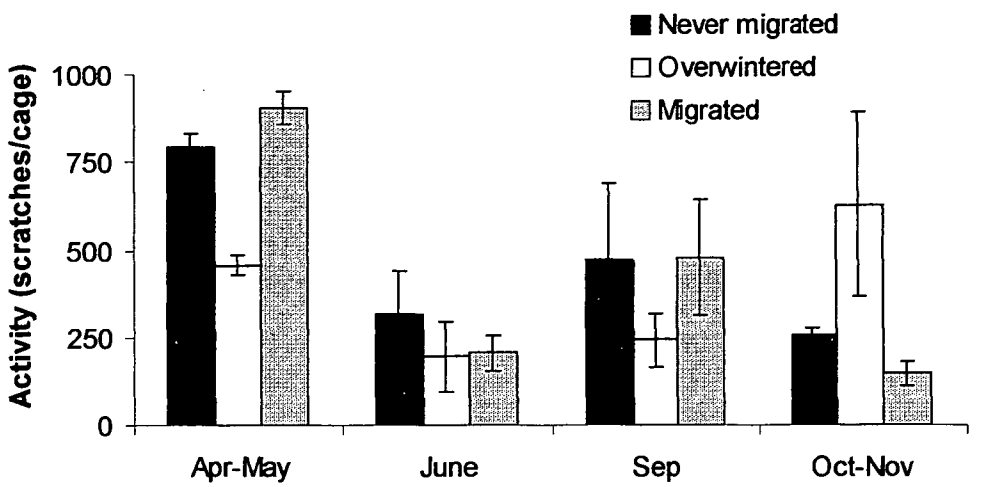

Figure 10. The effect of migratory history on the activity of swift parrots between months assessed in mesh-topped open orientation cages between 10 to 12 am on clear sunny days in Hobart, Tasmania. Sample sizes are: never migrated, n = 2; over-wintered, n = 2; migrated, n = 3. Error bars represent one standard error.

Migratory restlessness in covered cages - morning

There was no significant change in activity ($F_{(3, 19)}$ = 2.271, P = 0.113) in the periods examined (Figure 11). Birds that had previously migrated showed significantly greater activity ($F_{(1, 19)}$ = 5.251, P = 0.034) within the cages than birds that had never migrated. Neither the origin of the birds ($F_{(3, 19)}$ = 0.511, P = 0.483) nor cage position within the trials ($F_{(9, 16)}$ = 1.731, P = 0.203) had any significant effect on activity.

Migratory restlessness in covered cages - night

The activity of swift parrots in covered cones at night (Figure 12) in April (382.8 ± 115.51 scratches/cage) was not significantly different ($t_{(5)}$ = 0.252, P = 0.811) from activity at night in May (257.4 ± 113.80). There was also no significant difference

between morning and night activity in either April ($t_{(5)} = 0.916$, $P = 0.402$), or in May ($t_{(6)} = 1.233$, $P = 0.264$).

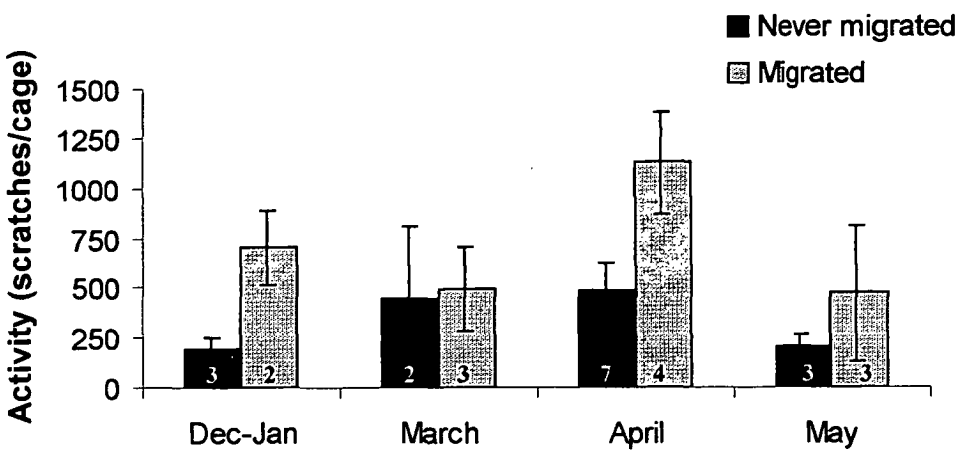

Figure 11. The effect of migratory history on the activity between months of swift parrots, assessed in covered orientation cages between 8 and 10 am in Hobart, Tasmania. Numbers inside the columns indicate sample sizes. Error bars represent one standard error.

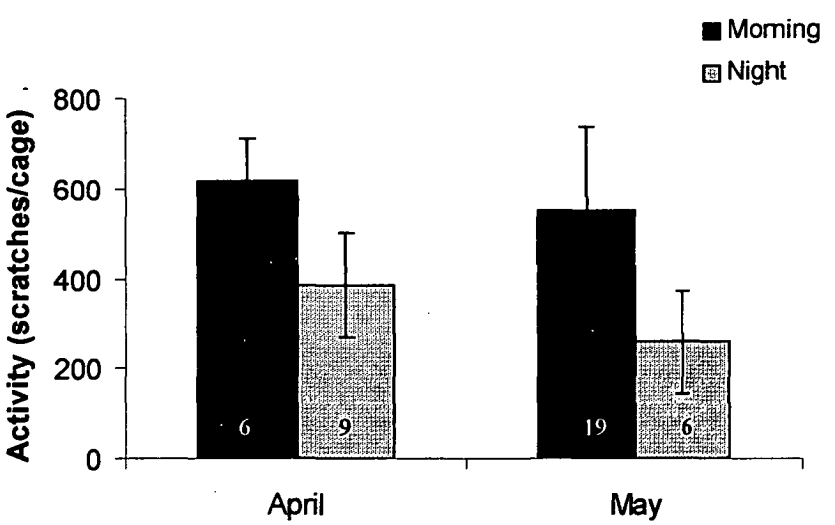

Figure 12. The activity between months of swift parrots in covered orientation cages, assessed between 8-10 am in morning trials and 8-10 pm in night trials in Hobart, Tasmania. Numbers inside the columns indicate sample sizes. Error bars represent one standard error.

DISCUSSION

Body weight and fat scores of wild swift parrots varied seasonally, with higher weights and fat scores in spring than in summer. This suggests that the changes in body weight and fat score are not associated with changes in preparation for migration. The storage of energy-rich fat to supply the demands of migration is more pronounced in species which migrate long distances and do not use stopovers, such as garden warblers, *Sylvia borin* (Bairlein and Gwinner 1994). Short distance migrations, like that of the swift parrots, will require proportionately less energy reserves (Bairlein and Gwinner 1994). The higher weights and fat scores seen in the swift parrots in spring are more likely to be associated with preparation for breeding and the abundance of food available in this season. The lower summer weights and fat scores may be explained by the depletion of energy reserves during the breeding season and/or the subsequent moult (Klasing 1998). Further study of these changes in birds in autumn and winter is required. In particular, changes in fat mobilisation and food intake in the weeks immediately prior to migration require study at a shorter time period than in this study.

Pectoral muscle condition changes rapidly in association with body mass and preparations for migration in long distance migratory birds (Bairlein and Gwinner 1994; Karasov and Pinshow 1998; Lindström et al. 2000). This increased muscle mass is thought to be primarily for the demands of flight, as the relative contribution of protein reserves to energy supply during migration is very low (~5 %) (Jenni and Jenni-Eiermann 1998). However, there was no seasonal pattern in the pectoral muscle condition of wild swift parrots. The short-distance migration of swift parrots may not require the muscular hypertrophy seen in these long distance migrants. Further, swift parrots remain highly active and nomadic in the non-migratory periods, which may account for the lack of observed change in muscle condition over seasons. Alternatively, the subjective assessment I used in this study may be too coarse to detect subtle variations and the use of quantitative methods such as ultrasonic measurement of pectoral muscle depth (Lindström et al. 2000) may be needed.

In a migratory warbler during the migratory period, migrants show elevated baseline corticosterone which may facilitate migratory fattening (Holberton 1999). Plasma corticosterone concentrations have been correlated with migratory preparation in passerines (Holberton 1999) and seabirds (Piersma et al. 2000), although direct causative relationships between migration and corticosterone concentrations remain to be established. The captive musk lorikeets, representing a resident and nomadic species, showed no distinct annual variation in plasma corticosterone concentrations. This pattern is dissimilar to captive starlings, where basal plasma corticosterone concentrations decreased during the period of the moult (Romero and Remage-Healey 2000), but is similar to the lack of seasonal variation seen in captive *Zonotrichia* sparrows (Marra et al. 1995). However, contrary to expectations, no seasonal changes in plasma corticosterone concentration were observed in the captive swift parrots. This suggests that no major physiological changes are required for migration in this species and is consistent with the lack of pre-migratory fattening observed. It is, of course, possible that patterns of plasma corticosterone concentration in wild swift parrots differ from those in the captive birds. However, in long distance migratory species, such as red knots, *Calidris canutus*, captivity does not conceal these rhythms (Holberton 1999), although the amplitude of variation in plasma corticosterone concentrations does decrease with continued captivity (Piersma and Ramenofsky 1998).

As observed in the captive birds, plasma corticosterone concentrations in the wild swift parrots showed little variation through the year. However, many values were considerably higher (~ 300 $ng.ml^{-1}$) than those observed in captive birds (~ 60 $ng.ml^{-1}$). It appears that these results were confounded by inevitable variations in time to sampling among these mist-netted birds. When plotted against time to sampling these values demonstrate the rapid elevation of plasma corticosterone that also occurs in short term stress responses in *Zonotrichia* sparrows (Astheimer et al. 1994) and starlings (Romero and Remage-Healey 2000). The confounding effect of acute stress on the plasma corticosterone concentration in many of the wild swift parrots means that it is not possible to present an accurate picture of changes in basal plasma corticosterone

concentrations through the annual cycle. However, the plasma corticosterone concentrations in wild birds sampled in less than five minutes are similar to the concentrations seen in the captive birds. This suggests that the patterns of plasma corticosterone seen in captive swift parrots are not confounded by acute stress responses. More study is needed of the plasma corticosterone concentration variations in wild birds. However, the lack of migration-associated variation in plasma corticosterone concentration, body weight, fat reserves and pectoral muscle condition observed in the swift parrots in this study suggest that little physiological preparation for migration occurs.

