Physiology

16

A. Gastrointestinal

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GASTROINTESTINAL PHYSIOLOGY AND NUTRITION

Most studies of nutrition and gastrointestinal (aka GI) physiology in birds have been conducted on domestic fowl. Birds of prey provide an interesting contrast to domestic fowl because of their carnivorous diets. This part of Chapter 16 summarizes our knowledge of anatomy, gastric secretion and motility, pellet formation and egestion, and the techniques available to study these aspects of raptor biology.

Gastrointestinal Physiology

Anatomical considerations. It is useful to have some notion of anatomy in order to better understand function. The GI tracts of raptors differ significantly from those of domestic fowl, with which most biologists are familiar (Fig.1; Duke 1978). Whereas turkeys have a well-developed crop, that of many raptors is poorly developed, and owls have no crop at all, only a simple enlargement of the esophagus. The crop is largely a food-storage area with little secretory activity, and is exceptionally well developed only in some vultures, whose crop allows them to consume up to 20% of their body weight in a single meal (Houston 1976). The stomach of turkeys, and virtually all other avian species except raptors and Ardeidae, consists of two pairs of alternately contracting muscles that grind food. The meat diet of raptors does not require strong mechanical grinding, and birds of prey have a simpler muscular stomach in which acid secretion and enzyme action start to break down the food. Digestion is continued in the small intestine, which also is the site of absorption. The pancreas fills the entire duodenal loop in turkeys, but occupies only half of the loop in owls, and is even smaller in hawks. There seems to be considerable variation in the total length of the small intestine between species of both raptors and owls. After correction for body-size differences, species such as falcons, which use a method of prey capture that requires extreme acceleration in flight, have a small intestine length about 50% shorter than that found in species such as eagles, buzzards, and kites that have less need for speed and agility when hunting (Barton and Houston 1994a). This may be an adaptation to reduce the overall weight of the digestive tract in those species which have an extremely active hunting strategy, and it does have the consequence of giving such species a reduced digestive efficiency and restricted prey selection (see later). Ceca in birds are highly variable in size, and usually are only conspicuous in certain plant-eating birds, where they are the sites of microbial fermentation of plant-cell

walls that cannot otherwise be digested (Klasing 1998). Thus, it is not surprising that they are absent in hawks. They are, however, well developed in owls (Fig. 1). It is not clear why Great Horned Owls (*Bubo virginianus*), which eat almost the same diet as Red-tailed Hawks (*Buteo jamaicensis*), have such a different cecal morphology. Perhaps because owls generally swallow their prey whole, the ceca are used to break down the plant material found in the gut contents of their prey. Cecal droppings of owls are readily distinguished from rectal excreta. In Great Horned Owls on a mouse diet, these droppings occur about once every three days (G. Duke, unpubl. data). This information might be used to determine how long an owl has been roosting at a particular site.

Gastric secretions and motility. Digestive secretions and intestinal absorption have received little investigation in raptors. Gastric secretions have been found to be more acidic (Duke et al. 1975) and to contain more pepsin (Herpol 1964, 1967; Duke et al. 1975) than gastric secretions of granivorous and omnivorous birds; and the pH of the gastric juice of hawks was found to be lower than that of owls (i.e., 1.7 versus 2.4, respectively) (Duke et al. 1975). In an extreme case, this strongly acidic environment enables the Bearded Vulture (*Gypaetus barbatus*) to feed mainly on bones — the only vertebrate known to be able to digest this unpromising diet (Houston and Copsey 1994).

GI motility (i.e., contractile activity) has received considerable attention (Duke et al. 1976b,c; Rhoades and Duke 1977). In more recent years, captive American Kestrels have been used to learn more about this subject (Duke et al. 1997).

Several methods may be used to study GI motility in raptors: (1) tiny strain-gauge transducers (SGT) surgically sutured to the outside surface of the GI tract (called the serosal surface) to monitor smooth muscle contractile activity (Duke et al. 1976b,c), (2) silver bipolar electrodes also sewn onto the serosa to detect electrical potential changes associated with depolarization (contraction) of smooth muscle (Duke et al. 1976c), and (3) radiography using image intensification (a modern type of fluoroscope) and viewing GI contractions on a video monitor or recording observations on video tape (Duke et al. 1976c, Rhoades and Duke 1977). Bioinformation detected by these devices can be recorded on a physiological recorder.

Swallowed foods collect in the crop of hawks and are slowly passed into the stomach. In owls, swallowed food items immediately fill the stomach and lower

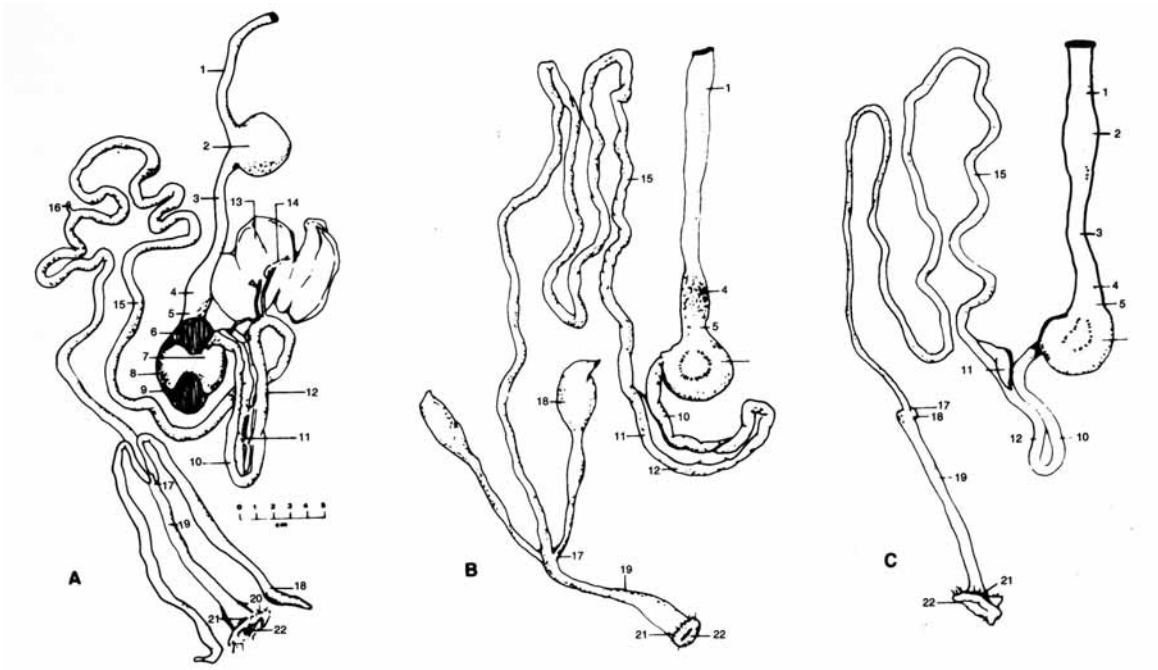

Figure 1. GI tracts of (A) domestic turkey, (B) Great Horned Owl, (C) Red-tailed Hawk. Included are (1) pre-crop esophagus, (2) crop, (3) post-crop esophagus, (4) glandular stomach, (5) isthmus, (6) thin craniodorsal muscle, (6a) muscular stomach of raptor, (7) thick cranioventral muscle, (8) thick caudodorsal muscle, (9) thin caudoventral muscle, (10) proximal duodenum, (11) pancreas, (12) distal duodenum, (13) liver, (14) gall bladder, (15) ileum, (16) Meckel's diverticulum, (17) ileocecocolic junction, (18) cecum, (19) colon, (20) bursa of Fabricus, (21) cloaca, (22) vent, greater curvature. From Duke (1978).

esophagus, and after 20 to 30 minutes the entire meal has been moved into the muscular stomach (Rhoades and Duke 1977). In Great Horned Owls, the motilities of the stomach and duodenum are coordinated and the gastroduodenal contraction sequence involves a contraction wave (called peristalsis) that moves first through the stomach, then on into the duodenum (Kostuch and Duke 1975). The peristaltic contraction is more apparent in the muscular stomach as a flattening or indentation moving around the greater curvature (Kostuch and Duke 1975, Rhoades and Duke 1977).

