

## CHAPTER 7. MAJOR MANIPULATIVE PROCEDURES

### A. Overview

This section concerns procedures most commonly used in conjunction with surgery (i.e., entry into the body cavity), such as restraint and the use of pharmaceuticals to alleviate pain, as well as recent innovations in surgical techniques. We also discuss euthanasia as an endpoint for manipulations that result in inadvertent or unavoidable pain that cannot be remedied.

Avian veterinary medicine has become highly advanced in recent years. Modern techniques are well presented in several texts and review documents: Harrison and Harrison 1986, Ritchie et al. 1994, 1997, Altman et al. 1997, Tulley et al. 2000, Hawkins et al. 2001, and Samour 2008. No researcher conducting invasive studies of birds should be without one or more of these books. However, no text, video, or anything that follows in these Guidelines suffices as a self-training manual. As is the case for all complex procedures, surgery should not be undertaken by novices. Training is essential and even those with adequate training should seek the guidance of a veterinarian prior to undertaking an invasive procedure. Any invasive technique is potentially dangerous. The subtleties (e.g., angle of introduction of a hypodermic needle, positioning of the subject, position of the investigator's hands) that allow experts to perform these procedures smoothly, rapidly, and with minimum distress to the subject are developed from long practice and may not be well communicated in text books, instruction manuals, or videos. An investigator should always seek direct instruction from an expert and should practice on appropriate models and under the supervision of a skilled practitioner or veterinarian.

These Guidelines present detailed material concerning frequently used procedures and commonly encountered problems in order to facilitate communication between investigators and the members of their Institutional Animal Care and Use Committees (IACUC), who may be more familiar with mammals than birds and with laboratory conditions than field conditions. This summary is not an attempt to catalogue all acceptable techniques. Rather, it is an attempt to establish a philosophy that will help all involved to determine the appropriateness of a given approach. The techniques discussed should be considered as examples.

The nature of these procedures and the potential for impairment of function, pain, infection, and death warrant a repetition of the fundamental “alternatives” principles. Though replacement with a non-animal model is rarely a viable alternative in wildlife research, the investigator should

consider reduction in the number of animals through careful planning of the statistical design of the investigation. The most pertinent consideration is the refinement of procedures. A non-invasive or less invasive procedure should always be considered, assuming that the alternate procedure can yield equally useful results.

## **B. Intended fate of subject**

The conditions governing the adoption of procedures may depend on the intended fate of the bird. There are four categories of subjects: wild birds in the field that are to be released immediately upon recovery; wild birds that have been brought into a laboratory and will be released after recovery in a holding facility; wild or captive bred birds that are to remain captive permanently or for an indefinitely long period after the procedure; and birds that will be euthanized.

For any animal that is to be released to the wild either immediately or following a holding period, the prime consideration should be that the procedure will have a minimal effect on the subsequent survival of the subject. It is important to consider the age and sex of the subjects as nestling and juveniles may respond differently than adults, and females may respond differently than males (e.g., Mulcahy et al. 2003).

For wild birds that are to remain captive permanently or for an indefinitely long period after the procedure or for birds that will be euthanized, less emphasis can be placed on survivability, at least in the short term. However, there should be no compromises on antisepsis and surgical standards or on fear and pain management.

## **C. Pre-surgery**

### ***General considerations***

Avian surgery is considerably different from mammalian surgery (Ritchie et al. 1994; Altman et al. 1997). In part, the differences are due to avian anatomy, especially the air sacs, respiratory system, and physiological differences, such as blood-pH and proclivity to fall into hypothermia. Birds tend to have high metabolic rates; as a consequence, pre-surgical fasting is not advised

for small birds and should be of a duration sufficient only to empty the crop in large birds (overnight for large birds, 4 to 6 hrs at most for small birds such as passerines; Curro 1998).

Any invasive procedure more complicated than a simple injection should be rehearsed with an appropriate model (mock-up, cadaver, or anesthetized subject), and the conservative limitations on techniques should be maintained until they can be performed quickly and smoothly. In addition to supervised practice of the invasive procedure, all other aspects of the procedure (e.g., capture, restraint, intubation, use of equipment) must also be practiced and specifics researched prior to performing the procedure. For example, the proper size of intubation tube is very important and will vary widely among birds.