Endogenous circannual rhythms of the migratory behaviours of orientation preference and migratory restlessness have been demonstrated in captive birds of both long and short distance migratory species (Berthold 1984; 1996; Gwinner 1996; Munro and Munro 1998; Wiltschko et al. 2001). Orientation preferences and migratory restlessness are thought to reflect the controls, respectively, of the direction and distance travelled during migration (Berthold 1996; Gwinner 1996). I expected to observe these behaviours in captive swift parrots during the periods of spring (August and September) and autumn (March to May) migrations.

There have been no previous studies of migratory behaviour in parrots. The behaviour of the parrots within the Emlen cages is different for that reported in passerines species (Berthold 1984; 1996; Gwinner 1996; Munro and Munro 1998; Wiltschko et al. 2001). The parrots did not simply hop towards a preferred direction and slide down the recording paper as passerines are reported to do. Instead, both swift parrots and musk lorikeets used their hook bill to grab the edge of the cage and scrabble with their feet in an attempt to escape the cage. With the proviso that there was no variation in the fitting of the cage lid, this behaviour should still reflect orientation preferences. Some parrots were able to cling to the mesh-topped lid, and thereby avoid contact with the recording paper. Future studies where visual orientation cues are required should use a transparent lid that does not provide such footholds.

The unexpected north-easterly orientation of swift parrots in open mesh-topped cages was also shown by the musk lorikeets. This strongly suggests that the results were confounded. The flaw in the experimental design was placing the orientation cages within auditory distance of the other birds being held in the aviary, which has confounded orientation experiments in yellow-faced honeyeaters *Lichenostomus chrysops* (U. Munro pers.comm.). Therefore, I must discount the results of those trials. The covered orientation trials are, however, considered to be free of confounding effects: in this experimental system no significant orientation preferences were observed in musk lorikeets, the non-migratory control species. Hence, it was surprising that the only strong directional preference shown by swift parrots was an easterly preference in March, when a northerly preference reflecting migration from Tasmania to the mainland of Australia was expected. These results are difficult to explain. It is possible that the swift parrots may be nocturnal migrants. In April, a strong northerly orientation was seen at night. However, these results provide only tentative support for this theory, and further studies are required over the complete migratory period. While diurnal variation in migratory restlessness is associated with the time of day, or night, of migration (Munro and Munro 1998), there are no studies examining diurnal variation in orientation preferences.

Migratory restlessness is not usually assessed simultaneously with orientation, but, instead, is assessed by locomotor activity within cages (Gwinner 1996; Munro and Munro 1998). In nocturnal migrants, such as warblers and flycatchers, migratory restlessness is greater at night (Gwinner 1996) and, although the numbers of studies are limited, activity increases during the day in diurnal migrants (Munro and Munro 1998). Swift parrots showed significant seasonal variation in activity within the orientation cones, with peaks associated with migratory periods, however this activity did not vary significantly between the morning and night experiments.

Detection of the migratory behaviours of preferred orientation and restlessness may be complicated in swift parrots by the observed variation between individuals in departure and arrival dates. Individual birds will show large variation in the timing and

duration of orientation preferences if the current models of the control of migratory behaviour (Berthold 1999) are valid in this species. This variation may be further complicated by the relatively short duration of migration in swift parrots. Although no reliable data are available, estimates suggest that the birds are able to cross Bass Strait in as little as six hours (Brown 1989). The persistence of migratory behaviour in swift parrots may, therefore, be significantly shorter than in long distance migrants.

The effect of the birds' migratory history on migratory restlessness suggests that some component of the restlessness in swift parrots is either learned or reinforced by having completed a migration. However, birds bred in captivity showed similar patterns of activity to those bred in the wild, suggesting that there is an endogenous rhythm to migratory restlessness in swift parrots. This is similar to orientation behaviour seen in captive regent honeyeaters that had never migrated (Cooke and Munro 2000). This suggests that captive-reared swift parrots should retain the behavioural mechanisms needed for migration if released into the wild. This is of considerable importance to the captive-breeding and re-introduction program for the orange-bellied parrot, and future studies should assess the inheritance of migratory behaviour in this endangered species.

The birds that did not show the expected patterns of migratory restlessness were originally obtained in poor health over-wintering in the north of Tasmania. It is possible that these birds represent a low proportion of the swift parrot population that does not migrate. However, both of these birds were obtained with clinical evidence of central nervous system damage, probably caused by malnutrition. An alternative explanation for their altered patterns of restlessness is that there was irreversible damage to the central nervous system, although no other clinical signs of nervous dysfunction were detectable following the birds' recovery. If these birds truly represent individuals with the genotype for over-wintering, their use in future captive breeding and re-introduction programs should be restricted, unless the decision to establish a non-migratory population of the swift parrots is contemplated. Reliable feed sources would have to be established in Tasmania throughout the winter for this strategy to be successful.

In summary, seasonal changes in body weight and fat reserves in wild swift parrots appeared to reflect the accumulation and subsequent depletion of reserves associated with breeding, rather than the demands of migration. Neither pectoral muscle condition nor plasma corticosterone concentrations showed the clear seasonal pattern of change expected in a migratory species. This is probably due to the relatively short nature of the migration and the highly mobile and nomadic nature of the species. However, this study has revealed changes in migratory restlessness, and, to a lesser extent, orientation preferences, that correspond to periods of expected migratory activity, suggesting the presence of endogenous rhythms driving the annual migration of the species. The implications of this study for the conservation of swift parrots will be discussed further in Chapter 12.

Chapter 11. Mortality in wild swift parrots

INTRODUCTION

Population declines occur by two demographic mechanisms, which are reduced breeding success and reduced survival (Green 1994). The majority of this thesis has concentrated on investigations into limitations on reproduction; however, this chapter details investigations into mortality of wild swift parrots. Knowledge of the causes of mortality and the demographics of survival within a population are necessary to understand the population dynamics of a species (Clobert and Lebreton 1991), and essential if population trends are to be predicted (Green and Hirons 1991).

Bird mortality due to collisions with artificial structures, such as windows, fences, motor vehicles and powerlines, has become increasingly recognised as a significant cause of wild bird mortality (Dunn 1993; Bevanger 1994; Mumme et al. 2000; Osburn et al. 2000). A study of bird deaths caused by window strikes in the USA estimated that there are 1-10 birds killed annually for every building in North America (Dunn 1993). There are few similar studies of Australian bird mortality. However, it is clear that such collisions are a regular cause of mortality of swift parrots in Tasmania (Brown 1989; Brereton 1996a).

In the first detailed study of wild swift parrot mortality, between 1981 and 1986, 37 birds were submitted as casualties to the National Parks and Wildlife Service in Hobart, mostly as a result of window collisions (Brown 1989). In the 1987/88 season, 17 birds (10 of which died) were submitted to the National Parks and Wildlife Service in Hobart after colliding with windows, fences or motor vehicles (Brown 1989). Predation on swift parrots was also assessed (Brown 1989). The remains of swift parrots were recovered from 57% (19/33) of the eyries of *Falco peregrinus*, the peregrine falcon, seven nests of *Accipiter fasciatus*, the brown goshawk, and three nests of *Accipiter cirrhocephalus*, the collared sparrowhawk (Brown 1989), although the number of *Accipiter spp.* nests examined was not recorded. Brown (1989) also includes an anecdotal report of predation of nestlings by *Dacelo novaeguineae*, the laughing kookaburra, and cat predation is mentioned as common, although no further information is provided. Brereton (1996a) estimated that about twenty swift parrots are submitted to the National Parks and Wildlife

Service in Hobart each year from window collisions, but no makes further reference to causes of mortality.

The aims of this study were:

a) to characterise the causes of mortality in wild swift parrots in their breeding grounds in Tasmania;
b) to characterise the nature of injuries sustained by swift parrots in collisions with windows, fences or motor vehicles; and
c) to assess the demographics of the wild swift parrot population involved in collisions and the effect of this cause of mortality on population survival.

The causes of mortality in captive swift parrots, over the course of this study are detailed in Appendix B. Understanding captive mortality is important if captive breeding is to held in reserve as a conservation management tool to ensure against extinction, such as the captive breeding and re-introduction program for the orange-bellied parrot.

MATERIALS AND METHODS

Collection of samples

Twelve dead wild swift parrots used in this study were birds that were submitted to National Parks and Wildlife Service prior to June 1998 and stored frozen. From June 1998 until January 2001, all swift parrots that were found and submitted to the National Parks and Wildlife Service were forwarded to the School of Zoology, University of Tasmania in Hobart as soon as possible. If such transfer could be done within three days of the date of death, then the birds were stored at 4°C until the post mortem examination was performed. Where immediate transfer was not possible, birds were frozen. To increase the number of carcases retrieved a request for assistance was mailed to all veterinary surgeons in Tasmania. A total of thirty-eight carcases were collected between June 1998 and January 2001. The gonads and gastro-intestinal tract from two additional specimens were obtained from Toowoomba in Queensland during the winter of 1998. Fifty-two wild birds were obtained overall.

Post mortem examination

The post mortem examinations were conducted systematically based on recommendations from avian veterinary references (Latimer and Rakich 1994; Dorrestein 1997). The birds were weighed (± 0.01 g) and pectoral condition (Chapter 10; Table 1) and fat score (Chapter 10; Table 2) were assessed. External morphological measurements were taken and the stage of moult was recorded. Any external abnormalities, such as haemorrhage, fractures, or bruising were noted. Plumage characteristics were recorded and used to assess the sex and approximate age of the bird (see Appendix A). Feathers were removed from the ventral body surface and the head to allow assessment of the skin and underlying muscles for bruising and punctures. The bird was then placed in ventro-dorsal recumbency and the abdomen opened. The abdominal incisions were continued cranially and laterally through the ribs and coracoid, allowing the sternum to be lifted off as a single piece and exposing the coelom (Figure 1).

