CHAPTER 6. MINOR MANIPULATIVE PROCEDURES

A. Overview

In the past two decades, the emergence in ornithology of immunology, endocrinology, and genetics have increased the need for the collection of blood and other fluids and feathers and tissues from wild birds. New technologies and methods make it easier to answer questions about every aspect of bird life using information obtained from samples taken from wild birds. Most of these procedures, such as experimental injections and implants of hormones and drugs, playbacks of tape-recorded vocalizations, and presentation of decoys, have long been fundamental tools for ornithologists and are considered minor manipulations to an individual bird. Minor manipulations are procedures that are considered less invasive than surgery and administering anesthesia. However, the term "minor" refers to the nature of the manipulation and not to potential impacts. The procedures as discussed in this section all have the potential to have serious impacts, including mortality.

There are a number of underlying themes for each of the procedures discussed. First, each technique or procedure should be appropriate to the scientific question and goals. Second, there is the need for training and/or practice with these techniques and training should be documented. Third, in many instances, veterinary oversight may be needed, and lastly, appropriate permits and approvals must be obtained prior to beginning research.

If these activities necessitate the handling of a bird, federal (and usually state) permits are required; for endangered species, permits are needed even if birds will not be handled (see Introduction and the Ornithological Council's Permits website). Submission of a research protocol for approval by an Institutional Animal Care and Use Committee is required for all of the procedures discussed here.

For all procedures covered here, handling time should be minimized, especially with breeding birds. If periods longer than a few minutes are routinely necessary, as they may be if the sampling procedure is complex, then a justification should be included in the IACUC protocol and be appropriate for the scientific objectives. In addition, researchers should be prepared to euthanize birds if they become injured during the handling and sampling process and the bird cannot be taken immediately to a veterinarian (or reputable animal rehabilitation center) and has a severe injury that, in a comparable injury, would likely induce considerable pain and distress

to a person (see section on Major Manipulations regarding appropriate euthanasia methods). The IACUC protocol should describe euthanasia methods that will be used.

B. Wild birds studied in captivity

Many studies entailing minor manipulations can be done in the field but others may require wild birds to be held in captivity. Wild birds used in captive studies should be as healthy and free of trauma as possible when initially brought in. Researchers should determine the appropriate housing and feeding conditions and should accommodate the social and behavioral needs of the birds (see section on Housing and Captivity). Consulting other researchers or zoos that have kept the same or similar species in captivity is advisable. Consult the American Zoo and Aquarium Association's taxon-specific housing and husbandry standards. Keeping a few birds in captivity before capturing all birds needed for the study can help in determining optimal conditions. A period of acclimation to captivity allows the birds to adapt to the new environment, ensuring that study results are not affected by stress responses to capture and captivity. It also gives the researcher time to identify and address health issues that emerge during the acclimation period. Studies show that passerines require three to four weeks to acclimate to captivity before experiments begin (Cockren, et al. 2008; Hull et al. 2007; Wingfield et al. 1982). Body mass usually declines after capture and plasma levels of metabolic and reproductive hormones are often abnormal. Wingfield et al. (1982) demonstrated that corticosterone decreased after an acclimation period of two to three weeks for White-crowned Sparrows (*Zonotrichia leucophrys*) kept in small cages with other individuals. However, Marra et al. (1995) found that corticosterone levels in birds acclimated for 35 days remained high compared to newly caught wild *Zonotrichia* sparrows. After three to four weeks, body mass returns to that of capture and hormone levels stabilize. It is known that handling stress raises the stress hormone corticosterone (Hull et al. 2007; Cockrem et al. 2008); studies have also shown that handling stress can affect the immune system in captive Red Knots (*Calidris canutus*) for a short period (Bueler et al. 2008) mostly leading to a redistribution of white blood cells in the body (Dhabher 2009).

Birds kept in captivity for a prolonged period of time may not be releasable. In many cases, federal or state permits or regulations prohibit release. In addition, the birds' survival may be affected by changes in body condition and behavior that occurred during the study. If permits allow release, birds should be conditioned for release as needed. For instance, they should be

maintained in a flight cage to allow the flight muscles to strengthen and should be given natural foods in a manner that resembles natural foraging conditions. Birds should be released into the same kind of habitat from which they were captured. The release of birds studied in captivity could pose a risk to wild populations. All birds must be checked for disease prior to release, particularly if they have been held in a facility where other species are present. If permits do not allow release, or if birds are otherwise not suited for release, they can be held for additional research if circumstances permit. Otherwise, euthanasia is necessary. In that case, contact a museum or teaching collection to make arrangements to donate the remains for future study. See the appendix on preparing birds for donation to museums.

C. Collection of blood samples

Effects of collection on survival and behavior

Specific methods for collecting blood samples from birds have been reviewed by Morton et al. (1993) and Campbell (1994). There are numerous resources available on proper blood sampling techniques, including an instructional video demonstrating blood collection that is available from the National Wildlife Health Center

http://wildlife1.wildlifeinformation.org/000VIDEOS/V2_BloodCollBirds/VideoBirdBloodCollectCDROM.htm

However, blood collection techniques, including the proper way to hold the bird, stop bleeding, and reduce stress, should be learned by working directly with an experienced mentor, such as a veterinarian or experienced avian researcher. Only after sufficient practice under the guidance of an experienced teacher should a researcher use these techniques without supervision. Sheldon et al. (2008) provides the most thorough literature review to date on the potential effects of blood sampling. There are considerable data gaps on the impacts of blood collection, such as the impacts on developing birds or birds of the tropics. In semi-captive or free-living species, collection of blood has been shown to not affect survival (Raveling 1970; Wingfield and Farner 1976; Bigler et al. 1977; Gowaty and Karlin 1984; Frederick 1986; Dufty 1988; Hoysak and Weatherhead 1991). Brown (1995) found that collection of blood samples from the jugular vein of 9-day-old Ring-billed Gull (*Larus delawarensis*) chicks had no effect on survival rates to 21 days of age and the rate of nest desertion by adults was also unaffected. Lanctot (1994) determined that withdrawal of up to 0.05 ml of blood from the jugular vein of Buff-breasted Sandpipers (*Tryngites subruficollis*) chicks within 24 hr of hatching had no effect on growth or

survival to fledging. Further, the occurrence of hematomas on the jugular in some chicks did not impair survival. In addition, Schmoll et al. (2004) found that blood sampling does not affect fledging success in Coal Tits (*Parus ater*). However, with chicks the risk of dehydration exceeds that of blood loss Schmoll et al. (2004), so investigators should provide birds with fluids, if possible. However, fluids that are too warm or cold could lead to additional problems, such as heat or cold stress. Too much fluid given intravenously can result in too great a reduction in packed cell volume (the portion of the blood comprised of red blood cells) or plasma total solids (Wicks and Schultz 2008).