Pellet formation and egestion. The formation and egestion of pellets is a unique gastrointestinal phenomenon in birds, and is particularly well developed in raptors and especially owls (Rea 1973). Analysis of food remains in pellets is a major aspect of many raptor studies (Mikkola 1983, Yalden 2003). Pellets are formed in the stomach from the indigestible bones, hair or feathers of prey (Reed and Reed 1928, Grimm and Whitehouse 1963, Kostuch and Duke 1975, Rhoades and Duke 1977). The prey remains in owl pellets reflect exactly the prey species eaten (Mikkola 1983). But pellet size varies considerably, and curiously has no correlation with the amount of food eaten (Erkinaro 1973). Raczynski and Ruprecht (1974) showed that some prey bones are digested, some skeletal parts more than others, and that food intake estimates based on pellet remains will underestimate the number of prey items swallowed (see also Chapter 8). Duke et al. (1996) also found considerable variability in parts of food items eaten, pellet size, and pellet egestion frequency in captive American Kestrels. Egestion involves both gastric activity and esophageal antiperistalsis (Duke et al. 1976c), and is considerably different from the mechanisms of vomiting in mammals with a simple stomach, or regurgitation of cud in ruminants (Duke et al. 1976c).

Monitoring of gastric motility in owls shows that food intake, or even the sight of food in hungry owls (Duke et al. 1976b), immediately causes a two- to threefold increase in gastric contractile activity. The first mechanical-digestion phase, with relatively rapid and vigorous motility, moves the entire meal into the muscular stomach, crushes or "macerates" it, and thoroughly mixes it with digestive secretions. The second, or chemical-digestion phase, has low amplitude and low frequency contractions that continue to mix gently ingesta with digestive secretions; most digestion is completed during this phase. During the third phase, fluid is evacuated from the stomach, and pellet formation and egestion occur (Fuller and Duke 1978). The length of these phases and the overall meal-to-pellet interval (MPI) varies directly with the amount eaten by an owl, and thus may be used to estimate meal size.

In order to learn more about other factors that regulate pellet egestion and thus alter the lengths of the three phases and influencing MPI, owls were jessed and attached to perches suspended over a sloping chute within a 1 × 1 × 2-m chamber. Pellets rolled down chutes into wire collecting baskets; a pellet landing in a basket depressed a micro-switch directly under the basket, thereby completing a circuit and activating a marker on a recorder located in another room. The exact time of the event was thus recorded.

Using this technique, six species of owls (Table 1) were fed as many laboratory mice as they wanted during a 30-minute period at two hours after dawn (0900)

Table 1. Mean meal-to-pellet intervals (MPI) in owls.[a]

Species	Number of Birds	Mean MPI ± SE (hour)	Number of Pellets
Eastern Screech Owl *(Megascops asio)*	2	11.86 ± 0.22	29
Great Horned Owl *(Bubo virginianus)*	4	13.25 ± 0.29	36
Snowy Owl *(Bubo scandiaca)*	2	12.02 ± 0.72	35
Barred Owl *(Strix varia)*	2	9.85 ± 0.44	25
Short-eared Owl *(Asio flammeus)*	1	10.22 ± 0.12	132
Northern Saw-whet Owl *(Aegolius acadicus)*	1	10.04 ± 0.32	4

[a] Data modified from Duke et al. (1976a)

daily. The length of the MPI was shorter in smaller-sized owls, but, more significantly, the MPI was directly related to meal size, indicating that the state of ingestion of the meal is important in regulating pellet egestion (Table 2; Duke et al. 1976b).

Experiments involving feeding Great Horned Owls on foods of different composition suggest that the presence of undigested food (proteins or fat) in the stomach seems to inhibit pellet egestion, which will not occur until digestion is complete (Table 3; Duke and Rhoades 1977). There also may be a stimulating effect of undigested material on the gastric mucosa, which contributes to pellet ejection. However, other factors also may be involved. Barred Owls (*Strix varia*) were found to have lengthened MPIs and smaller pellets when fed at a sub-maintenance level until they had lost 10% of their body weight. Analysis of the pellets disclosed that digestion of the meal was more complete in the hungry owls, indicating that the state of hunger may affect MPI (Duke et al. 1980). The constant sight of food may shorten MPI in Short-eared Owls (*Asio flammeus*) (Chitty 1938).

MPI in owls also may be influenced by environmental stimuli. When Great Horned Owls were fed as many mice as they wanted during a 30-minute period at either dawn or dusk, it was found that MPIs were directly related to meal size but that MPI's were longer for meals eaten at dusk than at dawn regardless of the size of the meal (Duke and Rhoades 1977). This is true for Short-eared Owls, too (Chitty 1938). Thus, the portion of the daily cycle during which gastric digestion and pellet formation occur may affect the MPI.

Kuechle et al. (1987) performed a field study using all of the basic information described above and adapting the techniques used therein for telemetry. In free-flying Barred Owls, movements were monitored via a tail-mounted transmitter and gastric motility was monitored via telemetry of signals from an implanted SGT to determine (1) time of ingestion, (2) time of egestion, (3) measurement of the lengths of phases in gastric digestion and thus, (4) estimation of the quantity consumed. Being able to distinguish movements associated with hunting and feeding from other types of movements is significant in understanding owl behavior, and an estimate of daily food consumption in a free-flying owl is very useful in understanding owl energetics.

In owls the MPI is directly correlated with the quantity eaten, but in hawks the major stimulus for pellet egestion is dawn, regardless of the quantity eaten (Balgooyen 1971, Duke et al. 1976b; Table 4). In a light-timed room with dawn set at 0700, the MPIs of hawks were 1 to 2 hours shorter when they were fed at 1100 than when they were fed at 0900. In another study involving Red-tailed Hawks in a room with dawn at 0700, feeding time was shifted from 0800 to 1600, and MPI changed from approximately 2200 to approximate-

Table 2. Mean meal-to-pellet intervals (MPI) as related to food consumption (grams DM/kg) in Great Horned Owls and Eastern Screech-Owls fed at 0900 daily.[a]

Species	Number of Birds	Meal Size	Mean MPI ± SE (hour)	Number of Pellets
Great Horned Owl *(Bubo virginianus)*	4	10	11.76 ± 0.46	4
		11 – 15	12.49 ± 0.35	11
		16 – 20	13.35 ± 0.51	12
		21 – 25	14.71 ± 0.52	9
Eastern Screech Owl *(Megascops asio)*	2	30 – 40	10.92 ± 0.25	9
		41 – 50	11.88 ± 0.28	13
		51 – 60	12.92 ± 0.41	6
		61 – 70	13.75	1

[a] Data modified from Duke et al. (1976a)

Table 3. Mean meal-to-pellet intervals for four Great Horned Owls fed (at 1500) two mice, two mouse skins, or two skins stuffed with various diets.[a]

Diet	Mean Mass of Meal (g)	Mean MPI ± SE (hour)	Number of Pellets
Two 25 g mice	50	15.52 ± 0.45	45
Two mouse skins (with skull)	15	15.26 ± 0.20	8
Two mouse skins plus two pellets [b]	25	8.19 ± 0.26	11
Two pellets only [c]	10	2.75 ± 0.29	5
Two mouse skins plus 35 g of horse meat	50	24.34 ± 1.02	10
Two mouse skins plus 9 g of suet [b]	24	33.74 ± 2.28	11

[a] Table modified from Duke and Rhoades (1977).
[b] Pellets, horse meat, and suet were sewn into the mouse skins with silk suture.
[c] Pellets were force-fed.

Table 4. Mean meal to pellet intervals (MPI) in hawks with dawn (lights on in the holding room) at 0700.[a]

		MPI (hour)			
Species	Number of Birds	Fed at 0900 Mean MPI ± SE	N	Fed at 1100 Mean MPI ± SE	N
Bald Eagle *(Haliaeetus leucocephalus)*	3	21.7 ± 0.4	10	20.9 ± 0.38	10
Northern Goshawk *(Accipiter gentilis)*	4	21.6 ± 0.83	9	20.6 ± 0.17	65
Broad-winged Hawk *(Buteo platypterus)*	2	21.7 ± 0.14	13	20.8 ± 0.13	5
Red-tailed Hawk *(B. jamaicensis)*	6	22.5 ± 0.09	72	20.4 ± 0.14	59
Roughleg *(B. lagopus)*	3	21.7 ± 0.08	79	-	-
Northern Crested Caracara *(Caracara cheriway)*	1	-	-	19.6 ± 0.08	14
American Kestrel [b] *(Falco sparverius)*	1	23.6 ± 0.06	10	-	-

[a] Data from Duke et al. (1976a).
[b] Dawn was approximately 0800.

ly 1800, respectively, a delay of only 4 hours, suggesting that the birds were "attempting" to egest as early in the day as possible (Fuller et al. 1978). It is theorized that whereas owls may hunt either at night or during the daytime, hawks require daylight for hunting (Fuller et al. 1978). Thus, hawks would benefit by egesting a pellet (i.e., emptying the stomach) early in the day, leaving the rest of the day for capturing and ingesting new prey. Hawks conditioned to eating late in the afternoon respond by shifting egestion time to just prior to the anticipated feeding time (Fuller et al. 1978).