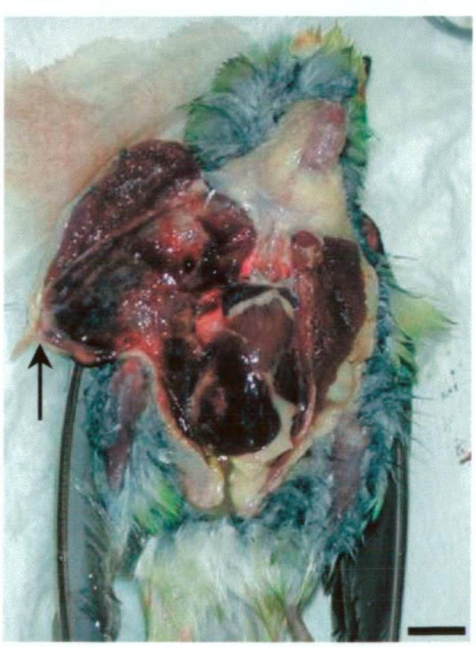

Figure 1. Exposure of the coelomic viscera by reflection of the sternum during post mortem examination of a swift parrot. Arrow points to the reflected keel. Scale bar represents 10 mm.

After visual appraisal of the organs *in situ*, the visceral mass, consisting of liver, spleen and gastro-intestinal tract, was removed. This was achieved by incising the distal oesophagus where it entered the proventriculus and lifting the visceral mass ventrally, incising the attaching air sac membranes and finally incising the cloaca at the vent. This exposed the heart, lungs, gonads and kidneys for appraisal. The skull and sinuses were opened to assess these areas for signs of trauma or disease. Sex was confirmed by examination of the gonads, which were measured and weighed. In male birds, a fine needle aspirate of the left testis was performed. The aspirate was smeared onto a microscope slide and stained with a modified Wrights stain to assess the stage of spermatogenesis (see Chapter 2 for details).

Samples of gut contents from crop and proventriculus were preserved in 70% ethanol. Smears of gut contents from crop, proventriculus, duodenum and intestine were made onto microscope slides and assessed for the presence of pollens (Chapter 6) and gastro-intestinal parasites.

In specimens that had not been frozen, samples of all organs were taken and placed into Bouin's preservative for 24 hours and then transferred to 70% ethanol, embedded in paraffin wax and cross-sectioned (6μm) using a rotary microtome. Sections were stained with haematoxylin and eosin and then examined microscopically. Histological preparation of these samples was carried out at the Department of Veterinary Pathology at Murdoch University. These were used to further characterise gross changes and aid in diagnosing the cause of death. Testicular samples were also used to assess the stage of spermatogenesis for comparison with the fine needle aspirates of testicular tissue.

RESULTS

Causes of mortality in wild swift parrots in their breeding grounds in Tasmania

The most common cause of death in wild swift parrots was trauma (71.1 %; n = 37). Three distinct traumatic causes of death were differentiated in this study, predominantly by the case history and post mortem findings. These were classified as window strike (n = 26), fence strike (n = 5) or motor vehicle impact (n = 6).

Predator attacks accounted for 7.7 % of the deaths (n = 4). There were two types of predator attack confirmed as causes of mortality. Cat attack resulted in the

deaths of two individuals. Both birds died of secondary bacterial infections between 24 and 48 hours after being rescued from cats' mouths; neither bird showed evidence of window strike injury prior to predation. There were also two confirmed cases of an attack by an unidentified raptor on swift parrots in urban areas. In both cases, the raptors were scared away from their prey after having caught it successfully. One swift parrot was dead and partially consumed when found; the second bird lived for six days before dying of a massive hepatic abscess.

Infectious agents accounted for 7.7 % of the deaths (n = 4) and were diagnosed by histology. All four of these swift parrots were found in the vicinity of a feeding table in Spreyton that attracts an abnormally high density of birds. Gross post mortem changes were not sufficient to identify this cause of mortality, except in the single case, where death of a juvenile swift parrot was caused by a pulmonary fungal infection, aspergillosis (Figure 2). In this case there were characteristic white fungal plaques in the air sacs and lung. One death was from proventriculitis with megabacteria and a septicaemic bacterial disease of unknown species. The third death in a banded bird was consistent with chlamydiosis (*Chlamydophila psittaci*). One juvenile bird died after a window strike but was infected with *Cryptosporidium* species in the bursa of Fabricius.

A cause of death could not be identified in 13.5 % (n = 7) of birds. This was either as a result of advanced tissue autolysis (n = 5) or a lack of diagnostic changes (n = 2) on both gross post mortem and histology.

The nature of injuries sustained by swift parrots in collisions

The gross post mortem changes that occurred in window and fence strikes were similar with the most common injuries corresponding to impact occurring on the sternum or to the head. Such injuries included: pectoral muscle bruising (n = 6); pulmonary congestion and haemorrhage (n = 13); cardiac haemorrhage and/or rupture (n = 4); fracture of the thoracic girdle (clavicle, coracoid and/or keel) (n = 5); rupture of oesophagus and/or jugular vein (n = 7); wing fractures (n = 4); liver and kidney haemorrhage (n = 4); head trauma (n = 10) and spinal injuries (n = 2). Some birds survived the initial impact only to die in captivity several days later as a result of secondary bacterial or fungal infections (n = 4).

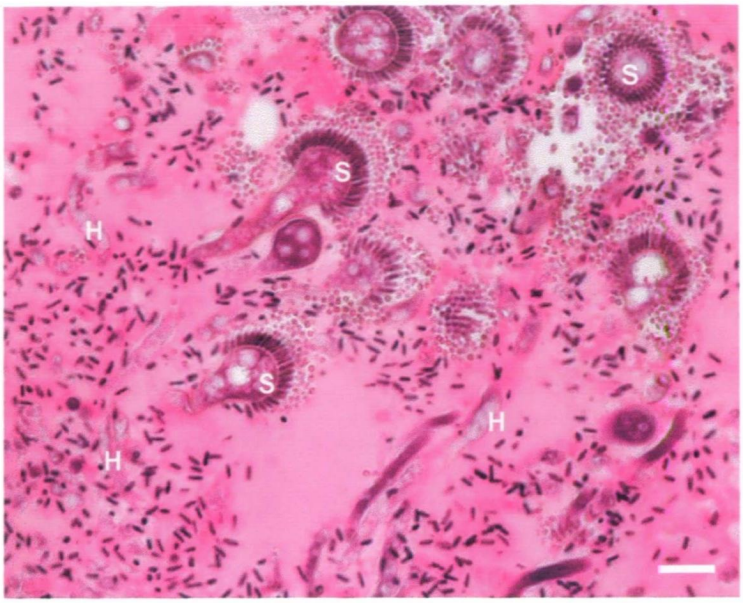

Figure 2. Histological appearance of a granuloma caused by *Aspergillus sp.* in the lung of a swift parrot. Note the sporogonia (S) and the septate hyphae (H). Scale bar represents 20 μm.

Motor vehicle impacts tended to result in more severe crushing injuries and included: crushed skull (n = 2); organ rupture (n = 4); cardiac haemorrhage and/or rupture (n = 2); pulmonary haemorrhage (n = 3); keel fracture (n = 3); leg amputation (n = 1); and spinal fracture (n = 1).

The demographics of the wild swift parrot population involved in collisions

Of the thirty-seven swift parrots confirmed to have died from window collisions, there were three juvenile females, eleven adult females, two juvenile males and twenty-one adult males. Therefore, the adult sex ratio was 1.9: 1.0 (males: females).

DISCUSSION

Swift parrot mortality due to collisions with artificial structures during the breeding season in Tasmania is removing a significant proportion of the healthy adult breeding population annually. Taking the highly conservative assumption that the

number of birds submitted approached the actual population mortality due to collisions, then approximately 1% of the estimated total population is affected annually. If this sample represents 10% of the actual population mortality due to collisions, then the rate increases to 10% annual mortality due to collisions of the estimated total population. The true figure probably lies somewhere between these extremes. The predominance of mortality due to traumatic collisions is partially due to the method in which this survey was performed. A bias inevitably results from an unavoidable reliance on the submission of dead birds by members of the public. This means that a specimen was more likely to be submitted if it was in an urban area and found intact. Therefore, deaths that occurred in rural or bush settings were unlikely to be noticed. Consequently, deaths from predation are likely to be highly underestimated in this study.

This study provides no insights into causes of death during the migratory periods, or the winter. Mortality during migration causes significant losses in long-distance migrants, especially among juveniles (up to 35 % mortality) (Owen and Black 1991). However, there are no comparable studies for short-distance migrants. The results of this study cannot, therefore, be extrapolated to give any indication of the annual mortality, because there is no reliable estimate of the percentage of birds killed that are submitted. Although public awareness of the endangered status of the parrot is high in Tasmania, there is very limited recognition of the bird itself, and some confusion occurs with the similar sized and coloured musk lorikeet.

The incidence of infectious disease in the wild swift parrots is notable for two reasons. Firstly, an incidence of a range of diseases was associated with a congregation of birds at a feeding station in Spreyton in the north of Tasmania. This study was not able to determine if the diseases were caused by the large concentration of birds at that site, and/or the poor hygiene practiced at the feeding station. An alternate explanation may be that these diseases exist in the wild population at a very low frequency and the number of birds congregating in the vicinity of the feeding table simply resulted in the greater probability of recovering the dead birds.

Importantly, this study found no evidence in wild swift parrots of several diseases that are endemic in other wild psittacine birds in Tasmania. In particular,

there was no evidence of infection with psittacine circovirus, a debilitating virus that is endemic in a range of Australian parrots (McOrist 1984; Raidal et al 1993). Recently, psittacine circovirus infection was diagnosed in wild musk lorikeets, *Glossopsitta concinna*, in the Hobart region (Gartrell et al. 2000b). These birds often feed in the same locations as swift parrots, so it seems unlikely that the swift parrots are not exposed to the virus at some stage in their migratory route. It is possible that the virus does not produce any histological changes in swift parrots and serological surveys may be required to detect exposure (Raidal et al. 1993). However, the virus does not appear to be causing mortality or recognisable disease in the adult population surveyed. A single case of psittacine circovirus has been reported in a nestling swift parrot (Raidal et al. 1993) that may have been taken from the wild (Raidal pers. comm.), and it is possible that the disease is a cause of nestling mortality.

Internal parasitic disease was also not present in the wild swift parrot population. In captivity, swift parrots are particularly susceptible to ascaridiosis (Appendix B; Laubscher 1999); however, no evidence of these parasites was found in wild swift parrots. I detected gastro-intestinal parasitism with the coccidia, *Eimeria dunsingi,* in wild musk lorikeets but not in the swift parrots (Gartrell et al. 2000b).

It is possible that the swift parrots' nomadic and migratory life history reduces the incidence of infectious disease in the population. The success of these types of disease is higher when repeated exposure of the host to an environment contaminated with the disease agents occurs (Gerlach 1994; Greiner and Ritchie 1994). The swift parrots' movements are such that the roosts and nest sites are rarely used annually and never continuously, thus potentially breaking the life cycle of many infectious disease agents.