### ***Aseptic technique***

High standards of antisepsis, which is the prevention of infection through the elimination of microbes, should be practiced routinely during invasive procedures. Proper training in and a need for “sterile technique” is key to any surgery or invasive procedure. The Guide for the Care and Use of Laboratory Animals (ILAR 1996) provides a broad discussion of the maintenance of antisepsis. Note the difference between disinfectants, which are agents used to reduce the number of microbes, and sterilization, which means the complete absence of all disease-causing organisms. A strong-enough disinfectant could sterilize a surface or an instrument if left in contact for a sufficient period of time. While no single procedure for sterilization is appropriate to all materials and all situations, precautions should always be taken to reduce the possibility of transmission of microbes. Sterile conditions are not required in the entire laboratory but commonly accepted practices that reduce the presence of microbes should be adhered to, including the use of a disinfectant on all surfaces. The surgical area should be specifically designated and set aside for that sole purpose; it should be scrubbed with a strong disinfectant, such as dilute sodium hypochlorite (household bleach, dilute 1/10), a quarternary ammonium compound, or an iodoform compound (followed by alcohol to remove the residue) before and after procedures. All organic debris from previous procedures must be removed prior to disinfection or sterilization. Special precautions, such as color coding and separate storage areas, must be taken to ensure that surgical instruments are used for that purpose only. They must not be mixed with necropsy, dissection, or skinning instruments. All non-disposable equipment should be sterilized by autoclave between uses.

The surgical site itself must be sterile, which is achieved through the use of a sterile drape and the use of sterile instruments. Aseptic technique in the laboratory setting requires that the surgeon thoroughly scrub and rinse hands and arms with proper agent (e.g., betadine, chlorhexidine, inactivated alcohol) allowing an appropriate amount of contact time prior to rinsing, wear sterile gloves, a face mask, and hair cover, as well as scrubs and a sterile surgical gown or apron. The surgical site should be free of feathers (may need to be gently pulled from where the feather shaft meets the skin in the vicinity of the planned procedure), disinfected with several cycles of gauze soaked with chlorhexidine or betadine, followed by alcohol, beginning with the area where the incision will be made and circling outward each time. Sterile drapes available from surgical supply houses should cover the surgical site (disposable drapes are available, versus reusable drapes that must be washed and autoclaved between procedures).

Though strict adherence to these aseptic protocols may be impossible in the field, certain basic practices must be observed. Specifically, the surgical site must be sterile and sterile gloves and instruments must be used. Surgical instruments are now sufficiently inexpensive that cost should not be a barrier to single-use where sterilization of instruments is not feasible.

The Guide for the Care and Use of Laboratory Animals acknowledges that modification of standard techniques might be desirable or even required in field surgery, but these modifications should not compromise the well-being of the animals. However, a sterile field around the surgical site is always necessary, as are sterile gloves and sterile instruments. It is obviously impossible to sterilize, or even disinfect, natural surroundings, but the general area, such as the surface upon which the bird rests, can be disinfected. For instance, unscarred, monolithic high-density polyethylene or polypropylene plastic boards (Oliver, pers.comm.) are suitable for surgical procedures because they can be disinfected (Ak 1994) by soaking in a 1/10 dilution of household bleach solution, quaternary ammonium compound, chlorine dioxide-based sterilant (Clidox<sup>®</sup>), or chlorhexidine (Nolvasan<sup>®</sup>). Alternatively, the board can be wrapped with pre-sterilized cloths or surgery drapes, disposable surgery drapes or paper covers, or plastic-backed absorbent cage liners that can be changed as soiled or between surgeries. A foam pad can also be used as a surface, and the pad or the plastic board can be nested inside a plastic garbage bag to further reduce the potential for airborne or other environmental contaminants. If possible, procedures should be carried out in some sort of shelter that reduces the possibility of wind-borne contaminants.

Sterilizing instruments under field conditions can be difficult so it is best not to re-use instruments that enter or touch the surgical site. Although a variety of chemical sterilizing solutions exists, the instruments must remain in the solution until needed. After removal from the solution, they must be handled aseptically and must be rinsed in sterile water and dried with a sterile towel, then used immediately. They cannot touch any non-sterile surface. For these reasons, field sterilization of instruments is impractical and therefore it is best to use disposable instruments and blades. Use of an assistant – who can access necessary items that may be outside the sterile zone – is also recommended.