Durham (1983) showed that in Red-tailed Hawks pellet egestion occurred at dawn each day even if the hawks had not eaten the day before or if they had eaten only meat without feathers, fur or bone. Thus, in hawks, egestion motility is not just the end result of having ingested, but is apparently an expression of a circadian rhythm. There are other differences between hawks and owls. Owls normally egest a pellet for each meal, while hawks may eat one to three meals before egesting a pellet (Duke et al. 1975, 1976b). The bones of prey receive little digestion in the stomachs of adult owls, whereas bones are virtually entirely digested in the falconiform stomach (Errington 1930, Sumner 1933, Glading et al. 1943, Clark 1972, Duke et al. 1975, 1976b). This is due to the lower pH in the stomach of hawks (Cummings et al. 1976). Nestling owls also digest bones.

The mechanism of pellet egestion in Red-tailed Hawks follows gastric and esophageal contractile activity very similar to that of Great Horned Owls (Durham 1983), with three clear phases of ingestion motility, chemical digestion and pellet formation, and egestion motility. It is likely that a telemetry study, as performed with Barred Owls, using Red-tailed Hawks or other hawks could provide very useful management information.

Ion and water balances. Little is known about ion and water balances in raptors, but the topic is relevant to management of captive birds. For birds weighing 60 g or more, which includes virtually all raptors, evaporative water loss from the respiratory surfaces and the skin in unstressed individuals can be offset by water produced via oxidative metabolism (Bartholomew and Cade 1963). The moisture in freshly killed prey thus can be used to meet (or partially meet) water loss associated with thermal stress, exercise, or both. Most raptors can be maintained in captivity, and even mate and lay eggs, in the absence of drinking water (Bartholomew and Cade 1957, 1963). Captive Great Horned Owls require 4.4–5.3% of their body weight per day as water (Duke et al. 1973). This intake is lower than that of all but one of 21 species tested by Bartholomew and Cade (1963), including roadrunners (*Geococcyx* spp.), a species adapted to life in an arid environment. Evaporative water loss amounted to approximately 45% of the water ingested with prey in Great Horned Owls (Duke et al. 1973).

Like many other birds, raptors are able to regulate salt and water losses via both the kidney-cloaca system and the nasal salt glands. Urine volumes in Red-tailed Hawks fed beef hearts averaged 30.2 ml/day with sodium and potassium concentrations of 38 and 61 mM/l, respectively. The nasal gland secretions of these birds contained 272 mM/l of sodium and 8 mM/l of potassium (Johnson 1969). Other studies of Red-tailed Hawks have indicated higher sodium and potassium concentrations in both urine (206 and 76 mM/l, respectively) and nasal secretions (380 and 20 mM/l, respectively); similar data were found for eight other falconiform species (Cade and Greenwald 1966). Although functional nasal salt glands are apparently present in all Falconiformes, they have not been reported in Strigiformes.

Nutrition and Food Metabolizability

Nutritional requirements. Small mammals and birds form the bulk of the diet in most raptors. The natural diets (qualitative requirements) of most birds of prey have been studied extensively; some examples are provided in Table 5. The biomass eaten is most important in understanding the energetics of the predator and its impact on the environment. Thus, not only the species of prey and the frequency it occurs in the diet, but also the weight of that prey item must be known. An extensive compilation of prey weights for 35 mammalian and 81 avian prey items was prepared by Steenhof (1983). This includes mean values, determined from a large number of samples in many cases, and separate means for adults (male versus female frequently) and juveniles.

Amounts that must be consumed to maintain a constant body weight under both field and laboratory conditions (quantitative requirements) are known for a few species (Table 5). Food consumption of an individual varies according to level of activity and ambient temperature. Activity is influenced by factors such as day length, prey availability, breeding and nesting, and disturbance. In general, consumption varies inversely with ambient temperature within species and with body size among species (Table 6), as well as directly with activity.

Unfortunately, little is known regarding daily or seasonal requirements for specific nutrients for raptors.

Table 5. Natural foods of some common North American raptors.[a]

		Percent of Diet				
Species	Ref.[b]	Small Rodents	Larger Mammals	Birds	Insects	Other
Northern Harrier (*Circus cyaneus*)	1	98.4	0.3	1.0	-	0.3
Red-shouldered Hawk (*Buteo lineatus*)	1	97.0	-	3.0	-	-
Red-tailed Hawk (*B. jamaicensis*)	1	95.5	1.4	3.1	-	-
Roughleg (*B. lagopus*)	1	98.1	-	1.9	-	-
American Kestrel (*Falco sparverius*)	1	90.3	-	9.9	-	-
Barn Owl (*Tyto alba*)	2	81.6	16.4	2.0	-	-
Eastern Screech Owl (*Megascops asio*)	1	3.4	-	6.3	0.3	-
Great Horned Owl (*Bubo virginianus*)	1	92.3	3.7	3.5	-	0.7
Burrowing Owl (*Athene cunicularia*)	2	12.1	0.7	1.3	85.9	-
Barred Owl (*Strix varia*)	3	53.2	7.8	24.2	4.8	10.0
Long-eared Owl (*Asio otus*)	1	100.0	-	-	-	-
Short-eared Owl (*A. flammeus*)	1	99.3	-	0.7	-	-

[a] Foods were determined by pellet analysis. Foods such as meat from a carcass and insect parts are thoroughly digested in falconiform stomachs and do not appear in pellets.
[b] References: 1 = Craighead and Craighead (1956), 2 = Marti (1969), 3 = Errington (1932).

However, the caloric and nutrient value of some wild and domestic rodents and birds are known (Bird and Ho 1976, Bird et al. 1982; Table 7). These data are useful in assessing the relative nutritive and energy value of wild prey.

The nutrient composition of vertebrate tissues is relatively constant, and as a food source their nutrient balance closely matches that required by birds (Klasing 1998), thus it is unlikely that any macro- or micro-nutrients are limiting in the diet for most species, although a few nutritional disorders have been described in raptors (Cooper 1978). The major difference between prey species is in the relative proportion of fat present, which varies not only between prey species, but also among individuals and between seasons within species. For example, some small passerines can store up to 50% of their body mass as fat prior to migration, making them energetically, high-quality prey.

Almost all raptors eat meat, which is relatively easily digested, and it might be assumed that all species would show similar digestive efficiencies. This, however, seems not to be the case (Barton and Houston 1994b). Digestive efficiency varies from about 75% to 82%, and this is correlated with the length of the digestive tract. Species with short guts tend to digest their food less efficiently than species with long guts, and consequently need to capture proportionately more prey each day. This may be associated with hunting strategy, for the species with short guts and poor digestive efficiency tend to be species which take a high proportion of birds in flight and need the ability to accelerate rapidly (Barton and Houston 1994a). For such species it may be advantageous to have a lightweight, low-volume gut, even if it results in poor digestive efficiency, because by being more agile they can capture more prey. It does, however, have the consequence that short-gut species are forced to feed on prey items with a high energy content (high body fat), and are unable to main-

Table 6. Food consumption at several ambient temperatures for some adult North American raptors kept outside for one year in Ogden, Utah U.S.A.