The nature of the injuries in the birds killed by impacts with windows and fences reveals that not all birds are colliding headfirst with these objects. The high frequency of sternal fractures, combined with pulmonary and cardiac haemorrhages indicates that many of these birds are striking the objects with their sternum. This suggests that the birds are seeing the objects and attempting to pull up prior to the strike. Hence, increasing the visibility of windows and fences should help to reduce

the mortality. Understanding the nature of injuries sustained in collisions is useful for veterinarians dealing with injured birds. Chest injuries are difficult to diagnose in live birds; it is recommended that this possibility should be considered when assessing and treating birds involved in collisions.

There was a marked male sex bias to the mortality of wild birds due to collisions (1.9:1). The same sex bias was found in wild swift parrots trapped by mist-netting (Chapter 2). While the trapping bias may be influenced by males being more territorial, the window collision data suggests that this is a true population sex bias. An adult male sex-bias is common in many species of monogamous bi-parental birds (Breitwisch 1989). However, the origin of the sex-bias in swift parrots was not identified in this study, suggesting that a previously unrecognised, but significant, mortality occurs outside the urban breeding habitat. A possible sex-biased cause of mortality that should be investigated further is predation. Females may be more exposed to predation due to their relative sedentariness during breeding; such female-biased mortality is seen in the threatened New Zealand parrots, the kaka, *Nestor meridionalis*, and the kakapo, *Strigops habroptilus* (Higgins 1999). There are no large nest-hole predators in Tasmania, however raptors may be drawn to the vicinity of nest-holes by the frequent arrival and departure of the male bird or the vocalisations of the chicks when hatched. Increased mortality of females during winter and migration is also possible and further study is needed.

Demographic studies of some endangered species have previously suggested that juvenile mortality is a key problem area in the life cycle (Perrins 1991; Owen and Black 1991). A study of motor vehicle collisions in the endangered Florida scrub-jay, *Aphelocoma coerulescens*, found that juvenile mortality in territories that included roads exceeded the production of yearlings (Mumme et al. 2000). Surprisingly, the results of my study in swift parrots were strongly biased towards adult mortality. This probably represents the low incidence of breeding that occurred in the wild during the three year study period. However, it must again be noted that predation was strongly under-represented in this study, and no assessment of migration mortality could be made. These two causes of mortality are much more likely to affect juveniles due to a combination of inexperience and reduced flight and foraging ability (Perrins 1991; Owen and Black 1991). Evaluation of both these

causes of mortality are technically difficult. Future studies of migration mortality in juveniles will only be possible with intensive banding projects (Owen and Black 1991) or when sufficient miniaturisation of satellite telemetry devices has been achieved.

In summary, this study has determined that the annual mortality of adult swift parrots due to collisions with artificial structures is likely to be having a significant effect on the swift parrot breeding population. However, this urban mortality does not explain the sex bias found in the wild population and a previously unrecognised, but significant, mortality must occur outside the urban breeding habitat. Further, the wild population of the swift parrots are relatively free of disease and disease is unlikely to be contributing to the swift parrot decline.

Chapter 12. General Discussion

It has become increasingly difficult to preserve habitats in their pristine state, and attempts to conserve a species by relying on habitat protection are unlikely to be successful without a detailed ecological knowledge of that species' requirements (Perrins 1991). The overall scientific aim of this thesis was to investigate the constraints on breeding success and survival of swift parrots as possible mechanisms of the population decline. The applied aim of this thesis was to assess the suitability of the current draft Recovery Plan (Brereton 2001) in ensuring the survival of the swift parrots.

The Swift Parrot Recovery Plan (Brereton 2001) focuses on habitat preservation and restoration as a primary focus for the conservation of this species. The rationale behind this strategy is that the swift parrot acts as a flagship species for the protection of forest ecosystems, particularly the Tasmanian blue gum and box-ironbark forests of south-eastern Australia, but also benefits other threatened and uncommon species including the forty-spotted pardalote, *Pardalotus quadragintus,* regent honeyeater, *Xanthomyza phrygia,* brush-tailed phascogale, *Phascogale tapoatafa,* squirrel glider, *Petaurus norfolcensis* and painted honeyeater, *Grantiella picta* (Brereton 1999). The swift parrots are an excellent indicator species for the overall health of this diverse array of habitats because of their nomadic and migratory life-cycle. They move sequentially through these habitats at periods that should correspond to the peaks of resource availability, and their nomadism allows them to adjust to temporary fluctuations in these resources. The population decline of the swift parrots suggests that large areas of the woodlands of Tasmania and south-eastern Australia have undergone such severe degradation and fragmentation that the flexibility of the swift parrots' movements can no longer compensate for local variations in resources.

The recovery strategy for the swift parrots is based on the assumption that the swift parrot population will be able to maintain its present numbers if the destruction and fragmentation of its habitat is reduced or stopped. In the long term, once restored habitat matures, it is hoped that the swift parrots will be able to increase their numbers. The results of this study strongly support the current direction of the

conservation effort, but raise some concerns as to the validity of these underlying assumptions. The overall objectives of the Recovery Plan are to change the conservation status of the swift parrots from endangered to vulnerable within ten years, and to achieve a demonstrable sustained improvement in the quality of swift parrot habitat to increase carrying capacity. However, in order to achieve these objectives, it will be necessary to understand the important features of the habitats used by the swift parrot. This investigations detailed in this thesis focussed not on the large-scale habitat degradations, but instead the features of the habitat used by the swift parrots, and particularly on the breeding habitat, that must be preserved to maintain the population.

The recovery actions listed in the Draft Swift Parrot Recovery Plan for 2001 - 2005 (Brereton 2001) are as follows :

"1. Identify the extent and quality of foraging habitat
2. Manage swift parrot habitat at a landscape scale
3. Reduce the incidence of collisions
4. Population and habitat monitoring
5. Community education and information
6. Manage the recovery process through a recovery team."

The implications of this research to the first four recovery actions are discussed below, as well as consideration of some alternative conservation measures not currently under consideration by the Recovery Team.

Action 1. Identify the extent and quality of foraging habitat
Action 2. Manage swift parrot habitat at a landscape scale

The results of this study suggest that the priority in the identification and management of the existing habitat should focus on identifying and preserving the flowering resources available within the breeding range of swift parrots, and, more particularly, on those trees which flower in the period between September to December. The Tasmanian blue gum appears to be important to swift parrots because of the readily available amounts of protein and energy that the swift parrots can

harvest quickly from its flowers. However, because of the annual variability in the timing and intensity of the eucalypts' flowering, it is a highly unreliable source of food. The trees partition their available resources between growth and reproduction (the production of nectar and pollen) (House 1997). The implication is that older trees, which are likely to partition more resources into reproduction and generally have larger canopies and more flowers (House 1997), should have priority for preservation. Priority should also be given to those habitats in close proximity to known nesting areas, and to those trees that are known to reliably flower biennially and within the critical period for the swift parrots' reproductive activity.

New plantings of Tasmanian blue gums are being established in south-eastern Tasmania, and outside the eucalypts' normal range in the north of Tasmania in order to provide some long-term safeguards for the swift parrots. The expected time for these trees to produce sufficient flowering to enable their use as significant foraging resources is 30-40 years (House 1997). It would be beneficial to ensure that the phenotype of these plants is one that will provide the maximal resources to the swift parrots in the critical period for reproduction (September to December) as regularly as possible. While it is recognised that flowering frequency is a product of environmental variables and genotype, the selection of blue gum genotypes that produce these traits should be investigated. The swift parrots' need for resources during the periods of incubation and chick-rearing also has implications for the restoration of grassy blue gum habitat. For this habitat to be of value to breeding swift parrots, it must be located within easy commuting distance from suitable nesting sites. The availability of nest-hollows should be assessed in years of abundant blue gum flowering, as demand for nest-hollows by the swift parrots should increase when constraints imposed by food resources are removed. If the congruence of good foraging resources and natural nest-hollows cannot be achieved, an alternate strategy to improve reproductive success may be to place nest-boxes in proximity of areas of anticipated flowering. Good success has been achieved with this technique in wild orange-bellied parrots, *Neophema chrysogaster*. In swift parrots, this strategy would involve a large input of resources and is suited as a last resort. It may also be used to allow closer study of reproduction in wild swift parrots.

This study also emphasises that habitats both in Tasmania and on the south-eastern mainland of Australia should not be preserved solely to ensure nectar provision. The specific requirements of the swift parrots differ between habitats. Pollen and insects, such as psyllids, are of major importance in providing protein for the swift parrots. The use of pollen to supply protein may explain the correlation between swift parrot numbers in Victoria in winter and the flowering golden wattles (Mac Nally and Horrocks 2000). I have observed foraging on golden wattles in both wild and captive swift parrots, but nitrogen balance experiments are required to determine if the parrots are digesting the pollen protein. This is a good example of how the specific requirements of the birds can influence their distribution.

Action 3. Reduce the incidence of collisions

Collisions with artificial structures were confirmed as a major cause of mortality to adult swift parrots in urban areas during the breeding season. Reduction of this mortality is needed, especially in years of poor flowering when the birds congregate in urban areas. However, this type of mortality did not cause the sex bias observed in the swift parrot population. This suggests that other, less obvious, causes of mortality may be acting on the swift parrot population, and in particular, on the females. The impact of predation at nest-hollows and migration mortality requires further investigation.

Action 4. Population and habitat monitoring

This study strongly emphasises the need for ongoing monitoring of both the swift parrot population and the habitat. In particular, it will be important to monitor the effects of climate change on the phenology of food resources as well as the distribution and loss of foraging habitat. Exclusively migrant populations, such as the swift parrot, may have lost the flexibility to respond to changes in the broad-scale environment (Berthold 1999). It is worthy of note that the two full migrant psittacines, the orange-bellied parrots and the swift parrots are both endangered (Garnett and Crowley 2000). Psittacine species which are resident in Tasmania throughout the year, (eg. *Glossopsitta concinna,* the musk lorikeet, and *Platycercus caledonicus,* the green rosella) are common and their populations appear to be stable.

This raises the possibility that migration is no longer a viable strategy for population survival given the recent anthropogenic large-scale disruption to these species' winter foraging grounds that has occurred in the south-eastern regions of Australia. Further, it is possible that the fully migrant species, the swift parrots and the orange-bellied parrots, have lost the flexibility to return to a partial migrant or resident strategy and that this has been a factor in their population declines.