However, in a statistically rigorous study using controlled comparisons of bled and non-bled Cliff Swallows (*Petrochelidon pyrrhonota*), Brown and Brown (2009) found that blood sampling reduced annual survival in the first year after sampling. Apparent survival of the 2,945 birds bled over the 20 years over study was reduced between 21% and 33%, depending on the amount of blood taken. The blood samples ranged from 0.3% to 1.2% of the birds' body mass. Non-bled birds included in the survival analysis were captured and handled at the same sites, at the same time, and in the same manner as the bled birds except that no sham non-bleeding was done, i.e., birds were not held in the posture used to bleed birds. The same four investigators undertook the blood sampling throughout the study period. Survival was also affected by the fumigation of colonies to control nest parasites; although survival rates were higher for birds in fumigated colonies, the effect of bleeding was greater among birds in the non-fumigated colonies.

Applicability of these results to other species may be limited by the fact that the blood samples in this study were taken with brachial venipuncture on a species that feeds on the wing and is in flight most of the day. Brown and Brown (2009) acknowledge the possibility that hematomas resulting from brachical venipuncture could be a problem for this very reason. Voss et al. (2010) also suggest that the birds in a less arid environment than that where the Bomberger and Brown study took place might recover more quickly from the fluid loss resulting from bleeding. A thorough commentary (Voss et al. 2010) also points out that the Bomberger and Brown (2009) study was the first on an aerial species, with higher mass-specific metabolic rates and a higher demand for oxygen from circulating red blood cells.

There may be sub-lethal or short-tem effects that alter forms of behavior or physiology that are not ordinarily assessed, particularly if multiple procedures of different types are being done on individuals. Repeated handling and blood sampling of birds has been shown to have sub-lethal

long-term effects (van Oers and Carere 2007). Captive Great Tits (*Parus major*) handled seven times and bleed give times had a higher rate of respiration and were more docile when handled thirty days later than were tits handled three times and bled once, suggesting that repeated manipulations can cause long-term behavioral and physiological changes.

Normal feeding and brooding activities, molt, and ability to migrate also have been shown to not be affected by blood collection (Wingfield and Farner 1976; Frederick 1986). Overall, the appropriate collection of blood samples from wild birds has not been shown to impair behavioral patterns or reproduction of wild birds.

Amount of blood

Earlier editions of these *Guidelines* specified that "a general rule of thumb has been no more than 2% of the body weight of the animal be collected in any 14-day period, or no more than 1% at any one time (McGuill and Rowan 1989). For a 10-g bird the maximum would be approximately 100μlof whole blood or one to two 70-μl capillary tubes; for a 50-g bird the maximum would be about 500μlof whole blood or 8 70-μl capillary tubes." A closer reading of McGuill and Rowan (1989) suggests that a single blood sample should comprise no more than 15% of total blood volume, in lieu of the rule-of-thumb then widely used in laboratory-based studies, which assumed that total blood volume comprised 10% of body mass and that 10% of the blood volume could be taken without harm to the animal. In fact, total blood volume may be less than 10% of body mass. Voss et al. (2010), noting the results of the Bomberger and Brown (2009) study of the impact of blood sampling on Cliff Swallows, suggest a more nuanced approach. They suggest, as did McGuill and Rowan (1989) that, "A more conservative method of determining safe blood sample volumes would involve calculating average blood volume for species specific lean body mass and limiting sample volume to less than 10% of total blood volume." Voss et al. (2009) acknowledge that 1% of body mass is an easily adopted metric for field use, but urge that with additional research similar metrics could be developed that are more conservative and appropriate to account for variation in seasonal and individual body composition. This more conservative approach may also provide a measure of safety for animals that are studied in the wild shortly after blood is drawn for, unlike laboratory animals, they will need to forage for food and escape predators shortly after release. Additionally, the more conservative approach may address the fact that some individuals will have lower blood volumes than others. A further consideration might be the foraging behavior of the species and the resultant demand oxygen from circulating red blood cells. Species with high metabolic rates,

such as hummingbirds, may be more susceptible to the effects of blood loss. Voss et al. (2010) also suggest that researchers take into account environmental variables that may affect survival after blood draws. Specifically, they suggest consideration of temperature and humidity which may make it more difficult for birds to compensate for the physiological effects of sampling. Finally, they also suggest decreasing the volume of blood taken when birds are facing additional stressors, such as heavy parasite loads or during energetically demanding periods such as migration or reproduction. In addition, they suggest adjusting the volume of blood taken to reflect the lean body mass of the individual rather than a species' average, which should be helpful where the average total blood volume of a species is not known.

Choice of methods

One of the most important considerations for blood sampling is the site from where the blood is collected (Sheldon et al. 2008). There are numerous things to consider first when choosing the method of blood collection, that includes: (1) how the blood be used for and the amount needed, (2) the need for whole blood, serum, or plasma needed, (3) the need for core blood rather than peripherally collected blood, (4) the proficiency of the collector for the various blood draw techniques, and (5) any structural, physiological, behavioral, or ecological considerations for the birds species to be sampled. These are all very important considerations that must be made before choosing an appropriate site for blood collection. The site of the blood collection is important not only for the different impacts to the birds; it may also impact the biological endpoints being measured. Additionally, as Sheldon et al. (2008) point out, different handling techniques are required depending on the site of blood collection. Blood collection techniques include the use of a syringe for obtaining blood from the jugular vein, occipital venous sinus, from the ulnar vein in the wing, or heart puncture (see also Dorrestein 1978; Vuillaume 1983). If the animal is not to be killed or incapacitated as part of the experiment, then the volume of blood to be withdrawn is an important issue (McGuill and Rowan 1989).

There may also be a scientific reason where core blood is necessary. Core blood is obtained through heart puncture by the furcular route or through the sternum. The scientific justification for employing this method should be very compelling because these techniques may result in severe debilitation or death, especially among smaller species, though the sternal approach resulted in no mortality in a number of taxa, including passerines (Utter at al. 1971). This may be the result of the structure of the heart; from the furcular region a needle may inadvertently

pass through the aorta or other major blood vessels before entering the heart and thus may be more likely to cause irreparable damage. It is difficult to judge where the needle enters the heart and to determine what kind of bleeding or damage may occur as a result of employing this technique. In terminal studies where birds are under anesthesia, exsanguination can be used both to draw blood and as euthanasia when used as an adjunct to other agents or methods (AVMA Guidelines 2007). This method should be performed only by highly experienced researchers or veterinarians.