				Amount Eaten per Day		
Species	Ref.	Diet	Body Mass (g)	Grams	Percent of Body Mass	Ambient Temperature (°C)
Bald Eagle (*Haliaeetus leucocephalus*)	2	mice	3870	219.8	5.6	27
Bald Eagle	5	mixed[c]	3922	344.8	8.8	–10
Bald Eagle	5	mixed[c]	3922	294.5	7.5	5
Bald Eagle	5	mixed[c]	3922	265.2	6.8	20
Northern Goshawk (*Accipiter gentilis*)	2	mice	1100	80.2	7.3	27
Broad-winged Hawk (*Buteo platypterus*)	2	mice	470	29.4	6.3	27
Red-tailed Hawk (*B. jamaicensis*)	2	mice	1320	75.5	5.5	27
Roughleg (*B. lagopus*)	2	mice	1020	48.0	4.7	27
American Kestrel (*Falco sparverius*)	2	chick	105	14.6	13.9	27
Common Kestrel (*F. tinnunculus*)	6	mice	204	24.3	11.9	14
Peregrine Falcon (*F. peregrinus*)	1	mice	680	60.6	8.9	27
Gyrfalcon (*F. rusticolus*)	1	mice	880	70.3	8	27
Barn Owl (*Tyto alba*)	3	mice	603	60.5	10	-[b]
Barn Owl	6	chick	262	28.3	10.8	14
Eastern Screech Owl (*Megascops asio*)	4	mixed	153	39.0	25.4	6
Eastern Screech Owl	2	mice	149	17.1	11.5	27
Great Horned Owl (*Bubo virginianus*)	2	mice	1770	71.2	4.0	27
Great Horned Owl	3	mice	1336	62.6	4.7	-[b]
Snowy Owl (*B. scandiaca*)	1	mice	1900	93.1	4.9	27
Burrowing Owl (*Athene cunicularia*)	3	mice	166	26.4	15.9	-[b]
Barred Owl (*Strix varia*)	2	mice	741	42.9	5.8	27
Barred Owl	4	mixed	625	67.0	11.8	4
Great Gray Owl (*S. nebulosa*)	4	mixed	1045	77.0	7.4	–10
Long-eared Owl (*Asio otus*)	3	mice	291	37.5	12.9	-[b]
Short-eared Owl (*A. flammeus*)	2	mice	432	50.0	11.6	27
Northern Saw-whet Owl (*Aegolius acadicus*)	2	mice	96	12.9	13.4	27

[a] References: 1= Duke et al. (1975), 2 = Duke et al. (1976a), 3 = Marti (1973), 4 = Craighead and Craighead (1956), 5 = Stalmaster and Gessaman (1982), and 6 = Kirkwood (1979).
[b] Data are mean values for birds kept outside for one year in Ogden, Utah.
[c] Chum salmon (*Oncorhynchus keta*), black-tailed jackrabbit (*Lepus californicus*), and Mallard (*Anas platyrynchos*).

Table 7. Partial analysis of nutrient levels in wild and domestic rodents, birds, and an insect.

		Rat[a]	Mouse[a]	Chicken[a]	Day-old Chick[a]	Sparrow[b]	Vole[b]	Grasshopper[b]
Number of animals		10	30	10	30	11	13	89
Average mass (g)		325.7	26.7	386.7	41.2	27	32	0.21
Dry matter % (freeze dried)		34.4	35.4	33.5	27.6	31.6	35.7	31.9
Crude fat (% DM)		22.1	24.9	26.9	24.2	15.9	6.01	6.03
Crude protein (N x 6.25% DM)		62.8	56.1	56.7	62.2	64.9	57.3	75.7
Ash (% DM)		10.0	10.4	9.5	7.4	10.6	10.1	4.8
Crude fiber (% DM)		2.4	1.7	2.0	0.8	0.43	3.85	-
Gross energy (kcal/g DM)		5.78	5.84	5.93	6.02	5.39	4.15	5.02
Calcium (%)	DM	2.06	2.38	1.94	1.36	2.94	2.85	0.31
	wet mass	0.69	0.84	0.65	0.38	0.94	1.02	0.098
Phosphorus (%)	DM	1.48	1.72	1.40	1.00	2.35	2.66	1.27
	wet mass	0.51	0.61	0.47	0.28	0.74	0.95	0.41
Ca:P ratio		1.39	1.38	1.39	1.36	1.3	1.1	0.2
Zinc (mg/kg)	DM	129.2	134.6	158.0	106.9	109.8	105.5	200.2
	wet mass	13.3	47.7	52.8	29.9	34.7	37.7	63.9
Copper (mg/kg)	DM	4.5	8.0	4.5	3.2	12.6	13.7	50.3
	wet mass	1.5	2.8	1.5	0.9	3.98	4.89	16.1
Manganese (mg/kg)	DM	7.5	11.7	9.0	3.0	11.4	14.9	25.1
	wet mass	2.5	4.1	3.0	0.8	3.6	5.32	8.01
Iron (mg/kg)	DM	175.7	239.1	146.8	121.8	592.0	332.3	331.4
	wet mass	58.9	84.6	49.1	34.0	187.2	118.7	105.8
Thiamine (mg/kg)	DM	13.3	-	8.5	16.0	-	-	-

[a] From Bird and Ho (1976)
[b] From Bird et al. (1982); House Sparrow (*Passer domesticus*), meadow vole (*Microtus pennsylvanicus*), red-legged grasshopper *(Melanoplus femurrubrum*)

tain their body weight if fed on prey with low fat levels (Taylor et al. 1991). This may explain why many falcons specialize on small passerines and are rarely found feeding on carrion or low-energy prey.

The ceca of owls apparently make little contribution to food digestion since metabolizability of a mouse diet was not significantly different between cecectomized and intact Great Horned Owls (Duke et al. 1981). Water balance also was unaffected by cecectomy.

Kirkwood (1981) calculated maintenance metabolizable energy (ME) based on food intake for several diets at several ambient temperatures for nine strigiform and 22 falconiform species using the linear regression equations ME = 110 $W^{0.679}$, where ME is expressed in kcal/day and W (weight) in kg. Data for Falconiformes and Strigiformes were pooled as separate regressions and were not significantly different. Wijnandts (1984) made similar calculations for 13 strigiforms and 26 falconiforms under caged conditions eating either mice or rats. Metabolizable energy also was calculated from published data on food consumption using a caloric value of 8.4 kJ/g for mice or rats and assumed metabolizability of 76%. Linear regression equations derived for falconiforms and strigiforms were ME = 9.722 $W^{0.577}$ (r = 0.918) and ME = 8.63 $W^{0.578}$ (r = 0.958), respectively, where ME is in kJ/bird/day and W is in g.

SUMMARY

We still have much to learn about the gastrointestinal physiology of raptorial birds. Prey availability (both population size and vulnerability), the nutritive value of the prey, and its metabolizability by raptors all must be considered in evaluating raptor energetics. In these birds with such uniquely carnivorous food habits, further research in this field should prove most fruitful. However, with the tragic passing of co-author Gary Duke, who led the world in the field of avian gastrointestinal physiology in 2006, and no one on the immediate horizon appearing to follow in his footsteps, it may be some time before significant advances in this field are again achieved.

ACKNOWLEDGMENTS

This chapter is dedicated to the memory of my co-author Dr. Gary Duke, the world's foremost authority on avian gastrointestinal physiology. The chapter would be all too brief without his significant contributions. Gary was a warm, caring human being who loved birds of prey and who was an excellent mentor to many of us in raptor studies. I thank Nigel Barton for his review of an earlier draft of this chapter.

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B. Hematological

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INTRODUCTION

Disease diagnosis and assessment of therapeutic efficacy often relies on hematological analysis (Howlett 2000, Cooper 2002). Rehabilitation centers can use hematological changes to help detect sub-clinical disease, in pre-release assessment of individuals, and as a prognostic indicator for new admissions. The health of raptor populations can be monitored similarly. Nutritional status, disease, or food supply differences among populations or immune suppression due to various stressors all can be detected using hematology (van Wyk et al. 1998, Cooper 2002).

Because raptors are at the top of many food chains, their health can reflect the health of entire ecosystems (Cooper 2002). Hematological alterations can indicate changing habitat quality and food availability or may imply exposure to pollutants or toxins (Hoffman et al. 1985, Mauro 1987, Bowerman et al. 2000, Seiser et al. 2000). Habitat loss and fragmentation have resulted in increased exposure of some raptor populations to parasites, which can alter host-parasite balances. Increased parasite pathogenicity may be implied by hematological alterations (Loye and Carroll 1995).

Recently, research has focused on determining the reference ranges that distinguish among species of raptors. A number of published references provide parameters by sex and age (Rehder et al. 1982, Ferrer et al. 1987, van Wyck et al. 1998, Bowerman et al. 2000). Computerized databases also are available, including ISIS (www.ISIS.org) and LYNX (Bennett et al. 1991).

The discussion of biochemical parameters is beyond the scope of this chapter. Several comprehensive references provide additional details in this area (Campbell 1994, Joseph 1999, Fudge 2000, Cooper 2002).

Sampling

Physiological variables affecting hematological testing. Blood sampling should be performed as soon as possible after capture and prior to other procedures, as the stress of capture and restraint can alter the leucogram (Wingfield and Farner 1982, Sockman and Schwabl 2001) and result in leucocytosis, heterophilia, lymphocytosis, or lymphopenia (Fudge 2000). Parga et al. (2001) advocated the use of the heterophil/lymphocyte ratio, rather than the absolute number of heterophils or lymphocytes, as a more sensitive indicator of stress in raptors, although the actual ratio may vary among species. Leucocyte numbers also may be altered with concurrent diseases (Howlett 2000, Parga et al. 2001).