The results of this study are not strong enough to predict the possible responses of the population of swift parrots to continued changes to the environment. The threats facing the species are the spatial and temporal limitation of resources. Spatial limitation occurs because destruction and fragmentation of foraging habitat in both the breeding and over-wintering ranges of swift parrots is likely to continue. The widespread distribution of the species during the winter period suggests that their mobility and nomadism will allow them to respond to the current diminution of resources in this period. However, the concentrated distribution of the species in the breeding period and the links between their reproduction and the large-scale flowering of the Tasmanian blue gum suggests that the breeding habitat is more critical. However, the temporal variations of this resource place further restrictions on the swift parrots. The Tasmanian blue gum flowers only biennially at best and the timing of flowering within a flowering year varies considerably. In 1999, there was heavy flowering of *E. globulus* in south-eastern Tasmania but the majority of flowering occurred in winter prior to the arrival of the swift parrots. It is currently unknown, whether this early flowering represents normal seasonal variations or is a response to global warming. If early flowering becomes a regular event, the endogenous migratory rhythms of the swift parrots may preclude its earlier arrival in its breeding grounds in Tasmania. Increasing spring temperatures may advance the plant phenology of food resources, particularly *E. globulus,* beyond the ability of the swift parrots to adjust their migratory schedules. In a European long distance migrant, the pied flycatcher *Ficedula hypoleuca,* adjustment to climate change is constrained by its date of arrival in its breeding grounds (Both and Visser 2001). Increasing spring temperatures over the past two decades have advanced tree phenology in its breeding habitat, and hence insect abundance, which has resulted in many bird species advancing their egg-laying date. However, the ability of the

migratory flycatcher to advance its breeding has been constrained by its endogenous migratory rhythms (Both and Visser 2001). The flexibility of migration in swift parrots should be studied further.

Continued monitoring of the wild swift parrot population for disease is recommended. The wild swift parrots were generally free of diseases that are endemic in other wild psittacine birds in Tasmania, including psittacine circoviral disease (PCD), which has been found in the orange-bellied parrots (P. Holz *pers. comm.*). It is possible that the swift parrots' nomadic and migratory life history reduces the incidence of infectious disease in the population. However, the congregation of the swift parrots in Tasmania during the breeding season means that if a disease epidemic takes hold in the wild, it is possible a large percentage of the population may be exposed. Those diseases that are mostly likely to have a catastrophic effect on the wild population are exotic viral diseases, such as the herpes virus that causes Pacheco's disease in parrots (Ritchie 1995). The transmission of disease from captive birds to wild populations has recently been confirmed by the diagnosis of PCD in wild South African Cape parrots, *Poicephalus robustus robustus* (Perrins 2000). Preventing the introduction of diseases like this requires strict quarantine controls on birds entering Australia and may be beyond our present diagnostic abilities (Ritchie 1995). Monitoring wild populations for the emergence of disease is currently lacking in Australian wildlife management (Ford et al. 2001).

Alternate conservation measures

Supplementary feeding

Supplementary feeding has been used to enhance the breeding success of some endangered species, including the kakapo (Higgins 1999), the Florida scrub-jay (Schoech 1996) and the orange-bellied parrots (Garnett and Crowley 2000). Both the initiation of reproduction and its successful completion in swift parrots are dependent on the availability of foraging resources. The period of egg production and incubation is a critical bottleneck for resources in the swift parrots. The male swift parrot is solely responsible for the provision of resources to the female during incubation and, in the first two weeks after hatching, to the female and the chicks. To maintain this high demand for food resources, nesting must occur in close proximity

to good foraging resources (Brereton 1996a). Theoretically, the provision of supplementary food in proximity to nesting sites could improve breeding success in the swift parrots. However, this study found an incidence of a range of infectious diseases was associated with a congregation of birds at a feeding station in Spreyton, in the north of Tasmania. This emphasises the need for hygiene at feeding stations. Further, the nutritional requirements of the birds should be taken into consideration when providing supplementary food, and this area requires further study as was evidenced by the problems with diet outlined in Chapter 7.

Captive breeding

There are no provisions in the Swift Parrot Recovery Plan for the use of captive breeding in the recovery of the swift parrot, as it is hoped that the effective management of the wild population will be sufficient to ensure the species survival (Brereton 1999). A small breeding program has been established at Adelaide Zoo, predominantly with the aim of enhancing community awareness of the status of the swift parrot. However, it will develop techniques that could form the basis of a captive breeding program (Digney 2001). Captive breeding programs are expensive to maintain and their effectiveness has been questioned (Restani and Marzluff 2001) despite some notable successes, such as in maintaining the population of the orange-bellied parrots. The World Conservation Union suggests captive breeding should be established if the population size falls below 1000 individuals (Restani and Marzluff 2001).

However, if captive populations are to be kept as a last redoubt for the swift parrot then more development of care and management techniques is needed. The swift parrot is not an easy species to maintain in captivity, and breeding in the captive colony at the School of Zoology was limited to captive-bred birds. This suggests that establishing well-managed captive populations should occur now, while the situation is not critical. The *caveat* on this suggestion is that the genotype of captive populations must be drawn from successful migrants, and constantly updated with infusions of genetic material from wild populations. If predictions about the effects of climate change on migratory species prove true (Both and Visser 2001), then the wild population will be exposed to increasing selection pressure on the

genotypes for migration. If captive populations are to be held in reserve for future replenishment or re-introduction programs, they must keep pace with continuing evolution (Frankel and Soule 1981). Our current knowledge of the behaviour and physiology of migration does not allow us to identify or track the evolution of migration in the swift parrot, or the other migratory psittacines.

The survival of re-introduced captive swift parrots to the wild needs to be studied, particularly in relation to the birds' ability to complete migration successfully. This study has determined that migratory behaviour most likely has a strong genetic basis and that captive reared birds should have the necessary behavioural mechanisms to allow migration. The success of such naive migration must be assessed before conservation effort is invested. Developing our understanding of migration in these species is an urgent research priority.

In conclusion, the population decline of the swift parrot is an important indicator of the degraded state of the dry forests and woodlands in south-eastern mainland Australia and Tasmania. The swift parrot is a robust species with few health problems in the wild population. The species' nomadic life history and ability to survive on a variety of food sources ensures they are able to overcome most local variations in food supply. However, there are key stages in their life history where they appear to be critically dependent on the variable food resource provided by the Tasmanian blue gum, in particular, during the initiation of reproduction and the rearing of young. The recovery of this species will be dependent on the successful management and restoration of the habitat in all of their migratory range, including, most critically, the grassy blue gum woodlands of south-eastern Tasmania.

Study of the swift parrot has provided unique opportunities to gain insight into a range of biological phenomena. The swift parrots' morphological and physiological adaptations to nectarivory allow greater understanding of the strategy of nectarivory in birds. The study of the migratory behaviour and physiology of the swift parrots allows the formulation of many questions about why migration is so rarely used by the Psittacidae. Finally, the swift parrots irregular, seasonal breeding, allows some comprehension of the ecological and evolutionary importance of the mechanisms by which environmental variables may synchronise, stimulate or inhibit reproduction.

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Appendix A.

Identifying the sex and age class of Swift Parrots *Lathamus discolor* by morphology: development of a field key

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(Submitted to Emu: Austral Ornithology)

RH: Sexing and ageing Swift Parrots by morphology

Abstract.

Accurate identification of sex and age class is crucial in order to maximise the information collected from wild birds. The differences between the sexes and age classes of Swift Parrots, *Lathamus discolor*, are subtle. The aim of this study was to develop a simple and reliable method of sexing and ageing of Swift Parrots by morphology. The recommendations of the only previous study of these differences were unreliable when applied to birds of known sex and age. I examined morphological features of Swift Parrots of confirmed sex and, in some cases, known age. There were significant differences between the sexes of adults in the appearance of an underwing bar, patterns of red feathering on the chest and neck, and iris colour. The head length, beak length, body length and wing chord of adult male Swift Parrots were significantly larger than adult females. Juvenile birds were recognisable by a brown iris until eight months of age and their beak length and wing chord were significantly shorter than adult birds. Despite the significant sex differences identified, serious problems remain in the development of a field key. Accurate sex identification in some individuals will require either visual inspection of the gonads or DNA analysis.

Introduction

Accurate identification of sex and age class is crucial in order to maximise the information that can be collected from wild birds. Accurate identification of sexes is also necessary for successful captive management, and to allow regulators to confirm that birds kept in captivity match their records. Although sex can be determined by endoscopy or DNA analysis these techniques are, respectively, either too invasive or too costly. A simple morphology-based key that would allow reliable determination of sex and age class would offer a great advantage, particularly to field studies. This is especially important in studies of rare species where identification of population structure is necessary for conservation management eg. population viability assessments (Lebreton and Clobert 1991).

The only previous study of morphological differences between sex and age classes in Swift Parrots was presented by Bartram (in Higgins 1999). In this study of museum specimens, Bartram concluded that:

1) Ageing - Three age classes were identified: juveniles, immatures and adults. Juveniles had more pointed tips to their primary wing feathers and differed in the colour of their bill, iris and legs, although the age at which adult colouration was achieved was not determined. Immature birds were considered to be those with adult plumage but still possessing the underwing bar, and lacking red on the centre of the belly and lower breast. Juveniles and immature birds of both sexes were smaller than adults in tail and bill length ($p < 0.01$) and always had an underwing bar.

2) Sexing - Adult females were duller than adult males, although most differences were subtle. A yellow underwing bar was present in adult females, but was always lacking in adult males. The bill of adult females was usually duller and darker than that of adult males, and the iris paler yellow. Adult females were also smaller than males in wing chord, tail length and bill length ($p < 0.01$). Juveniles were difficult to sex: some juvenile females lacked red on the throat, whereas this was always present in males; female immature birds were duller than males.

However, a number of problems were identified when we applied this scheme to captive birds of known sex. Firstly, we found the colour differences in the beak and iris were unreliable for differentiating between adult males and adult females. The relative measure of the dullness of the plumage of females was only apparent

when a bird of the other sex was available for direct comparison. The use of the presence or absence of the yellow underwing bar to distinguish between adult and immature males was also unreliable; some mature male birds retained either a full or partial wing stripe into their third year. Bartram's ageing scheme also exhibited circular logic. The basis of the classification of immature adult males was that these birds have adult plumage but possess an underwing bar; then concluded that all immature males had an underwing bar. I also found that attempting to distinguish sex based on the morphological measurements used by Bartram (in Higgins 1999) was unreliable. While the mean measurements of wing chord, tail length, bill length and bill width in males and females were significantly different, the degree of overlap in the range of these measurements meant that determination of the sex of individual birds remained unreliable. Further, I noted that morphological measurements of feather lengths could be influenced by feather wear and breakage. This was particularly noticeable in recently fledged juveniles where the tails were often shortened due to breakage within the nest-hollow. It is unclear whether this contributed to the significantly shorter tail length seen in Bartram's study of museum specimens.