When larger amounts of blood are required, the jugular vein is commonly preferred. Hematomas do not occur as readily there as with the brachial vein. Hematomas that occur as the result of taking blood represent an additional immediate loss of blood that is an important consideration if maximal blood samples are being taken. Also, a hematoma can be painful, especially if it over or adjacent to a joint.

For jugular bleeding in general, it is important the correct technique is applied and following the blood draw pressure is applied to the vein to prevent continual bleeding. To somebody proficient in this technique there are rarely problems. For a jugular blood draw, the collector must be very proficient. No beginners should attempt to do this. Training in the field on research subjects is inadvisable; it is better to learn in a controlled environment, perhaps obtaining birds from a pet shop (see section on quarantine in captive housing before bringing these birds into the lab if research subject are housed or will be housed in the lab). An appropriate size of needle and syringe should be used. For example, for small species weighing less than 10 g, a 30 gauge needle with a 0.3 cc total volume tuburculin syringe allows for the measuring of blood drawn to in increments of 0.01 ml. Blood samples where more than a drop or two of blood is required are much more quickly obtained from the jugular vein than from the brachial or femoral vein and likely result in much less stress to the bird (when performed by an appropriately trained and experienced individual). Hands-on training and initial supervision from someone experienced in this technique should be a requirement for this technique.

Given the small amount of blood required for many studies, investigators may prefer to obtain small amounts of blood from the ulnar (wing) vein or from vessels in the tibio-tarsi. Either one will yield a suitable blood sample. In larger species a syringe and needle is appropriate. For smaller species (i.e., less than 100 g) it is recommended that the vein be punctured with a 26 gauge or smaller needle and blood collected directly into microhematocrit capillary tubes. Using the microhematocrit tube for blood collection, it is recommended that quantities of one-third to

one-half capillary tube (70 μl for birds less than 7 g, one tube for birds seven7 to 15 g, and two tubes for larger birds. However, this may be too much blood collected if repeated sampling is completed or the birds are stressed in other ways. Proper training for bleeding from the ulnar vein is important. It is easy to hit an artery and get excessive bleeding. If the needle is not used properly a hematoma can occur. New researchers should obtain training from someone with extensive experience to ensure proper collection of venous blood with minimal stress. Nestlings may be more susceptible to hematomas and the tibio-tarsal.

Toe-clipping is acceptable in the field only for very small birds such as hummingbirds if only a small amount of blood is required. It is generally necessary to clip only the toenail (Leonard 1969). Although toe-clipping may have the added benefit of identifying previously sampled birds, it is not an accepted procedure for marking birds.

Stopping bleeding

The normal clotting time for most bird species has not been determined, but can be considered to be about 5 min. Therefore it is imperative not to prematurely remove the pressure being applied to the incision site.

Investigators should always be prepared to stop the bleeding if clotting does not occur spontaneously and quickly. On occasion, bleeding will stop on its own but this should not be the assumption. Soft, direct pressure sort should be applied to the puncture site. A cotton ball may be used but the cotton may stick to the clot and pull the clot away when the cotton is removed, causing bleeding to resume. A non-adhesive bandage pad can be applied in lieu of cotton.These pads are slightly stiff, preventing good contact with the site, especially for smaller birds. In addition, the area can be tilted to be raised to be above the heart. Corn starch or flour can aid in blood clotting. Veterinarians sometimes use a styptic powder such as Kwik-Stop® which is useful for stopping bleeding from toenail clips but aviculturists have raised concerns about using styptics on soft tissue or bleeding feather shafts. It is unclear if this concern emanates from the minor stinging sensation caused by stypic; no literature suggesting toxicity or other specific adverse reaction has been found.

It is better to prevent hemorrhage from the brachial vein than to try to control it. Before a needle is withdrawn from the vein, proximal occlusion of the vein must be released and pressure must be provided over the insertion site before the needle is withdrawn. Pressure is increased as the

needle is withdrawn. Too much pressure may completely empty the vein of blood, which will prevent clotting. The proper amount of pressure can only be learned with experience.

Blood samples

Once taken, the blood (and other tissue) samples should be properly preserved for survival under field conditions and protocols should be established for handling of the blood sampling prior to collecting blood to ensure that blood is not wasted. Because avian erythrocytes are nucleated and better DNA techniques have been developed, one drop of blood is now sufficient for most genetic studies. New techniques are being developed every year to make DNA analysis cheaper and more field-friendly (Quintana et al. 2008). For instance, until 2009, genetic sex identification analyses relied on between-sex variation in intron size in two genes on the sex chromosomes, but in about 50% of bird species, intron size does not vary between the sexes (Griffiths et al. 1998). In 2009, a new technique was developed that uses multiplexed PCR and that should work for all avian species (Han et al. 2009). If only DNA is needed, researchers should consider feather sampling as discussed below.

Repeated sampling and time of sampling

Measuring stress hormones, immune function, or other physiological endpoints has become an important tool for assessing health and for measuring the effects of various environmental and anthropogenic stressors in wild birds. Such studies often require repeated sampling and may cause lasting effects on the bird or may affect the value of the biochemical indicator being measured (Davis 2005, Pérez-Rodríguez et al. 2007, van Oers and Carere 2007).

Repeated sampling over a short time has been used successfully in smaller species (Marra and Holberton 1998; Wilson and Holberton 2004) and has not been shown to cause negative impacts. The capture, handling, and restraint comprise the baseline stressors; it is assumed that all birds will regard capture and handling as stressful (Wingfield 1994). Between samples, many birds can be held in cloth bags, which allow adequate ventilation but prevent injury if the bird struggles. These bags should be placed in a secure, safe place in the shade and sheltered from direct negative effects of weather. Paper lunch bags can also be used as a disposable alternative that can help to reduce the possibility of disease transmission between individuals

and populations. Bags are not an appropriate form of confinement or restraint for species with long necks, long bills, log legs, or towhees that may dislocate their legs in the bags [see discussion in Capture and Marking]. In captivity, wild birds survive well after repeated blood sampling (even at three to five-day intervals), and body mass remains normal (Wingfield et al. 1982; Stangel 1986). However, hematocrits can be reduced by repeated blood sampling (Aramaki and Weiss 1961; Fair et al. 2007) and the combined volume of blood taken during a stress series should not exceed the equivalent of 1% of body mass per sample. With care, serial blood samples may be taken from the same site such as the ulnar vein without creating multiple puncture wounds. Serial collection of blood samples by heart puncture should not be attempted. Care should also be taken to ensure that breeding birds are not withheld from their nests for too long. A 30- to 60-minute period before or after bleeding is not a problem, unless the individual becomes separated from a flock, or could potentially lose a territory. Investigator discretion is advised if environmental conditions, daylight, or weather have changed during the holding period.