Researchers should be aware that other physiological factors might affect hematological parameters. The following variables should be considered when plan-

ning hematological testing, so that efforts can be made to minimize their impact: (1) erythrocyte production may decrease with increasing ambient temperatures and may vary with season (Hunter and Powers 1980, Rehder et al. 1982); (2) hematocrit may be increased by high androgen levels and decreased by high estrogen levels; (3) molt decreases hematocrit in both sexes (Sturkie 1976, Rehder et al. 1982); (4) up until fledging, hematocrit and hemoglobin levels increase with age (Rehder et al. 1982, Bowerman et al. 2000); (5) some studies have reported differences in hematocrit between sexes, whereas others have found no correlation (Sturkie 1976, Rehder et al. 1982, Dawson and Bortolotti 1997); (6) Rehder and Bird (1983) demonstrated diurnal variation in hematocrit and red-blood-cell (RBC) count of Red-tailed Hawks (*Buteo jamaicensis*); and (7) certain sedative and anesthetic drugs also can cause leucogram or hemogram changes (Mauro 1987, Fudge 2000). All of this leads us to recommend consistency in sampling.

Venipuncture procedure. Birds that weigh less than 500 g usually are sampled with a 25-gauge hypodermic needle and a 1- or 2-ml syringe, whereas a 23-gauge hypodermic needle is best used for birds of more than 500 g (Cooper 2002). Veins of very small birds can be nicked with a scalpel blade and blood collected in a capillary tube (Dawson and Bortolotti 1997). Butterfly catheters may lessen the effect of bird movements during sampling (Cooper 2002). Smaller-gauge needles increase the risk of hemolysis. The use of larger needles increases the risk of hematomas (Fudge 2000). Excessive negative pressure may collapse veins (Jennings 1996). Adding anticoagulant to the syringe prior to venipuncture may dilute the blood sample, although some authors advocate this if clotting of samples is a problem (Rehder et al. 1982, Cooper 2002). Because of inconsistent results, we do not recommend sampling from talon clipping (Campbell 1994). Regardless of technique, the phlebotomy site must be prepared aseptically to prevent bacterial contamination (Fudge 2000).

Avian blood volume ranges from 6 to 12% of body mass and no more than 5–10% of the total blood volume should be removed. This equates to approximately 0.5–1% of total body mass (Campbell 1988, Fudge 2000). Smaller volumes should be removed from unhealthy or stressed birds (Cooper 2002).

When repeatedly sampling the same bird, allow for sufficient time for erythrocyte replenishment between sampling (Mauro 1987). The average life span of an avian erythrocyte is 28 to 45 days (Rodnan et al. 1957). That said, American Kestrels (*Falco sparverius*) bled at 10% of blood volume weekly for 20 weeks showed no decrease in hematocrit (Rehder et al. 1982).

Inappropriate restraint can result in blood-vessel laceration and prolonged bleeding. Hematoma formation may considerably increase the total blood volume removed. To reduce this risk, pressure should be applied to the phlebotomy site for at least 1 to 2 minutes after venipuncture, and the bird should not be released until hemostasis is complete.

Normal venipuncture sites. The jugular vein is the largest accessible vein, although hematoma formation there can be a problem in inexperienced hands (see below). The right jugular generally is larger than the left and also has an overlying apteria (featherless area). A popular alternative is the basilic vein, which crosses the elbow ventrally. If the bird is struggling vigorously, it can be difficult to sample, and wing trauma (including fractures) can occur. It is not always easy to find in smaller raptors. Large hematomas can develop after iatrogenic tissue trauma or insufficient post-sampling pressure. A third location for blood sampling is the median metatarsal vein, which is found proximal to the tarsometatarsal joint. There is less chance of hematoma formation in this vein due to the anatomy of the surrounding soft tissue (Fudge 2000).

After 25 years of blood-sampling American Kestrels at the Avian Science and Conservation Centre at McGill University in Montreal, the large jugular vein has become the preferred sampling site. With one person holding the bird's head stable and in an appropriate position to expose the vein, hematomas are rarely encountered using this method (I. Ritchie and D. M. Bird, pers. comm.).

Sample preparation. One or two blood smears should be made at the time of collection using non-anticoagulated blood. Avian blood cells are fragile and rough smear techniques can result in large numbers of unidentifiable (smudge) cells (Jennings 1996, Fudge 2000). If protected from moisture, air-dried, unfixed blood smears may last up to 72 hours (Howlett 2000).

Human pediatric blood tubes, which are available commercially, are quite suitable for raptor blood. The tubes are available as ethylenediaminetetraacetic acid (EDTA), heparin, and plain-gel, and when filled to the line, provide the correct sample to anticoagulant ratio. Hemolysis can be reduced by precise sampling and removing the needle from the syringe prior to filling the sample pots with blood (Fudge 2000). Heparinized capillary tubes may be capped with plasticine and stored.

Choice of anticoagulant. The laboratory where the analyses will be performed should be contacted prior to sample collection for preferred anticoagulant, storage, and other processing information.

Blood for hematological analysis usually is collected into EDTA. Note that erythrocytes may lyze within 24 to 48 hours of exposure to EDTA. This is particularly marked in some non-raptors (Campbell 1994). Heparin may affect the affinity of blood cells to Romanowksy stains and cause clumping of leucocytes and thrombocytes (Jennings 1996, Howlett 2000). Fudge (2000) found that citrated blood provided better cell integrity for automated analysis.

Sample Storage and Processing

Time from sampling to processing should be as short as practical, and samples that are not analyzed immediately should be kept cool (approximately 4°C). One person should do the blood smear staining and interpretation to minimize variability. Wright's, Giemsa, and modified Wright's-Giemsa stains all provide good cell morphology, although new staining techniques also seem to do well (Campbell 1988, Fudge 2000, Samour et al. 2001, Cooper 2002, Kass et al. 2002).

Factors affecting analysis. Sample clotting can occur due to slow sample collection, tissue trauma, inadequate sample mixing and overfilling of anticoagulant sample pots (Jennings 1996). Hemolysis and lipemia can alter a number of hematological and biochemical parameters, including total protein levels (Joseph 1999, Cooper 2002). Inaccurate cell identification can occur with blood smears made from old or anticoagulated blood, exposed to formalin fumes or stained with expired stains (Fudge 2000). Failure to detect hemoparasites can result from poor-quality smearing or staining techniques, operator inexperience, or from the use of poor-quality microscopes (Cooper 2002).

Relevant international agreements, including CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora), and country and local laws must be considered before transporting samples internationally (Cooper 2002).

Hematological Parameters

Listing "normal" hematological values for each species is beyond the scope of this chapter. Researchers should consult relevant peer-reviewed publications and databases for specific information. General information can be found in Samour (2000), Cooper (2002), and Redig (2003).

Total plasma protein. Although sometimes considered a biochemical parameter, analysis of total plasma protein (TPP) is required for complete interpretation of the erythron, especially in instances of anemia. Protein electrophoresis and fibrinogen determination also may be performed.

The erythron. Evaluation of the erythron involves determining hematocrit or packed cell volume (Hct or PCV - l/l), hemoglobin (Hb - g/l) and the RBC count (x 10^{12}/l) followed by calculation of mean corpuscular volume (MCV), mean cell hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). For additional information consult Howlett (2000) and Fudge (2000).

The accepted hematocrit range for raptors is 35–55 l/l (0.35–0.55) (Fudge 2000, Cooper 2002). Lower values have been obtained from apparently healthy birds (Rehder et al. 1982). Abnormalities must be interpreted in conjunction with TPP and fibrinogen levels (see Table 1). A reticulocyte count of 5–10% is considered physiologically normal.

Anemia can be characterized as regenerative or non-regenerative, based on the numbers of reticulocytes. Some hematologists maintain that there is no sat-

Table 1. Changes in the Erythron. (Based on Joseph [1999] and Fudge [2000].)

Condition	PCV	TPP	Fibrinogen	TPP:Fibrinogen
Dehydration	Increased	Increased	Increased	>5
Polycythemia	Increased	Normal	Normal	1.5–5.0
Anemia	Decreased	Normal	Normal or increased	Depends on cause
Infection or Inflammation	Normal	Normal	Increased	<1.5

isfactory method of obtaining an objective reticulocyte count in raptors; instead they rely on the subjective analysis of stained blood smears, noting the degree of polychromasia and the numbers of rubricytes, pro-rubricytes, and rubriblasts, if present (M. Hart, pers. comm.). Others are comfortable using a vital stain such as new methylene blue, or Wright's stain, to preferentially stain reticulocytes (Fudge 2000). In the presence of anemia, a reticulocyte count of <5% indicates a poor regenerative response, whereas a count of >10% indicates a good regenerative response (Cooper 2002). Polychromasia greater than 1–5% also can indicate an appropriate regenerative response. Anemia can be classified according to etiology or erythrocyte morphology (see Tables 2 and 3). Concurrent dehydration may mask the signs of anemia.