The aim of this study, then, was to develop a simple and reliable method of sexing and ageing of Swift Parrots by morphology. Ideally such a method should be reliable in the hands of a person inexperienced in the species, and applicable in the field.

Methods

Seventy-three dead Swift Parrots were dissected. These birds were either wild birds killed by accidental collisions with windows, cars and motor vehicles, or birds that had died in captivity. Visual inspection and histological examination of the gonads determined the sex of each bird, but age was unknown. Birds were grouped as either adults or juveniles based on the iris colour. Juvenile birds are reliably identifiable by their brown iris colour (Higgins 1999). The sex of thirty-six captive Swift Parrots was determined by endoscopic examination of the gonads under general anaesthesia, and the age of each bird was recorded where known. From both groups of birds, the following characteristics were determined for each individual.

Categorical characteristics

Underwing bar

Three categories for the appearance of the underwing bar were identified.

Full underwing bar = a yellow bar of varying thickness extending across the remiges from the first to the seventh primary.

Partial underwing bar = a yellow bar evident on less than seven of these primary remiges.

No underwing bar = a complete absence of the underwing bar on any primary remiges.

Neck pattern

The pattern of the red neck feathers was divided into two categories.

Collar = the pattern formed when the red feathers extended laterally in a half-circle from the base of the red neck.

Bib = the lack of the half-circle extending from the red of the neck.

Red chest flecking

The colour of the chest feathers was divided into two categories.

Flecking = presence of any red feathers upon the chest or abdomen. Flecking covered a spectrum from two or three red feathers to nearly 50 % of the feathers being red.

No flecking = the absence of red feathers on the chest or abdomen.

Iris colour

Four categories of iris colour were identified; pale yellow, golden and orange were seen in adult birds. Only three adult birds were found with orange irises, so these birds had to be excluded by statistical analysis. Brown irises were seen in all birds (n = 6) less than 8 months of age.

Under-tail covert colour

There was considerable individual variation in under-tail colour. The patterns were broadly classified into five groups based on the predominant pattern: green, red, red with yellow fringe, orange and mixed colours.

Numerical characteristics

All body measurements, except weight, were measured with Vernier callipers (± 0.2 mm). Weights were measured using Salter scales (± 0.1 g). Head length was measured from the poll to the furthest extent of the closed beak. Beak length was measured from the first feathers above the cere to the tip of the beak. Forehead blue extent was measured from the first feathers above the cere to the tip of the most caudal blue feather on the forehead. Body length was measured from the crown of the head to the tip of the pygostyle, with the neck extended and the body flattened. The tail was not included in this measurement to prevent variations associated with feather wear and breakage. Wing chord was measured from the folded proximal tarsus to the tip of the first primary feather.

Statistics

Categorical data were reduced to frequency tables and analysed by Chi-square analysis. Numerical data were analysed by ANOVA using Systat 7.0 for Windows. No transformations prior to analysis were necessary.

Results

Categorical characteristics - adult birds

The differences between the sexes in the frequency with which these categorical characteristics occur is presented in Table 1. Although there are significant sex differences, there was considerable overlap between the sexes in all characteristics except underwing bar. There were significant differences between the sexes in relation to the underwing bar ($X^2 = 37.28$, df = 2, $p < 0.005$), the neck pattern ($X^2 = 10.19$, df = 1, $p < 0.005$) and the occurrence of red chest flecking ($X^2 = 6.73$, df = 1, $p = 0.01$). Only two males and one female bird had orange coloured irises; data from these birds were excluded from Chi-square analysis as their expected frequencies made the analysis unreliable. A significant sex difference occurred in the frequency of occurrence of yellow and golden iris colour ($X^2 = 4.94$, df = 1, $p = 0.025$). Results of the analysis of under-tail colour had greater than 20 %

of expected frequencies with n < 5 and Chi-square analysis was therefore unreliable, although some sex differences are apparent in the data.

Table 1. Observed frequency distributions (% of total for gender) of selected morphological characteristics of adult male and female Swift Parrots. * represents a significant difference between the sexes (P < 0.05).

Characteristic		Observed frequency Male (n= 62)	Female (n = 28)
Underwing bar*	Full	22.6	89.3
	Part	17.7	10.7
	None	59.7	0
Neck Pattern*	Collar	56.5	19.2
	Bib	43.5	80.8
Chest flecking*	Yes	46.8	16.7
	No	53.2	83.3
Iris colour*	Golden	69.0	37.5
	Pale yellow	31.0	62.5
Under-tail colour	Green	0	47.1
	Red	35.0	11.8
	Red with yellow fringe	50.0	23.5
	Mixed	5.0	11.8
	Orange	10.0	5.9

Categorical characteristics - juvenile birds

Only nineteen juvenile birds of known sex were available for this study. This low sample size precluded reliable categorical data analysis (Ott and Mendenhall 1985). Descriptions of under-tail colour were only recorded for three male and three female juvenile birds, making conclusions based on this data unreliable. The observed frequencies of the selected characteristics in these juvenile birds are presented in Table 2. Brown irises were seen in all birds (n = 6) less than 8 months of age.

Table 2. Observed frequency distributions (% of total for gender) of selected morphological characteristics of juvenile male and female Swift Parrots.

Characteristic		Observed frequency Male (n= 8)	Female (n = 11)
Underwing bar	Full	62.5	100.0
	Part	37.5	0
	None	0	0
Neck Pattern	Collar	50.0	10.0
	Bib	50.0	90.0
Chest flecking	Yes	25.0	0
	No	75.0	100.0
Under-tail colour		(n = 3)	(n = 3)
	Green	66.6	0
	Red	33.3	0
	Red with yellow fringe	0	0
	Mixed	0	100
	Orange	0	0

Numerical characteristics - adults

The mean measurements of adult male Swift Parrots were significantly larger than those of adult females in relation to head length, beak length, body length and wing chord. No significant differences ($P > 0.05$) were detected between the sexes of adult Swift Parrots in weight, forehead blue extent and tarsal length (Table 3).

However, despite the significant difference in the mean values for head length, beak length, body length and wing chord, there was considerable overlap was present in the ranges of all the variables measured (Figure 1).

Table 3. Comparison between the sexes of numerical characteristics examined in adult Swift Parrots (N = 61). Measurements are given as means (± one standard error). N.S. indicates no significant difference between the sexes ($P > 0.05$).

	Female (± one s.e.)	Male (± one s.e.)	F	P
Head length (mm)	33.2 (± 0.27)	33.8 (± 0.12)	3.873	0.05
Beak length (mm)	14.7 (± 0.19)	15.3 (± 0.14)	4.401	0.042
Forehead blue extent (mm)	12.9 (± 0.58)	14.1 (± 0.35)	2.485	N.S.
Body length (mm)	119.7 (± 1.64)	128.9 (± 1.45)	14.935	<0.001
Tarsal length (mm)	16.0 (± 0.42)	16.6 (± 0.16)	2.136	N.S.
Wing chord (mm)	121.8 (± 0.99)	125.1 (± 0.76)	5.404	0.024
Weight (g)	60.2 (± 2.63)	61.9 (± 1.62)	0.259	N.S.

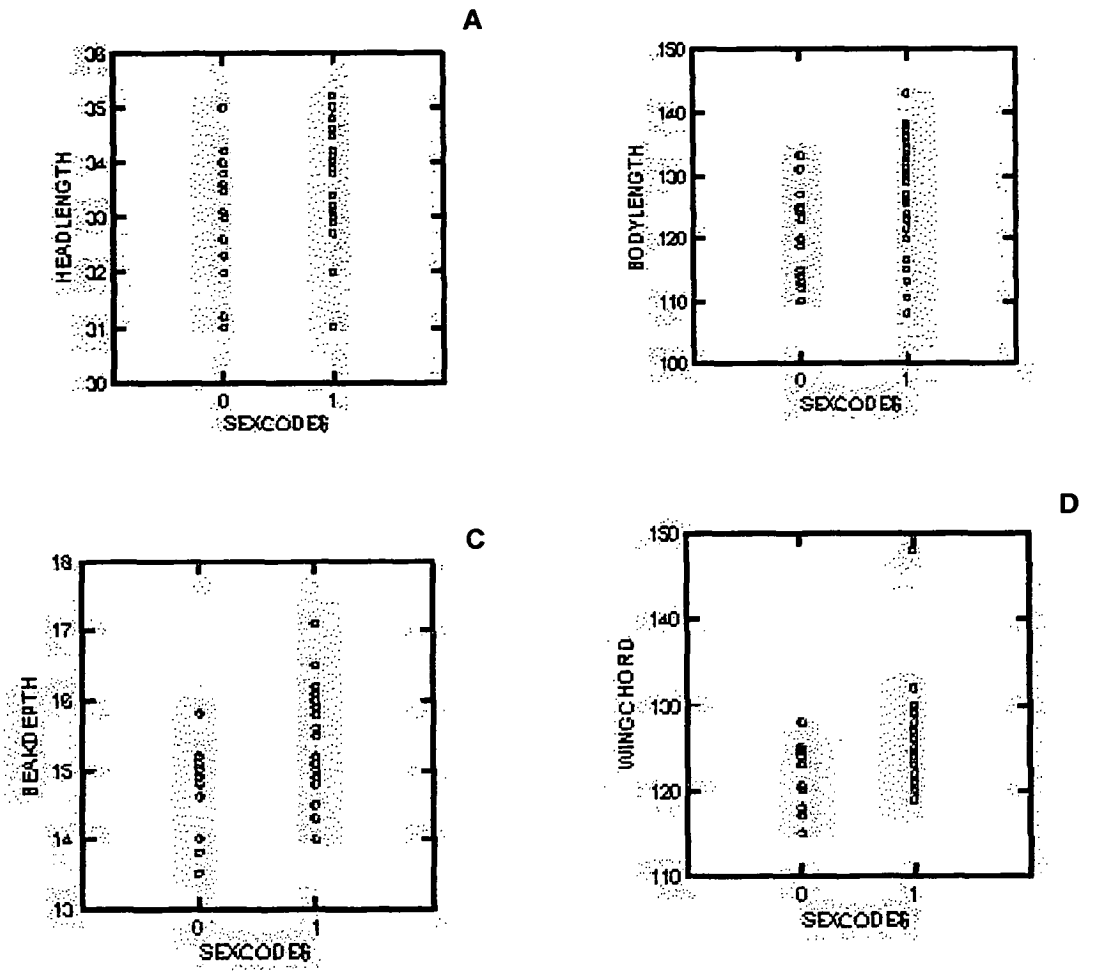

Figure 1. Scatter-plots of the distribution of: a) head length (mm), b) beak length (mm), c) body length (mm), d) wing chord (mm) in adult female (sexcode = 0; n = 18) and male (sexcode = 1; n = 41) Swift Parrots.