Time of sampling can affect the value of the hormone being sampled. To ensure that blood samples are reliable estimates of precapture corticosterone concentrations (Romero and Romero 2002), a small initial blood sample should be collected within two to three minutes of disturbance, including time spent capturing and handling the bird prior to blood collection (Chloupek et al. 2009, Lynn and Porter 2008, Romero and Reed 2005, and Romero and Romero 2002). Comparing the rate of corticosterone secretion over a predetermined period can be an indicator of the strength of the acute adrenocortical response (Wingfield 2005).

Alternate means to obtain blood or material for genetic studies, stable isotope analysis, and contaminants

Other means to obtain DNA from birds include feathers (Smith et al. 2003, Quintana et al. 2008) and eggshells for maternal DNA (Egloff et al. 2009). Feather pulp is commonly collected for genetic investigations (De Volo et al. 2008; Freedman et al. 2008; Hogan et al. 2008

Recently, a less invasive technique has been developed that utilizes blood-feeding insects (Heteroptera, Triatominae) obtained from incubating birds (Becker et al. 2006); blood collected using this method was found to contain similar corticosterone levels as other methods (Arnold et

al. 2008). Any technique that involves the movement and possible introduction of non-native species into a geographic area should be carefully evaluated for unintended consequences.

Fecal sampling provides a non-invasive alternative to blood for measuring stress hormone levels but lack the time precision of blood hormones and require extensive validation to correct for feeding rates, gut passage time and other factors (Romero and Romero 2005).

D. Collection of other tissues

Research in physiology and genetics often requires tissue biopsy. A biopsy involves the removal of cells or tissues for examination and the most commonly sampled (other than blood) are adipose tissue, muscle, liver, and gonad. Many of the procedures used could be considered surgery and a veterinarian should be consulted in the planning of these procedures. Samples taken should involve the minimal amount of tissue necessary for scientific validity. Depending on the tissue to be sampled, analgesia or anaesthesia may be required to effectively and humanely obtain the necessary sample and the researcher may need to consider incision closure options (e.g., surgical glue or sutures). In addition, care should be taken during the procedure to keep any invasive techniques sterile. Refer to relevant sections within Major Manipulations for details on aseptic technique, pain management, and incision closure . The survival ability of birds that are released following a biopsy procedure should not be compromised.

Various studies (Baker 1981Westneat et al. 1986; Westneat 1986b) show that muscle biopsy has little effect on body condition or survival in either wintering or breeding birds. After prompt handling and release, the birds often returned to normal foraging and breeding activity. Males often sing within minutes of release, and even nestlings that were biopsied showed no debilitation, begged for food, and were fed normally. Even biopsy of the pectoralis major muscle was found not to hinder flight (Westneat 1986b). However, Frederick (1986) determined that incubating adult White Ibis (*Eudocimus albus)* subjected to biopsy of the pectoral muscle abandoned their nests, with the resulting loss of their nestlings. Other ibises subjected only to blood sampling did not abandon their nests. Taking muscle biopsies may not cause mortality of birds, but may affect reproduction, dispersion, recapture rates, and short-term changes in body mass (Westneat 1986, Westneat et al. 1986).

Surgical liver biopsies are taken from birds for studies of contaminants and for diagnosis of disease. Degernes et al. (2002) give a thorough description of anesthetic and surgical techniques for obtaining liver biopsies in the field but observed that biopsied adult birds abandoned their nests.

Feathers are also increasingly used for stable isotope analysis (Hobson and Wassanaar 2008). Plucking a few remiges or retrices is usually an innocuous procedure, but care should be taken not to remove so many feathers as to impair flight or other essential functions; this is less of a problem in a captive subject. The removal of growing feathers can result in bleeding, and release of the bird should be delayed until bleeding has stopped. Additionally, the energy requirements and consequences of replacing plucked feathers during different time period is not completely understood. Down feathers can be successfully removed from nestlings without serious effect (Stangel et al. 1988, Evans et al. 2009).

Pathogens such as avian influenza may persist in the cloaca or oropharyngeal tract, which can be swabbed to obtain samples. Collecting swab samples from birds is not considered to have a major impact on birds but care must be taken in handling the bird and completing the technique quickly, especially for the orophyaryngeal swabs which can cause discomfort. Due to the large number of researchers now swabbing wild birds, there are several online training videos for collecting swab samples. See
<http://wildlifedisease.nbii.gov/media/Sample Collection04010301_256k.zip>.
However, videos are no substitute for training by experienced researchers. Lastly, handling of birds should include proper human health and safety precautions that may be recommended when pathogens that have the potential to cause serious disease in humans may be present (Ornithological Council 2006).

E. Collection of food samples

Obtaining information on a species' diet in the field is often an important component of ecological and nutritional studies. Historically, dietary analysis involved sacrificing birds to enable direct observation of stomach contents (Duffy and Jackson 1986; Barrett et al. 2007). In some cases collection of fecal samples and regurgitated pellets can provide most, if not all, of the information needed. However, in some species fecal material is not useful (e.g., frugivores). In other cases, such as marine birds at sea, it is not possible to collect fecal samples, although many will regurgitate stomach contents soon after capture. If birds are to be sacrificed to obtain

stomach contents, investigators must obtain scientific collecting permits and select the most appropriate euthanasia technique (see Section on Major Manipulations). In addition, arrangements should be made to assure that the specimen is donated to a teaching institution or a museum collection or otherwise made use of for scientific study.

Several techniques available for dietary analyses do not require sacrificing birds: neck ligatures, fecal analysis, pellet analysis, stomach pumping or flushing, emetics, and biochemical methods (Rosenberg and Cooper 1990). Some seabirds may regurgitate stomach contents soon after capture. However, others may not, and the use of other techniques may be necessary.