Hematocrit and TPP will decrease with chronic undernourishment (Ferrer et al. 1987, Cooper 2002). Unfortunately, the severity and duration of food deprivation required to cause these changes are uncertain. In Common Buzzards (*B. buteo*) starved for 13 days, these parameters changed only when feeding was resumed (Garcia-Rodriguez et al. 1987). Conversely, American Kestrels typically die after five days of starvation (Shapiro and Weathers 1981). Dawson and Bortolotti (1997) found that hematocrit was not accurate in predicting nestling survival in American Kestrels. Body size, species ecology and developmental stage also influence an individual's ability to withstand sub-optimal nutrition.

The leucogram. Detailed anatomy and function of leucocytes is not discussed here. The reader should con-

Table 2. Classification of anemia according to erythrocyte morphology. (Based on Fudge [2000].)

	Type of anemia		
	Normocytic normochromic	Hypochromic microcytic	Hypochromic macrocytic
PCV	Decreased	Decreased	Decreased
MCHC	Normal	Decreased	Decreased
MCV	Normal	Decreased	Increased
Polychromasia	None to slight	Increased	Increased
Anisocytosis	None to slight	Normal to increased	Normal to increased
Possible causes	Generally non-regenerative, reduced RBC production	Iron deficiency, chronic blood loss, chronic disease	Acute blood loss, early stages of lead toxicity

Table 3. Classification of anemia according to etiology. (Based on Campbell [1994, 2000], Fudge [2000], and Howlett [2000].)

Insufficient erythrocyte production	Acute or chronic blood loss	Increased erythrocyte destruction
Malnutrition	Blood-sucking ectoparasites	Hemoparasitism
Chronic disease including mycobacteriosis and aspergillosis	Gastrointestinal parasitism	Bacterial septicemia
Chemicals (lead and aflatoxicosis)	Trauma	Acute aflatoxicosis
Iron and folic acid deficiencies	Rupture of organs or neoplasms	Toxemia
Some neoplasms		

sult Campbell (1988, 1994) and Fudge (2000) for additional information.

Although reference ranges should be established for individual species, it is generally true that vultures and eagles tend to have higher white blood cell (e.g., WBC x 10^9/l) counts than hawks, falcons and owls (N. Forbes, pers. comm.). Both the total and the differential leucocyte count should be obtained. The differential leucocyte count should be expressed both as an absolute count and as a percentage. Consult species-specific reference ranges for normal values. As a guide, in most owl species, the lymphocyte percentage ranges from 40–70%, while in most other raptors, the heterophil is the most common cell (Joseph 2000, Cooper 2002). It is believed that the eosinophil differential can range from 10 to 35% in healthy raptors (Joseph 2000). Conversely, Samour et al. (1996) found that eosinophils were closely associated with parasitism and were not present in such proportions in "normal" individuals. Table 4 lists some leucocyte abnormalities and potential etiologies.

Hemoparasites. Blood parasites are found in many raptors, with incidences varying geographically and among parasite and host species (Joseph 1999). Hemoparasites can cause increased TPP levels, leucocytosis, anemia or death (Garvin et al. 2003, Redig 2003). Table 5 lists hemoparasites in raptors and details regarding pathogenicity, as well as vectors and diagnoses. Pierce (1989) provides a color reference to hemoparasites.

Table 4. Changes in the leucogram of raptors. (Based on Campbell [1994, 2000], Fudge [2000], and Howlett [2000].)

Leucogram changes	Potential etiologies
Leucocytosis	Bacterial infections, including mycobacteriosis, stress, trauma, toxicity, fungal infections including aspergillosis, leukemia
Leucopenia	Overwhelming bacterial infection causing depletion of bone marrow, viremia, depression of bone marrow
Heterophilia	Bacterial infections, including mycobacteriosis, stress, fungal infections including aspergillosis, toxemia
Herteropenia	Overwhelming bacterial infection, viremia, bone marrow suppression, deficiency diseases
Toxic heterophil changes (cytoplasmic basophilia, vacuolization and degranulation, karyorhexis, karyolysis)	Septicemia, viraemia, toxemia, severe infection
Monocytosis	Infection, including mycobacteriosis and aspergillosis, chronic disease, tissue necrosis
Lymphocytosis	Some infectious and metabolic disorders, some neoplasms
Lymphopenia	Stress, uremia, immune suppression, some neoplasms, viremia
Reactive lymphocytes	Infection, including salmonellosis and aspergillosis
Eosinophilia	Parasitism, including hemoparasites, tissue damage, hypersensitivity (questionable)
Eosinopenia	Corticosteroids, stress
Basophilia	Tissue damage, parasites (inconsistent), hypersensitivity (questionable), chronic disease
Fibrinogen (increased)	Infection, inflammation, hemorrhage
Fibrinogen (decreased)	Liver failure
Thrombocytosis	Rebound response to hemorrhage, response to excessive thrombocyte demand (including phagocytosis)
Thrombocytopenia	Excessive peripheral demand or depression of production (e.g., severe septicemia)

Table 5. Hemoparasites of raptors. Based on Cooper (2002), Gutierrez (1989), Joseph (1999), Lacina and Bird (2000), Redig (2003), Remple (2003), Samour and Peirce (1996), Samour and Silvanose (2000).

Species	Site of infection and incidence	Extent pathogenic	Vector and transmission	Diagnosis
Leukocytozoon spp. (Hemosporidia)	Peripheral RBC and WBC — relatively common in a number of species, seasonal incidence	Generally non-pathogenic. May cause illness and occasional deaths due to anemia in young, debilitated or heavily infested birds	Simuliid black flies	Blood smears — non-pigmented gametocytes in RBC cytoplasm. Occasionally found in muscle, heart, spleen, kidney and liver tissues on histology
Hemoproteus spp. (Hemosporidia)	Peripheral RBC — more common in Strigiforms	Generally non-pathogenic. May cause illness and occasional deaths due to anemia in young, debilitated or heavily infested birds	Hippoboscid flies and Culicoides midges	Blood smears — pigmented gametocytes occupying >50% RBC cytoplasm
Plasmodium spp. (Hemosporidia — 34 spp.)	RBC, WBC, thrombocytes and reticulo-endothelial cells. Disease reported in falcons, especially Gyrfalcons (*Falco rusticolus*) and gyr-hybrids	Pathogenicity varies. Clinical signs: anemia, thrombosis, dyspnea, acute death	Culicine, Aedes occasionally Anopheles mosquitoes	Blood smears — pigmented gametocytes, trophozoites and schizonts in RBC, WBC and thrombocytes. May displace nucleus from central position. Unfixed spleen and liver sections
Microfilaria	Free in plasma — sporadic reports in variety of species	Uncertain	Uncertain	Blood smears
Babesia spp. (piroplasm)	Peripheral RBC — a few reports only in Prairie Falcon (*Falco mexicanus*), Saker Falcon (*F. cherrug*), Barn Owl (*Tyto alba*), Bearded Vulture (*Gypaetus barbatus*)	Pathogenicity controversial. Poor performance, possible death	Ticks, including *Ornithodorus concanensis*	Blood smears
Atoxoplasma spp. (coccidia)	Mononuclear leucocytes — reported in Spotted Owl (*Strix occidentalis*)	Uncommonly reported	Ingestion of sporulated oocysts	Reddish intracytoplasmic inclusions indenting leucocyte nucleus
Trypanosomes (flagellated protozoa)	Free in plasma — reported in a variety of species	Not known to be pathogenic	Blood-sucking arthropods	Blood smears. Examination of buffy coat

Management Considerations

Hematological parameters respond to physiological or environmental alterations within hours to weeks. Determining these parameters is easy and inexpensive and can indicate perturbation of the individual, or the population and, in some instances, the ecosystem. If abnormalities are identified, more specific tests (including biochemical, serological and toxicological analysis and polymerase chain reaction [PCR]) should be performed.

The correct interpretation of hematological values requires well-established "normals." Unfortunately, there are gaps in understanding here. Many species have poorly defined "normal" ranges and information regarding basic physiological changes accompanying undernourishment is lacking for many raptors. It also should be noted that databases, published reference ranges, and laboratory reference ranges may have been obtained by different methods and from different numbers of animals in various clinical states and, therefore, may not be directly comparable.