Numerical characteristics - juveniles

In the juvenile birds (n = 9) there were no significant differences between the sexes in any of the characteristics examined. Juvenile birds had significantly smaller beak lengths (n = 53, df = 1,49, F = 4.142, P = 0.047) (Figure 2); and wing chords (n = 65, df = 1,61, F = 5.842, P = 0.019) (Figure 3) than adult birds.

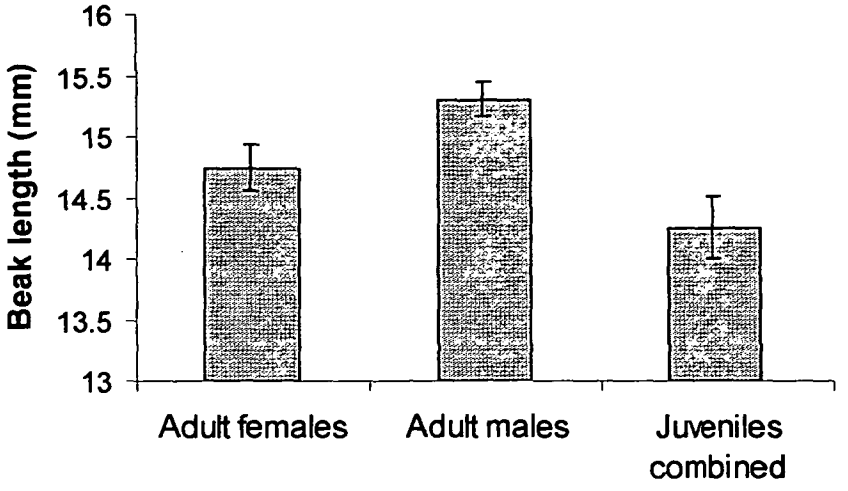

Figure 2. Mean measurements of beak length in adult female, adult male and juvenile Swift Parrots. Error bars represent one standard error from the mean. All three groups are significantly different ($P < 0.05$) from each other.

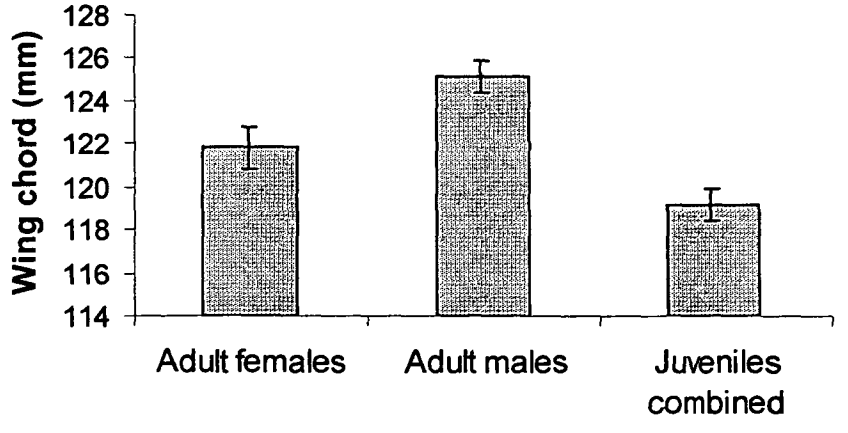

Figure 3. Mean measurements of wing chord in adult female, adult male and juvenile Swift Parrots. Error bars represent one standard error from the mean. All three groups are significantly different ($P < 0.05$) from each other.

Discussion

This study has confirmed that there are discernible morphological differences between adult males and females of Swift Parrots, and between adults and juveniles. Adult male Swift Parrots are, on average, larger than their female counterparts. They are also more likely to exhibit increased red feathering in their neck pattern, on the chest and abdomen and, to a lesser extent, in the under-tail feathers. In aviary birds, the red feathering on the chest of some birds increases with successive moults (Laubscher 1999). This study also confirms that the most reliable indicator of juvenile status is the presence of a brown coloured iris, which is present until at least 8 months of age. Adult iris colour did vary, but was never brown.

The mechanisms that produce variation in adult iris colour as seen in Swift Parrots are also largely undetermined. The three birds that displayed orange iris colour were all collected in the north of Tasmania at an artificial feeding station. It is possible that either diet or genetics is responsible for this infrequently seen colour. The relative homogeneity of the population of Swift Parrots has not been assessed and further study is required.

There were sex differences in the neck pattern and chest feathering of Swift Parrots; specifically an increased frequency of red feathering in males. In passerine birds, red pigmentation of feathers is associated with dietary carotenoids and is an indicator of reproductive fitness (Olson and Owens 1998). However, in the psittacines, dietary carotenoids are metabolised prior to deposition and are not easily altered by dietary manipulation (Hencken 1992). If red feathering is used as a fitness signal in Swift Parrots, its expression may be balanced by increased visibility, resulting in greater predation risk. Further studies are needed to determine the role of red feathering in Swift Parrots and other psittacine birds.

The sex difference in the mean sizes, the tendency for males to have more vivid plumage and a possible population sex bias (Chapters 2 and 11) towards males of Swift Parrots suggest that female choice dominates pair-bonding. Captive male birds were noted to undergo a limited moult of the cere and facial feathers prior to the breeding season. This would account for the increased vividness of the male plumage at this time and suggests that colouration of the facial feathers is an

important secondary sexual characteristic. Further studies are needed to assess the ultraviolet properties of these feathers, as birds' vision extends into the ultra-violet range (Güntürkün 2000); UV luminescence is a secondary sexual characteristic in other parrots (eg. *Melopsittacus undulatus*) (Burkhardt 1989).

However, the degree of overlap in the range of all the characteristics examined means that, while sex differences exist, there are no simple characteristics or measurements that will reliably allow accurate sexing of all individual birds. Multi-variate analyses of this type of data have been used to detect sex and species differences in birds using computer modelling programs such as SHEBA (Rogers 1995) and MULTIMIX (Young and Kearvell 2001). Such methods are not, however, suitable for field-based methods of sexing.

Based on this study and the results of the study of museum specimens by Bartram (1999), I therefore propose the following key for assigning sex and age based on morphology in Swift Parrots (Table 4). Serious problems remain in identifying sex in some adult birds, particularly adult females and adult males with an underwing bar. While Bartram (1999) proposed that some of these birds could be classed as immature males, my study suggests that some male birds retain an underwing bar into their third year. Differentiating these males from female birds still relies on the examiner having enough familiarity with the species to discern the subtle differences in the relative dullness of the female plumage. No method for identifying the sex of juvenile birds was determined. Therefore, this key will allow reliable allocation of sex and maturity in most specimens of Swift Parrots. However accurate sex identification in some cases will require either visual inspection of the gonads or DNA analysis.

Table 4. Key for determining the sex and/or age class of Swift Parrots by morphological features.

Morphology		Sex and/or age class
1. Is the iris brown?	- Yes →	**Juvenile bird** (<8 months of age). No reliable features for sexing.
	- No. Go to 2.	
2. Is the underwing bar absent?	- Yes →	**Adult male bird** (probably greater then 2 years of age)
	- No. Go to 3.	
3. Is a partial underwing bar present?	- Yes →	**Probable adult male bird** (age uncertain)
	- No (full underwing bar present). Go to 4.	
4. Is the under-tail colour predominantly green?	- Yes →	**Probable adult female bird**
	- No. Go to 5.	
5. Is the under-tail colour completely red?	- Yes →	**Probable adult male bird**
	- No. Go to 6.	
6. Are red feathers present on the chest and is the neck pattern a collar?	- Yes →	**Most likely adult male**
	- No. Go to 7.	
7. Are the facial feathers bright and vivid in colour?	- Yes →	**Possibly adult male**
	- No →	**Possibly adult female**

Appendix B. Potting mix constituents

6 parts composted fine pine bark
4 parts coarse washed river sand
1 part peat moss
pH is adjusted to around 6 with the addition of dolomite lime at a rate of 2.7 kg m^{-3}.
Major elements are supplied via the slow-release fertiliser Osmocote®. Low P formulations are used for native plants:
3-4 month Osmocote® N:P:K 19: 2.6: 10 @ 1 kg m^{-3}
8-9 month Osmocote® N:P:K 17:1.6:8.7 @ 2 kg m^{-3}
Micromax® (for trace elements) @ 0.5 kg m^{-3}
Ferrous sulphate @ 0.5 kg m^{-3}
Osmocote and Micromax are products of Scotts-Sierra Horticultural Products Co., USA

Osmocote® (9 month formulation)	Composition (% of total mass)
Nitrate nitrogen	8.1
Ammonium nitrogen	8.9
Phosphorus	1.6
Potassium as potassium sulfate	8.7
Sulfur as sulfates	5.9
Organic resin coating as vegetable oils (resin contains unnamed cyclic diene)	9.0

Osmocote® (4 month formulation)	Composition (% of total mass)
Nitrate nitrogen	7.5
Ammonium nitrogen	10.5
Total phosphorus	4.8
Potassium as potassium sulfate	9.1
Sulfur as sulfates	4.6
Organic resin coating as vegetable oils (resin contains unnamed cyclic diene)	7.5

Mineral composition of mineral supplement used in potting mix derived from ferrous sulfate, manganese sulfate, zinc sulfate, copper sulfate, sodium borate and sodium molybdate.

Micromax ® Micronutrients	Composition (% of total mass)
Sulfur (Combined)	12
Boron	0.1
Copper	0.5
Iron	12
Manganese (Total)	2.5
Molybdenum	0.05
Zinc (Soluble)	1

Appendix C. Pesticide assays

Pesticide levels found in the brain tissue of an adult male swift parrot that died after displaying neurological dysfunction. Analytical Services Tasmania carried out the assays. No detectable levels of pesticides were found.