Neck ligatures on nestlings

Use of neck ligatures to obtain food samples from nestlings may occasionally be justified. Neck ligatures can be used in nestlings but not adults due to the need for easy recapture. In such cases, the investigator should be careful to ensure normal blood circulation and tracheal function. Different ligature materials can have different levels of efficacy and. For example, plastic cable-ties are more effective and less dangerous (50% less fatalities) than are pipe cleaners (Mellott and Woods 1993). An additional variable to consider is nestling age (Johnson et al. 1980; Poulsen and Aebischer 1995). If the nestlings are too young, researchers risk damage as a result of physical handling; if the nestlings are too old, researchers run the risk of having the nestling fledge with the ligatures still in place. In many cases, other, less-constrained methods might provide similar results (e.g., fecal analysis, Poulsen and Aebischer 1995). Consideration also should be given as to whether the procedure will result in food deprivation such as will jeopardize survival. Not only are nestlings deprived of their meal, but parents can alter their feeding behavior in response to ligature presence (e.g., Robertson 1973) and have found to aggressively pull on the ligatures on nestlings (Little et al. 2009). McCarty and Winkler (1991) used artificial nestling puppets constructed of the skins from salvaged nestlings to entice adult Tree Swallow (*Tachycineta bicolor*) to provide food items; this technique also worked with nestling puppets made of clay.

Fecal analysis and pellet analysis

Fecal analysis, either of opportunistically collected droppings or of droppings of held birds (Parrish et al. 1994), has the advantage of being non-invasive but is limited to assessing components of bird diets that survive the digestive process, such as insect exoskeletons. In some bird groups, fecal analysis can yield comparable results to more invasive techniques, such as stomach pumping (e.g., shorebirds). In others, however, fecal analysis may not be useful because feces either may not contain identifiable material (e.g., frugivores) or feces may not be deposited in an accessible location (e.g., birds at sea).

Pellet analysis has been used in a wide range of species, including seabirds (reviewed in Barrett et al. 2007) and raptors (Redpath et al. 2001). Like fecal analysis, pellet analysis is non-invasive. However, it is a largely opportunistic approach to dietary analysis that might not lend itself to statistical or experimental design rigor (Rosenberg and Cooper 1990).

Stomach and crop flush

Wilson (1984) and Ryan and Jackson (1986) have developed a pump to flush stomach contents. A bird's stomach is filled with warm water administered via a plastic tube. The bird is then inverted over a bucket and its stomach palpated to induce regurgitation. The pump gave qualitative and quantitative results (at least in larger birds) comparable to those obtained from sacrificed birds and had no apparent ill effects in several studies (e.g., Robertson et al. 1994). Jahncke et al. (1999) used a stomach pump based on Wilson's (Wilson 1984) design to sample diets of Peruvian Diving-Petrels (*Pelecanoides garnotii*); five of 220 birds (2.3%) died during handling although the precise cause of death was not reported.

Hess (1997) used stomach flushing with a saline solution on Red-cockaded Woodpeckers (*Picoides borealis*) with no significant effect on adult survival. Gionfriddo et al. (1995) had similar success with House Sparrows (*Passer domesticus*), implying that stomach flushing may be a viable technique for granivores and other birds with muscular gizzards. A key to success for both of these studies was the careful usage of plastic tubing to deliver the flushing solution and not puncture the airsac or digestive lining. Knowledge of the species to be studied is important in assessing whether it may respond adversely to any regurgitation technique. Ryan and Jackson (1986) found in several seabird species that the percent of food items recovered in a single pumping was correlated with how full the stomach was ranging from approximately 70%

to 100% obtained. Grebes of any species should not be subjected to any regurgitative techniques because of the feathers in their crops; however, the pellets from grebes can be used for diet analysis (Jordan 2005).

Emetics

When fecal analysis and stomach flushing/pumping are either not practical or advisable, emetics may be administered to obtain crop contents. For the purposes of this discussion, we consider emetics to be any chemicals other than sodium chloride or saline solution used to induce regurgitation. There are three major considerations in the application of emetics: type, dosage, and method of delivery (e.g., direct or via plastic tubing). Investigators have a wide range of chemicals to choose from, including tartar emetic (antimony potassium tartrate), apomorphine, and ipecac. Due to the potential effects of some emetics, this technique should be considered with only the most rigorous scientific justifications.

Tarter emetic is the most widely used of these options, despite reports of high mortality (Zach and Falls 1976; Lederer and Crane 1978). There are two problems with the use of tartar emetic: it is toxic if absorbed into the bloodstream and it is difficult to determine the correct dose (Prŷs-Jones et al. 1974). Too low a dose may fail to induce vomiting, thereby allowing the emetic to be absorbed into the bloodstream (Herrera and Hiraldo 1976, cited in Diamond et al. 2007); too high a dose may result in severe trauma or shock (Prŷs-Jones et al. 1974).

Poulin et al. (1994) tested the effectiveness of tartar emetic on 3,419 birds from 137 species in 25 families. The base dosage was 0.8 ml of a 1.5% solution of antimony potassium tartrate per 100 g of body mass delivered through a flexible plastic tube. Analyzable samples were obtained from 79% of tested birds and 2% of tested birds died. Mortality of sensitive species was lessened by dosage reductions. Mortality rates did not differ between birds that did regurgitate and those that did not. Poulin and Lefebvre (1995) tested 2,656 birds of an additional 137 species. Unlike Poulin et al. (1994), base dosages were adjusted downwards for birds weighing less than 10 g and upwards for birds over 40 g. Analyzable samples were obtained from 73% of tested birds and 2.6% of birds tested died.

Durães and Marini (2003) tested the effectiveness of tartar emetic on 369 individuals from 44 species. Analyzable samples were obtained from 70% of tested birds and 10% of tested birds died prior to release. The birds were maintained in a dark, ventilated box until regurgitation,

were checked periodically, and were kept up to a maximum of one hour. In order to evaluate more precisely the effects of tartar emetic, birds were released only when they showed no signs of side effects (drowsiness, disorientation) and could fly normally. Mortality was significantly higher during the early morning hours, leading the authors to suggest that emetic sampling should occur later in the day after most birds have had sufficient time to rebuild reserves expended during the previous night. Durães and Marini (2003) also noted a significant difference in the mortality rates of birds that did not regurgitate (85% of fatalities) and those that did (15% of fatalities). This latter finding highlights the critical nature of dosage accuracy when using tartar emetic.

Carlisle and Holberton (2006) tested the relative effectiveness of fecal analysis versus tartar emetic administration in assessing diets of migratory songbirds. Regurgitated samples allowed for a faster assessment of diet than fecal samples did (i.e., fewer samples were required). However, if enough samples were collected, fecal analysis eventually produced a similar assessment of diet. Recapture rates of birds treated with tartar emetic were less than half that of untreated birds.