CONCLUSIONS

Although raptor hematology is now a crucial part of clinical veterinary medicine, its use as a management tool in wild populations remains limited. Application to population and conservation medicine has been hampered to date by a scarcity of "normal" values, incorporating age, sex and physiological variables. As more research is conducted, the use of hematological techniques will increase.

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C. Reproductive

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INTRODUCTION

The reproductive anatomy and function of raptors have attracted little attention to date. Basic information, such as the presence and location of sperm-storage tubules and the duration of the fertile period, is still unknown for most species. This paucity of knowledge not only acts as a limiting factor for improved reproductive success in captive breeding programs, but also renders it more difficult to understand the reproductive ecology of wild raptors.

The increasing number of endangered raptor species is being accompanied by a growing interest in their biomedicine and captive propagation. More raptors are coming under the scrutiny of microscopes and modern laboratory techniques, and we hope that this will spark a greater interest in resources and research dedicated to studying reproductive physiology of birds of prey. Meanwhile, readers should consult the limited available studies undertaken in various species used as models, such as the American Kestrel (*Falco sparverius*) (Bird and Buckland 1976, Bakst and Bird 1987), the general review on raptor physiology by Duke (1986), as well as those on the reproductive systems of domestic birds (Johnson 2000, Kirby and Froman 2000) and wild birds (Gee et al. 2004, Samour 2004).

FEMALE REPRODUCTIVE SYSTEM

Reproductive Tract

Ovaries, follicular growth, and ovulation. Unlike the majority of birds, raptors commonly have two functional ovaries (Domm 1939). The phenomenon has been recorded in many species of raptors (Venning 1913, Wood 1932, Boehm 1943, Snyder 1948), but seems more prevalent in accipiters than in Strigiformes (Fitzpatrick 1934).

When growing follicles, females experience a significant increase in body weight. Inability to accomplish this gain may prevent full ovarian growth and egg-laying (Newton 1979, Hardy et al. 1981). Recent studies of Barn Owls (*Tyto alba*) indicate that the onset of reproduction is not triggered by body condition (i.e., an increase in body fat). In fact, the perceived increase in body weight prior to breeding is more likely due to water accumulation as a result of changes in protein metabolism (Durant et al. 2000). Interpretation of the "need" to put on extra body fat as an energy-safe strat-

egy ought to be reconsidered. The rapid growth phase of follicles usually takes 5 to 14 days, during which follicles highest in the growth hierarchy incorporate vitellogenin and low-density lipoprotein in an estrogen-receptor mediated event. In the Golden Eagle (*Aquila chrysaetos*), total fecal estrogen levels progressively increase during the rapid growth phase (Staley 2003), presumably in relation to the increasing activity of the external theca cells of prehierarchal follicles.

Similar to other birds (Wingfield and Farner 1978, Johnson 2000), ovulation of the first egg in Peregrine Falcons (*F. peregrinus*), Golden Eagles, and Asian Imperial Eagles (*A. heliaca*) takes place soon after the estrogen maximum, and coincides with a peak of progesterone and cortisol (J. Blanco, unpubl. data). Both serum luteinizing hormone (LH) and progesterone also peak early in ovulation.

Oviduct. Similar to what has been described for other birds (Gee et al. 2004), the raptor oviduct consists of five distinguishable regions: infundibulum, magnum, isthmus, shell gland, and vagina. The size and mass of the oviduct increase parallel to the ovary early in the breeding season, as regulated by steroid hormones. The presence of sperm storage tubules at the uterovaginal region is poorly documented in raptors. These microscopic structures in the folds of the cervix mucosa have been observed in the American Kestrel (Bakst and Bird 1987; Fig. 1) and in other raptor species (Blanco 2002). These tubules determine the fertile period by maintaining sperm viability and continual release to the site of fertilization.

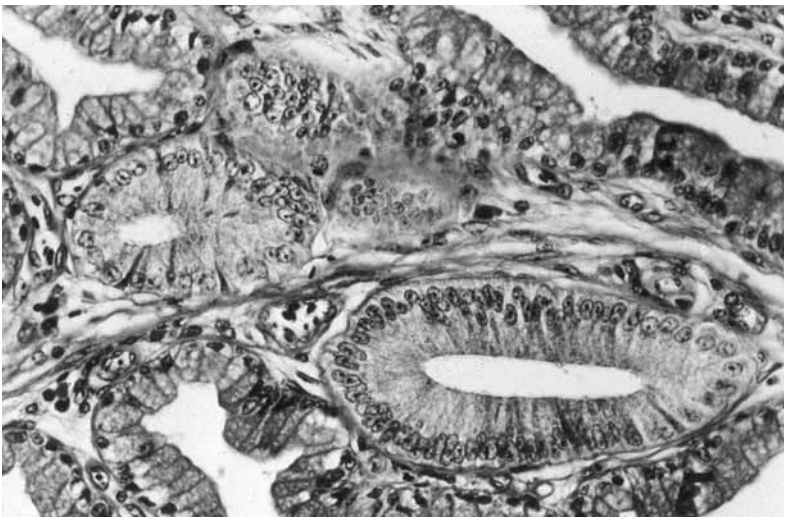

Figure 1. Sperm storage glands were first discovered in American Kestrels (*Falco sparverius*) and are likely found in most other raptors.

Eggs

Egg physiology and variation in eggshell and membrane characteristics. After ovulation the ovum is engulfed by the infundibulum (the site of fertilization), and next descends through the oviduct. The process usually lasts for two or more days, depending on the size of the birds, and involves the addition of numerous layers that conform to the egg. Cuticle, crystallization layers (external, palisade, and mammilary) and eggshell membranes can be differentiated easily in the eggs of raptors. The morphology and size of eggshell pores vary among species (Blanco 2001) and, together with the outer crystallization layer, may be of taxonomic interest.

Falconiformes have been reported to produce more massive eggs than Strigiformes of similar body size (Saunders et al. 1984). Interestingly, body mass is positively correlated with egg width in free-ranging Eleonora's Falcon (*F. eleonorae*) (Wink et al. 1985) and Black Kites (*Milvus migrans*) (Viñuela 1997), but not in captive American Kestrels (Bird and Laguë 1982a). Both inter-annual and intra-seasonal variation in egg-laying dates have been recorded in both captive and free-ranging populations of Peregrine Falcons (Burnham et al. 1984) and Golden Eagles (Blanco 2001), with a significant decrease in length, breadth and initial mass with time.

Certain external factors including stress (Hughes et al. 1986), ambient electromagnetic fields (Fernie et al. 2000a), organochlorine compounds and metabolites (for review see Hickey and Anderson 1968, Ratcliffe 1970, Cooke 1979, Wiemeyer et al. 2001, Chapter 18), heavy metals (Ohlendorf 1989, Blanco 2001), and PCBs (Lowe and Stendell 1991, Fernie et al. 2000b) can induce shifts in eggshell thickness and ultrastructure, as well as in ultrastructure and fiber organization and pattern of the shell membrane.

Clutch size and replacement. Clutch size often is influenced by phylogeny and individual factors including size and age (Brommer et al. 2002). From a global perspective, the number of eggs laid varies latitudinally in some falcons in Australasia (Blanco 2001), as well as longitudinally in several eagles and *Milvus* kites in that region (Olsen and Marples 1993).

The ability to replace clutches has been used as a management tool (see Chapter 23) to augment both captive and wild populations of raptors (Bird and Laguë 1982a). In captive and wild American Kestrels, replacement clutches had fewer eggs than first clutches, but did not differ in fertility, hatchability, and fledging success (Bird and Laguë 1982a,b; Bowman and Bird 1985).

MALE REPRODUCTIVE SYSTEM

Paired reproductive tracts in male birds of prey lie along the dorsal body wall and consist of a testis, epididymis, and a straight ductus deferens, which differs from the highly convoluted version found in some domestic species (J. Blanco, unpubl. data). Spermatogenesis depends on follicle-stimulating hormone (FSH), testosterone, the activity of Sertoli cells and their interaction with the spermatogonial stem cells. Seasonal testicular growth usually takes up to 45 days in the majority of raptor species, a period longer than ovarian growth in the female. FSH and LH, as well as testosterone, are essential for spermatogenesis. The process of spermiogenesis, and the duration of the transport through the excurrent ducts are unknown, but it is clear that fluid is absorbed to concentrate sperm and to become seminal plasma. Seminal plasma differs from blood plasma in electrolyte and protein composition (J. Blanco, unpubl. data). The importance of this process is not well understood, but is likely related to sperm motility more than fertilizing ability, since testicular sperm are able to penetrate the inner periviteline membrane *in vitro*.