Analyte	Level in brain tissue (mg/kg)
Aldrin	<0.01
alpha-BHC	<0.01
alpha-Endosulfan	<0.01
Azinphos-methyl	<0.10
beta-BHC	<0.01
beta-Endosulfan	<0.01
Bromophos-ethyl	<0.10
Chlorpyrifos	<0.10
cis-Chlordane	<0.01
Coumaphos	<0.10
DDD	<0.01
DDE	<0.01
DDT	<0.01
Diazinon	<0.10
Dichlorvos	<0.10
Dieldrin	<0.01
Disulfoton	<0.10
Endosulfan sulfate	<0.01
Ethion	<0.10
Fenthion	<0.10
HCB	<0.01
Heptachlor	<0.01
Heptachlor epoxide	<0.01
Lindane	<0.01
Malathion	<0.10
Mevinphos	<0.10
Parathion	<0.10
Parathion-methyl	<0.10
Phorate	<0.10
trans-Chlordane	<0.01

Appendix D. Causes of mortality in captive swift parrots

INTRODUCTION

Reports of mortality in captive swift parrots are not well documented. Anecdotal reports from avicultural texts describe the sudden death of recently captured wild birds in good condition, but no further investigation is recorded (Lendon 1979; Hutchins and Lovell 1985). There are also reports of fledgling birds dying during periods of high temperature when breeding has been attempted on the mainland of Australia (Lendon 1979; Hutchins and Lovell 1985; Laubscher 1999). A recent avicultural publication on swift parrots contained anecdotal reports of common diseases seen in captive birds held in European aviaries; these included chlamydiosis and ascaridiosis (Laubscher 1999).

Understanding captive mortality is necessary if captive breeding is to be held in reserve as a conservation management tool to ensure against extinction. The aim of this appendix is to describe the swift parrots' susceptibilities to disease in captivity. This appendix details causes of mortality in captive swift parrots in the research colony at the School of Zoology, University of Tasmania, Hobart, from 1998 to 2001. It also reviews mortality in private aviaries and at Healesville Sanctuary, Victoria between 1978 and 1997.

MATERIALS AND METHODS

Mortality of captive swift parrots in Tasmania

I dissected captive swift parrots that died in the research colony (n = 19), or in private aviaries in Tasmania (n = 7) to identify causes of death. All the birds from the research colony were examined within 24 hours of death, and were stored at 4°C prior to post mortem. The birds from private aviaries had all been stored frozen prior to submission. Infectious agents, or the pathological changes consistent with these organisms, were identified on gross post mortem and histology. Occasional special tests were used to confirm the diagnosis. The post mortem technique and ancillary diagnostic testing used are described in Chapter 11, Materials and Methods.

Review of captive swift parrots from Healesville Sanctuary

I obtained and reviewed records of post mortems of swift parrots (n = 25) that died at Healesville Sanctuary between 1978 and 1997. Post mortem examinations were carried out by veterinarians at Healesville Sanctuary and histopathology was carried out by the Victorian Institute of Animal Science, Attwood, Victoria.

RESULTS

Mortality of captive swift parrots in Tasmania

The causes of mortality identified in captive swift parrots were either infectious agents (39 %, n = 10), or suspected toxicities (46 %, n = 12). The cause of death could not be identified in four birds (15 %). The types of infectious organisms identified and the characteristic findings associated with them are summarised in Table 1.

Table 1. Infectious agents causing mortality in swift parrots held in captivity in Tasmania and the characteristic findings and ancillary tests used for diagnosis.

Infectious disease	N	Post mortem findings	Histological findings	Ancillary tests
Bacterial infections	5	Necrotic enteritis and/or hepatomegaly and splenomegaly	Heterophilic inflammation associated with presence of bacteria	Gram stains
Ascaridiosis	2	Dilated intestines full of nematodes	Cross sections of nematodes in intestines, villous atrophy	Nil
Mycotic pneumonia	1	Mucoid abscess encompassing right air sacs and lung	Heterophilic exudation with debris aggregation and branching, non-septate fungal hyphae	Nil
Megabacteriosis	1	Dilated proventriculus with white mucus	Proventriculus not sectioned	Microscopy of proventricular wet smears
Chlamydiosis	1	Cloudy air sacs, hepatomegaly, splenomegaly	Severe granulomatous splenitis, hepatitis and enteritis with intra-lesional chlamydial inclusions	Giemsa stains (Figure 1) Serology of affected researcher

There was a high incidence of deaths (n = 11) in the captive colony due to a suspected toxic agent, the identity of which was not confirmed: these deaths and the associated investigation are discussed in detail in Chapters 7 and 8. A single death from confirmed zinc toxicity occurred prior to these events. There was a single case of chlamydiosis (Figure 1). There were four captive birds for which a cause of death could not be determined due to either advanced autolysis of tissues (n = 2), or a lack of diagnostic changes (n = 2).

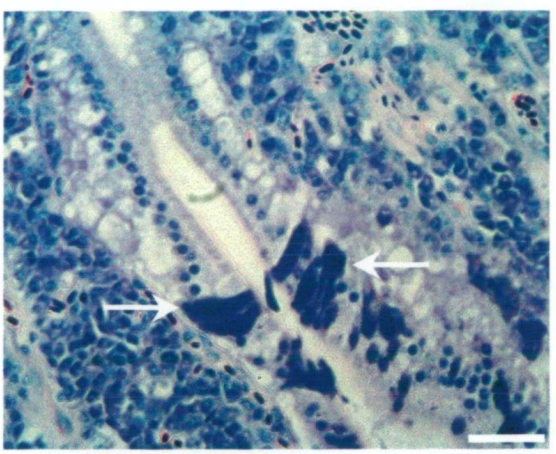

Figure 1. Giemsa stain of the intestinal mucosa of a juvenile swift parrot infected with *Chlamydophila psittaci*. Arrows point to intestinal epithelial cells saturated with Giemsa positive chlamydial inclusions. Scale bar represents 20 μm.

Mortality of captive swift parrots at Healesville Sanctuary

The causes of mortality of captive swift parrots in captivity at Healesville Sanctuary were classified as infectious (68 %; n = 17); traumatic (16 %; n = 4); reproductive disorder (4 %; n = 1); or renal disease (4 %; n = 1). A cause of death was not established for two birds (8 %). Diagnosis of death due to infectious causes was made on post mortem examination, histology, and occasionally, in the case of suspected bacterial infections, culture of the causative agent. The types of infectious agents and the characteristic findings associated with them are summarised in Table 2. There were four birds killed by trauma. Two of the birds were nestlings being hand reared that died of hyperthermia when a brooder malfunctioned. The other two

birds were found with fractured limbs or haemorrhages that were consistent with collision with the cage wire. A single adult female bird, found dead in the nestbox, was diagnosed with egg peritonitis and a prolapsed oviduct with an egg present in the oviduct. A single adult male bird was found to have yellowish kidneys with focal accumulations of urates at post mortem. Histology revealed renal tubular intra-luminal calcified deposits and tubular necrosis, but no causative agent was identified. There were two other cases where a cause of death could not be established; one because of autolysis and the other because of a lack of significant findings at post mortem and histological examination.

Table 2. Infectious agents causing mortality in swift parrots held in captivity at Healesville Sanctuary, Victoria and the characteristic findings and ancillary tests used for diagnosis.

Infectious disease	N	Post mortem findings	Histological findings	Ancillary tests
Aspergillosis *(Aspergillus fumigatus)*	8	Caseous granulomas in air sacs and lungs	Necrosis with fibrino-haemorrhagic exudate and granulomatous inflammation Branching septate hyphae and sporangia	Fungal culture
Bacterial enteritis *(Klebsiella sp. Proteus sp. Escherichia coli Pseudomonas sp. Clostridium sp.)*	9	Wasted body condition No other characteristic findings noted	Heterophilic inflammation associated with presence of bacteria	Gram stains Bacterial cultures

DISCUSSION

The results of the survey into mortality in captive swift parrots indicate that when the birds are held in confinement they are susceptible to a range of toxic and infectious diseases. Toxicoses are difficult to diagnose and the detail of the captive environment must be carefully assessed to detect potential toxins. I suspect that in the Tasmanian colony the incidence of the suspected toxic disease was exacerbated by the high protein diet the birds were fed (Chapters 7 and 8). The nutritional

requirements of the species requires further investigation if captive management is to be used in conservation efforts.

The liquid nectar diet fed to the captive birds may predispose them to bacterial gastroenteritis as evidenced by the high incidence of bacterial infections seen both in Tasmania and at Healesville Sanctuary. Prevention of this type of disease is dependent on attention to hygiene (Gerlach 1994; Clubb and Flammer 1994). Similarly, the high incidence of aspergillosis at Healesville Sanctuary was probably a combination of high environmental contamination with *Aspergillus* spores and immunosuppression of the captive birds (Bauck 1994; Oglesbee 1997). The birds that were in captivity at Healesville Sanctuary were all injured wild birds that could not be rehabilitated. Increased susceptibility to aspergillosis occurs with stress due to shipping and confinement of injured wild birds (Oglesbee 1997). Prevention of aspergillosis involves reducing environmental contamination by regular cleaning and disinfection, reducing stress to the birds where possible and the prophylactic treatment of at-risk birds (Bauck 1994; Oglesbee 1997).

A zoonotic disease of importance that was identified in the captive population was chlamydiosis, caused by the obligate intra-cellular bacterial parasite *Chlamydophila psittaci*. The zoonotic potential of this disease was realised with the confirmed infection of two visitors to the aviary. Both visitors suffered classic syndromes of high fever, joint pain, headaches, shortness of breath and general debilitation. Serology confirmed both cases, and the illness responded within 24 hours to treatment with doxycycline. In the captive swift parrots only a single death due to chlamydiosis occurred, but as the aviary was promptly treated with prophylactic doxycycline, further mortality was probably prevented. The infection was unusual in its severity, both in the short duration of illness prior to death and the high numbers of organisms seen on histology. The replication of the organism within the intestinal mucosa (Figure 1) would undoubtedly have resulted in massive contamination of the aviary environment.

In conclusion, a range of toxic and infectious diseases complicates the captive management of swift parrots. This is a difficult species to maintain in captivity, and further research is recommended if captive breeding and/or re-introductions are to be held in reserve as a conservation management tool to ensure against extinction.