It is important to remember that the administration of emetics can have indirect effects that may not be immediately apparent during sampling. For example, Carlisle and Holberton (2006) report that all recaptured tartar-treated birds had lost mass. They went on to perform a dosage experiment on captive Dark-eyed Juncos (*Junco hyemalis*). Eighteen individuals were included in this experiment: six received the full mass-specific dose (0.8 ml of a 1.5% solution of antimony potassium tartrate per 100 g of body mass), six received one-half the recommended dose, and six received one-quarter the recommended dose. All 18 individuals were alive 15 to 20 min post-treatment (the standard pre-release holding time); 17 of the individuals were dead within 30 min.

Johnson et al. (2002) tested for another indirect effect of emetics using resighting rates of migratory songbirds during the non-breeding season. Johnson et al. (2002) captured 18 Black-throated Blue Warblers (*Dendroica caerulescens*), nine of which were administered tartar emetic (0.8 ml of a 1.5% solution of antimony potassium tartrate per 100 g of body mass delivered orally through a 1.5-mm-diameter flexible plastic tube) and released. Of the nine treated birds, only one was resighted within the following week; seven of the nine control birds were resighted over the same time period. Johnson et al. (2002) also captured 74 other warblers from different species, 61 of which were treated with tartar emetic. Even with the lower

sample size for untreated birds, the resighting rate of untreated birds was statistically higher than the resighting rate of treated birds and although the direct conclusions remain unknown, the comparative resighting rates do suggest an impact.

Valera et al. (1997) tested the emetic effectiveness of apomorphine and reported effectiveness and mortality rates similar to those reported in tartar emetic studies. In this study, only nestlings suffered mortality; however, nestling mortality rates resulting from apomorphine application were lower than those reported as a result of ligature usage (Johnson et al. 1980). One advantage that apomorphine may have over tartar emetic is that it can be applied repeatedly to the same individual, seemingly without negative effects. This is not the case for tartar emetic (Zach and Falls 1976).

Diamond et al. (2007) reintroduced the use of ipecac as an emetic. Initially recommended by Kadochnikov (1967, cited in Diamond et al. 2007), ipecac has not been used to good effect, likely due to inadequate dosage. In the Diamond et al. study (2007), ipecac and tartar emetic were used in assessing diets of Kenyan birds; the tartar emetic dosage used was 0.025 ml per g body weight of a 1% solution in water and the ipecac dosage used was 0.1 ml per g body mass of a 1:20 solution by volume of an ipecac tincture in water. There was no difference in emetic efficacy. Three of the 63 birds (4.8%) treated with tartar emetic died; none of the 93 birds treated with ipecac died. Diamond et al. (2007) reported on an additional 44 wood-warblers in New Brunswick treated with ipecac; all individuals successfully regurgitated and there was no evidence of negative effects on the birds. In addition to being less toxic than tartar emetic, ipecac's effectiveness is less reliant on the amount of emetic delivered to the birds. Given that ipecac appears to be at least as effective as tartar emetic, it should be considered the emetic of choice for diet analysis.

Biochemical methods of dietary analysis include stable isotope analysis and quantitative fatty acid signature analysis. Quantitative fatty acid signature analysis is primarily suitable for assessing the diets of marine organisms and relies on the fact that predators tend to store the fatty acid signatures of their prey items in their adipose tissues (reviewed in Barrett et al. 2007). These tissues can be non-destructively sampled using biopsy (discussed below). Fatty acid analysis allows for a more precise determination of diet than stable isotope analysis but using the two in concert could provide a powerful dietary assessment tool.

F. Force feeding

Nutritional investigations may require force feeding of experimental subjects (usually in captivity). Tube feeding using a soft rubber or atraumatic metal feeding tube of proper size and volumes of food that are appropriate for the size of the bird is safe and effective. Murphy and King (1986) found that force feeding by inserting a tube down the esophagus was injurious in some cases. Food has to be fed as a slurry and regurgitation can result in choking (especially in small species). Holding the bird upright will help to prevent regurgitation. Intubation may also injure the esophageal wall. If done improperly, the tube can damage the choana (slit in the roof of the mouth) or the trachea. Food in the trachea will be aspirated into the lungs and can cause pneumonia or death from suffocation. As an alternative, Murphy and King (1986) suggested feeding pelleted food by placing pellets directly into the pharynx with forceps, thereby inducing reflexive swallowing. Mortality is reduced to near zero and regurgitated pellets do not result in choking; however, the use of pellets takes much longer than tubal feeding. Researchers may seek training from aviculturalists who have extensive experience tube feeding young parrots and other birds.

G. Cloacal lavage

Studies of the mode and timing of insemination are important for analysis of population trends, transfer of genetic information, and mating systems. Cloacal lavage, of both males and females, is a technique to acquire information concerning sperm production and transfer (Quay 1985, 1986, 1987). However, Immler and Birkhead (2005) also found that sperm can also be found in fecal samples appropriate for answering many questions. Cloacal lavage is also used to investigate pathogens in lower digestive system of birds (Brown et al. 1993; Yashkulov et al. 2008). Cloacal lavage uses distilled water or saline in a quantity appropriate for the size of the species. The water is administered via a disposable plastic pipette that is introduced into the cloaca. As much as possible of the lavage is sucked back into the pipette and transferred into a specimen tube. The technique is sometimes extended by the implantation of cloacal microspheres (Quay 1988). Considerations should be made for the potential pain and discomfort for this procedure and should be performed by a properly practiced person.

H. Injections and insertion of implants

Injections of appropriate solutions or implants, whether subcutaneous, intramuscular, intracoelomic, or intravascular, may usually be made with very little effect on survival or normal bird behavior. Some solutions may be irritating or dangerous to the subject if they are not properly injected. Implants may migrate or become inactive if they are not properly inserted. It is strongly recommended that new techniques are evaluated on captive individuals before used with wild birds. The personnel performing the procedures of injections or implants, whether subcutaneous, intramuscular, intraperitoneal, or intravascular should be properly trained. In the United States, the Federal Food, Drug, and Cosmetic Act provides that drugs administered legally to animals must be approved by the Food and Drug Administration or recognized by experts (e.g., the agency) to be generally safe and effective. The Animal Medicinal Drug Use Clarification Act provides that an approved drug must be used if available, but there are few drugs approved for use in birds. Veterinarians under certain conditions may legally use approved human and animal drugs in an extra-label fashion. Therefore, extralabel use should take place under the supervision of a veterinarian and adequate records of extralabel drug useage must be maintained (http://www.avma.org/reference/amduca/extralabel_brochure.pdf).