Male Gametes

Semen production period, seminal quality, and factors of influence. Semen production period varies among species and individuals, but usually last for nearly three months. Bird and Laguë (1977) described an average period of 74 days for captive American Kestrels with a maximum of 103 days. Longer periods were found for Peregrine Falcons (95 days; Hoolihan and Burnham 1985) and eagles (up to 110 days; Blanco 2002).

Semen production in American Kestrels held in Montreal, Canada begins at about 12 hours and 45 minutes of daylight, and declines considerably at about 15 hours and 45 minutes (Bird and Buckland 1976).

Ejaculate characteristics vary greatly among species and individuals, and with collection method (Bird and Laguë 1976, Boyd et al. 1977, Weaver 1983), male reproductive condition, nutrition (Randal 1994), certain pollutants (Bird et al. 1983) and climate (Bird and Laguë 1977). Concentrations ranging from 31,000 to 40,000 spermatozoa per mm^3 and volumes between 3 and 14.6 µl have been reported for the American Kestrel (Bird and Buckland 1976, Bird and Laguë 1977, Brock 1986). Expectedly, ejaculate volume increases with species size. Semen volume in Peregrine Falcons can be as high as 95 µl (Hoolihan and Burnham 1985), with cell concentrations ranging from 26,000 to 81,000 sperm per µl.

Sperm production varies seasonally; sperm concentration increases early during the breeding season, peaks in mid-season, and declines thereafter. This pattern varies longitudinally. Numbers of spermatogonia, spermatids and abnormal spermatozoa are more likely to be present in the ejaculate both early and late in the season when testes are no longer at their maximum size and when testosterone levels are lower than normal. This is related to the need to ensure maximum sperm quality at the time of maximal frequency of copulation prior to egg laying (Blanco et al. 2002).

Urine contamination of semen and subsequent sperm damage is frequent during collection using forced-massage techniques (Bird and Laguë 1977). Fox (1995) provides a useful description, including an illustration of the various contaminants in raptor semen. The use of modified dilutents may help reduce deleterious effects (Blanco et al. 2002). *Escherichia coli* is the most prevalent bacteria contaminating raptor semen. Samples need to be evaluated with caution before artificial insemination to avoid the risk of ascendant salpingitis (Blanco and Höfle 2004).

Artificial insemination. Artificial insemination with fresh semen has been successful in a variety of non-domestic avian species including raptors. This technique has been used as a management tool in several captive breeding projects using fresh diluted semen (Temple 1974, Samour 1986). In the American Kestrel, fertility rates using artificial insemination are similar to those achieved by natural mating (Bird et al. 1976).

Sperm cryopreservation. Semen collected by massage techniques has been cryopreserved and progeny obtained in several species (Gee 1983, Gee et al. 1985, Brock 1986, Parks et al. 1986, Samour 1988, Gee and Sexton 1990, Brock and Bird 1991, Knowles-Brown and Wishart 2001, Wishart 2001). Comparative studies on sperm tolerance to different osmotic conditions, cryoprotectant concentrations and cooling rates indicate considerable variation, even between closely related raptor species (Blanco et al. 2000). Different freezing rates and protocols are described in Brock et al. (1983) and Knowles-Brown and Wishart (2001).

Glycerol and the alternatives, dimethyl sulphoxide (DMSO) and dimethyl acetamide (DMA), have been used widely in sperm cryopreservation in non-domestic species. Sperm from the falcon type (Brock and Bird 1991, Gee et al. 1993) have been successfully cryopreserved using either 13.6% glycerol; 6%, 8%, or 10%

DMSO; or 13.6% DMA. Evaluation of fertilizing ability has been mostly based on progressive motility and fertility after artificial insemination (Gee et al. 1985, Brock and Bird 1991, Gee et al. 1993). In the American Kestrel, motility after thawing averaged 41% and 13% using glycerol and DMA, respectively (Brock and Bird 1991). Post-thaw fertility rates have been obtained following artificial insemination using glycerol as cryoprotectant in the Peregrine Falcon (33.3%) (Parks et al. 1986) and the American Kestrel (11.8%) (Brock and Bird 1991).

Photoperiodism, Reproductive Hormones, and Endocrine Disruptors

The influence of photoperiodism on levels of gonadal hormones in birds generally is well understood, but most of our knowledge of this phenomenon in raptors is based on the use of artificial lighting to induce reproductive activity in captive pairs (Willoughby and Cade 1964, Bird et al. 1980). We know nothing about natural circadian rhythms in raptors, but data collected on other bird families are likely relevant and applicable.

Nelson (1972) and Swartz (1972) were among the first to elucidate the need for photoperiodic stimulation to induce northern-nesting raptors like Gyrfalcons (*F. rusticolus*) and Peregrine Falcons to breed in captivity (i.e. the farther north they originate from, the longer the photoperiod they require). If extra day-length in the form of artificial lighting is to be used, the changes in day-length should be made as gradually as possible to reduce physiological shocks (Bird 1987). At least one successful attempt using artificial photoperiodic changes has been made to induce American Kestrels to undergo an out-of-season breeding period between two consecutive successful spring breeding periods (Bird et al. 1980). An attempt to hasten sexual maturity in kestrels using photoperiod encountered mixed success (Ditto 1996). Such procedures could be used to increase the output of offspring in endangered species breeding programs or to accelerate the turnover of data in experimental research involving captive raptors.

The vast majority of our knowledge about raptor reproductive endocrinology has relied upon blood sampling and plasma-hormone determinations. In the female, plasma corticosterone, progesterone, estradiol 17β and estrone are highest during courtship and egg laying (Rehder et al. 1984, 1986), whereas high levels of androgens, including testosterone, were associated with aggression, territoriality, courtship, nest-building, testicular development, and spermatogenesis in the male (Temple 1974; see also Rehder et al. 1988). Information on plasma levels of lutenizing hormone in American Kestrels can be found in Ditto (1996). More recently, fecal steroid monitoring, which has been used to study seasonality in hormone levels (Bercovitz et al. 1982), the effects of human disturbance (Wasser et al. 1996), steroid excretion lag time (Wasser et al. 1996), and sex determination (Bercovitz and Sarver 1988), shows potential as a safe non-invasive source of information regarding hormone levels.

Exposure to extreme temperatures can limit avian reproduction (Mirande et al. 1996). Drastic temperature fluctuations often reduce semen production (Kundu and Panda 1990), as well as egg-laying and copulation frequency (Bluhm 1985).

The impacts of organochlorine chemicals on reproduction of birds of prey have been well documented (see Chapter 18). Studies indicate that these chemicals also act as endocrine disruptors. For instance, preliminary data by Bowerman et al. (2003) suggest that hormone disruptors, not necessarily estrogen or androgen mimics and their antagonists, are associated with reproductive and teratogenic effects in Bald Eagle (*Haliaeetus leucocephalus*) populations in the Great Lakes Basin. Alterations in reproductive behavior in captive breeding American Kestrels were induced by exposure to Dicofol, one of the last organochlorine pesticides to be banned from use in the U.S. (MacLellan et al. 1996). Other organochlorine chemicals that impact upon reproduction in birds of prey through hormone disruption come in the form of industrial by-products and include polychlorinated biphenyl ethers (PCBs). Captive American Kestrels exposed to PCBs developed more frequent aggressive courtship interactions and experienced clutch abandonment (Fernie et al. 2003); alterations in brood patches also have been observed in PCB-exposed kestrels (Fisher et al. 2006). Most recently, attention has focused on the alarming increase in residue levels of polybrominated diphenyl ethers (PBDEs) in food chains world-wide, arising from the use of brominated flame retardants applied to many household products (Chapter 18). Using the American Kestrel as a model test species, a number of reproductive effects have been documented thus far (cf. Fernie et al. 2006).

SUMMARY

Captive-propagation programs have been extremely useful in maintaining genetic diversity and restoring

wild populations of endangered raptors. However, captive breeding success requires knowledge of a species' reproductive behavior, physiology and endocrinology. In addition, species-specific differences in anatomy, gamete or physiological parameters may complicate the task of maintaining captive breeding populations of raptors. Further research is needed to unravel some of the major questions including the spatial requirements and factors involved in the control of reproduction in endangered raptors. Finally, an improved knowledge of the reproductive physiology of raptors will help us better understand the impacts of chemicals released into their environment on their reproduction and, ultimately, their survival.

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