Injection of experimental substances is widespread in research on birds. Subcutaneous and intramuscular injections are simple in the laboratory and cause little trauma. Intravenous injections require some acquired skill. Intracoelomic injections require more stringent justification because some drugs may irritate the viscera or the possibility of mechanical or chemical damage of the viscera. Also, drugs intended for injection into the coelomic space are easily deposited into the respiratory sytem of abird, given the unique airsac anatomy of birds.

No study has directly investigated the impacts of injections on individual survival. However, hundreds of published field studies involving injections, especially subcutaneous injections, support the conclusion that these injections appear to have little effect on survival. This undoubtedly varies for the type and amount of injection given. Due to number of types of injections that could be given, a review of each type is not given here. For longer-term studies, repeated injections are sometimes necessary, requiring multiple captures at frequent intervals. This in itself may cause serious disruption of normal activities. For these reasons, implants in silicone rubber tubes, pellets, or mini-osmotic pumps may be used to provide long-term administration of the experimental substance (up to several weeks). Whenever possible, such

implants should be made subcutaneously because intracoelomic implants are often encapsulated by connective tissue. Implants inserted under the skin of the flank or sides of the thorax are most effective and are easy to remove after the experiment is terminated. Implants placed under the skin on the back may rupture the skin, allowing infection. It is important that the size of the implant should be such that it does not place pressure on the skin, regardless of location. Implants under the skin of the neck are also not advised as they can penetrate the thoracic cavity, resulting in severe respiratory distress. Custom-made, mini-osmotic pumps are available for odd-sized animals or for administering substances for prolonged periods from companies that also provide free training videos for the use of these pumps in rodents that can be applicable to birds. As with all invasive procedures, the area of operations and instruments should be as sterile, with separate sets of sterile instruments used for each implant surgery. See the section on Major Manipulations for a thorough discussion of sterile procedures.

Timing of implant placement is also important in some cases. Treatment of free-living birds with hormones usually has no debilitating effect, but some treatments, such as the sex steroids, can disrupt the normal temporal progression of reproductive and associated events. The impacts of additional sex steroids on birds can be varied and includes suppression of immune function (Duffy et al. 2000; Castro et al. 2001). Hence, every effort should be made to remove the implant after the experiment. In species that breed at high latitude or altitude, the short breeding season allows only a short time for molt. If these functions are disrupted by implants, death may result due to poor plumage and delayed migration. If these outcomes are likely under the conditions of the particular study then the investigator should remove the implanted devices from controls and experimental birds either by removal of the implant or removal of the bird from the wild. However, experimental subjects with control implants, or implants from which all hormone has diffused, do survive over winter at the same rate as individuals without implants (Wingfield 1984). Further, the stress of recapture may cause problems that interfere with results and cause impacts to the birds. A crucial element in assessing appropriate actions in all of the above is whether the risk induced by the experiment applies primarily to individuals or to the population.

The field of immunology in wild birds has grown significantly in the last decade, requiring injections of immunizations or mitogenic substances to measure immune response. Similarly, doubly-labeled water is a common injection in energy studies in birds. Wilson and Culik (1995) found that seabirds injected with doubly-labeled water differed in their foraging parameters (e.g., dive depths, dive angles and foraging ranges) from the non-injected birds. The authors suggest

that the relatively large volume of liquid injected intramuscularly causes discomfort which lasts for at least two days, which dissuades birds from engaging in normal foraging behavior and they suggest this problem may be alleviated by multiple small intramuscular injections or intra-peritoneal injections. Other implants include radio or satellite transmitters placed subcutaneously (Berdeen and Otis 2006) or in the coelom of birds. These are discussed at length in the section on Capture and Marking.

I. Determination of egg viability

Certain experimental procedures require an estimation of the number of eggs within a clutch that have viable embryos and the age of embryos. Breaking eggs has obvious deleterious effects on reproductive success but can be scientifically justified in some cases. Alternative means should be pursued when available. Two alternative, non-invasive, and inexpensive techniques are candling and flotation.

Candling, or transillumination, involves placing an egg in front of a light source and assessing or measuring the amount of dark space within the field of view (Westerskov 1950; Weller 1956). More modern approaches to field candling and photography allow for clear delineation of embryonic development and are designed to minimize the amount of egg handling time and how long the adult is away from the eggs, an animal welfare consideration with this technique (Young 1988; Lokemoen and Koford 1996).

Floating involves placing eggs in water or other aqueous solution at ambient temperature; egg viability and embryo age determinations are based on whether or not the eggs float and the orientation of floating eggs (Westerskov 1950; Fisher and Swengel 1991; Walter and Rusch 1997). For example, if the egg is more than 10 days old and does not float, there is no viable embryo. Devney et al. (2009) present results that suggest that floatation in salt- rather than freshwater may provide more accurate results, at least for colonially nesting seabirds. Egg flotation is useful for eggs with shells too thick or too heavily pigmented for candling. The primary concern with the use of floating techniques is that they may decrease hatchability by allowing excess water to permeate the egg. However, Alberico (1995) tested the effect of egg flotation on hatchability of clutching in 131 American Avocet (*Recurvirostra americana*) and Black-necked Stilt (*Himantopus mexicanus*) nests and detected no significant effect.

Recently, Reiter and Anderson (2008) tested the relative efficacy of egg floatation and egg candling in estimating incubation day of Canada Goose (*Branta canadensis*) nests. Both techniques overestimated incubation day early in incubation and underestimated incubation day late in incubation. Although egg flotation provided less biased results, both techniques provide a level of precision required for robust estimation of nesting parameters.

Electronic devices, such as doppler stethoscopes and audiocartridges, that can detect the embryonic heart beat or movements of the embryo within the shell may also be useful but have limited field potential (Mineau and Pedrosa 1986; Tazawa et al. 1991).

J. Playback of recorded vocalization and the use of decoys

Playback of conspecifics or a recording of the study subject's own voice is a common research technique. Predator calls, particularly those of small nest predators such as pygmy owls, are often used to attract passerines. Studies of the impact of playback are sparse, but inferences about the nature of possible impacts can be made from the fact that birds are enticed to move in response of the recorded sound. For instance, nesting birds may leave eggs or hatchlings unattended. Documented physiological responses include increased plasma testosterone levels in Spotted Antbirds (*Hylophylax n. naevioides*) (Wikelski et al. 1999). Mennill et al. (2002) determined that male Black-capped Chickadees (*Poecile atricapillus*) enticed to engage in song contests with playback lost paternity in their nests when songs simulating aggressive males were played for six minutes.

Playback has long been used as a tool to assess species presence and abundance to assess species presence and abundance (Johnson 1981; Proudfoot and Beasom 1996; Turcotte and Desrochers 2002) and to manipulate behavior in ethological and behavioral ecology research. More recently, it has been used to study reproductive activity (Gunn et al. 2000; Doran et al. 2005). Unfortunately, these studies rarely assess the potential negative impacts of the use of playback. Playback of recorded vocalizations to free-living birds causes little disturbance if the duration of the playback is kept within reasonable bounds (normally less than 30 min) (Turcotte and Desrochers 2002; Hahn and Silverman 2007; Celis-Murillo et al. 2009). Playback may distract subjects from activities that are essential to reproductive success. Playback (both conspecific and predator vocalizations) during the breeding season has been shown to negatively affect pair bond status and breeding success in some birds, such as owls (Springer

1969; McNicholl 1978) cited in (Proudfoot and Beasom 1996) and songbirds (Baptista and Gaunt 1997). Unless required for an experiment, speakers should not be placed close to a known nest location. Overuse of both conspecific and predator playback during the non-breeding season, especially on cold winter days, can result in birds wasting valuable foraging time responding to playback.

Live decoys are frequently used in conjunction with playback and require particular attention in the field. Animal welfare concerns are just as important to the decoys as to the wild birds. This topic is discussed thoroughly in the section on Capture and Marking. Generally, though, the use of live decoys to lure hawks to a mist net or trap should be carefully monitored. Avoid stressing the decoy by providing shelter from direct sun and by providing a refuge to escape from the raptor. A live decoy should be observed constantly by the investigator. Except for very short periods of use, live decoys must be provided with food and water (Evrard and Bacon 1998). Birds used as live decoys should be habituated to housing in a cage under field conditions for at least a day prior to onset of the experiment.

Decoy traps can be readily designed for both terrestrial (e.g., Burtt 1980) and aquatic systems (e.g., Anderson et al. 1980). Evrard and Bacon (1998) tested the efficacy in capturing ducks of four different trap designs: swim-in bait traps, swim-in bait traps with live decoys, floating bait traps, and decoy traps. The two trap types with decoys were more effective than traps without live decoys. Evrard and Bacon (1998) checked on and fed the decoy ducks daily. Despite daily checking, several decoy (exact number not given) and trapped ducks fell victim to mink and raccoons. The mortality rate was higher than previously reported for other studies using decoy traps (e.g., Anderson et al. 1980; Sharp and Lokemoen 1987), perhaps due to difference in predator communities. In addition to losses to predators, Sharp and Lokemoen (1987) reported one decoy fatality in which a decoy got its bill wedged in the side of the trap and drowned. Sharp and Lokemoen (1987) also make the important observation that farm-raised ducks fared much better during their time as decoys than did wild-caught decoys.

K. Artificial eggs

The use of artificial eggs is invaluable to many ornithological studies, allowing reduced risk during trapping and providing for the development of eggs of special value (e.g., in the

maintenance of threatened populations). Artificial eggs composed of various materials, including wood, paper maché, plastic, and clay, have elicited normal nesting responses. However, egg recognition varies widely among species. In some species, individuals recognize the unique patterns of their own eggs (e.g., Antonov et al. 2006; Prather et al. 2007). For others, egg recognition mechanisms may be very general (Moskát et al. 2003). When artificial eggs are used briefly, such as during trapping, a general approximation of real eggs should suffice. However, when it is intended that artificial eggs be incubated for days or weeks, extreme care should be given to the mimicry of the original egg shape, size, pattern, and weight (e.g., Marchetti 2000). Birds that are uncomfortable sitting on surrogate or artificial eggs may desert the nest, resulting in a loss of data.

L. Experimental manipulation of plumage

Experimental approaches to altering the external appearance of a bird fall into two broad categories: alteration of size and shape of feathers and other body parts, and the alteration of plumage coloration. A common experimental approach to the manipulation of feather size and shape has been the alteration of tail structure in the context of testing hypotheses and predictions arising from sexual selection theory. Perhaps most famously, Andersson (1982) experimentally shortened and elongated the tails of Long-tailed Widowbirds (*Euplectes progne*) to test female preferences for male secondary sexual characteristics. Under captive conditions such manipulations are not traumatic unless, as a result, the experimental subject has difficulty feeding and drinking. Under natural conditions, however, it is important to ensure that such manipulations do not impair flight or other types of locomotion. Certain types of long tails, primarily those that appear to exist as a result of sexual selection pressures (e.g., widowbirds), compromise a bird's flight ability (Balmford et al. 1993; Norberg 1995). Experimentally increasing already unwieldy tails may compromise an individual's health and fitness. Tail manipulations can also affect a bird's ability to move in complex habitats; Romero-Pujante et al. (2005) reported that experimental manipulations of tail length compromised the ability of Bearded Tits (*Panurus biarmicus*) to move about in reedy marshes, its primary habitat.

The most common contemporary approaches to plumage color manipulation of feathers on birds are the hiding or removal of an obvious trait (e.g., using black dye to hide the epaulets of Red-winged Blackbirds [*Agelaius phoeniceus*]) and the manipulation of the plumage coloration gradients towards the extremes of natural trait expression (e.g., using marking pens to alter

House Finch [*Carpodacus mexicanus*] plumage) (see Hill 2006a,b for a complete treatment of bird coloration). More recently, researchers have begun raising captive birds on pigment-free diets (e.g., carotenoid-free diets; McGraw and Hill 2001), thereby creating a cohort of birds with minimal coloration that can be colored to suit experimental needs. The advantage to this approach is that researchers can initially manipulate bird plumage color without resorting to external applications while maintaining subject health (e.g., McGraw and Hill 2000). Changes in plumage coloration do not appear to influence predations rates on altered birds (Stutchbury and Howlett 1995) .The primary animal welfare concern with regard to plumage color manipulation is to avoid the use of marking or coloration chemicals that may be toxic to birds. In this respect, the use of marking pens is preferable to shoe polish, hair dyes, or colored oils. Given that birds use their bills to self-preen, simply avoiding the mouth, nostrils, and eyes while applying dyes known to be toxic (as recommended in Rodgers 1986) is not sufficient.

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