### ***Physical restraint***

Invasive procedures require restraint and sometimes immobilization to minimize stress and the chance of unintended injury to the subject. The precise nature of the restraint depends on the procedure and the species (Fowler 1978, 1995) and can be achieved using either physical or chemical means. Chemical restraint will be discussed below under *Pain Management*.

For smaller birds, variations of handling techniques used in banding are adequate (Donovan 1958) to provide physical restraint of individuals prior to administration of pharmaceuticals. To minimize potentially damaging movement during recovery from invasive procedures, small- to medium-sized birds can be enclosed in cardboard or fabric tubes or comparable devices. See the section on Capture and Marking for precautions to take when holding birds in this manner. Large species can often be calmed by enclosing the head in an opaque hood (Maechtle 1998) or the body in a fabric sleeve (Bolen et al. 1977). Dark hoods are also useful for reducing struggling and stress during pre-surgical evaluation and post-operative recovery. Care should be taken that the restraint does not interfere with ventilatory movements of the abdomen and thorax or impede respiratory air flow. In addition, hoods or other external coverings should not be adhered or attached to the bird so strongly that in an outdoor procedure, the bird may awake and flee with the apparatus still attached.

Physical restraint must not create a situation that may induce hyperthermia or hypothermia. Temperature-controlling equipment such as ice, fans, or warming pads may be needed (e.g., Rembert et al. 2001). Minimizing external stimuli such as vocalizations or other noise or rapid changes of light or temperature helps ensure successful restraint and recovery. All handling and

restraint equipment must be cleaned and disinfected between every animal and procedure to minimize the potential spread of disease.

One effect of handling and physical restraint that often goes unnoticed or unmeasured is a physiological response to the activity. For example, Hood et al. (1998) measured corticosterone levels in Magellanic Penguins (*Spheniscus magellanicus*) following capture and subsequent restraint and found that corticosterone levels were higher in birds that had been held and restrained for longer periods. Given the wide-ranging effects of stress hormones on bird behavior, physiology, and reproductive success, additional care must be taken to minimize the amount of time a bird is handled and restrained during major manipulations. In another example, Greenacre and Lusby (2004) examined the effects of handling and restraint on body temperature and respiratory rate in 17 Hispaniolan (*Amazona ventralis*) and Blue-fronted Amazons (*A. aestiva*). Body temperatures rose by 2.3 °C within 4 min, and respiratory rates almost doubled within 15 min. The authors concluded that birds restrained for more than 4 min must be monitored for signs of overheating, such as open-mouthed breathing and tachypnea (increased breathing rate).

As some species may be dangerous to the handler, proper restraint should include protection for the handler as well as the bird to prevent accidental injury to the bird during defensive maneuvers. Heavy gloves are appropriate for handling raptors, psittacines, and other birds with strong and sharp talons or beaks. Safety goggles should be worn when handling birds with long beaks and ear protectors or plugs when working near species capable of loud calls.

#### **D. Pain management**

Many birds show little to no behavioral evidence of pain or discomfort from punctures or incisions over much of the body, especially in the bare skin areas between the feather tracts (Green 1979; Steiner and Davis 1981); the head and bill, scaled portions of the legs, and vent area are exceptions. For discussions of the complex topic of pain in animals, see Bateson 1991, Elzanowski and Abs 1991, Gentle 1992, and Andrews et al. 1993. The evident psychological component of pain can be aggravated or suppressed by fear. In addition, various species respond to traumatic experiences differently, and either restraint or disorientation may elicit a more evident response or a response of greater magnitude than that provoked by such physical injuries as punctures or small incisions. Unfortunately, an animal's fear of the unknown cannot

be lessened by assurances. For this reason, analgesics and anesthetics may be used reduce the total stress of a procedure, as long as their application does not decrease a subject's chances of survival.

The lack of an apparent pain response and the potentially stressful affects of anesthesia and prolonged handling have led past investigators to perform some surgical procedures with little or no anesthesia and to close incisions without sutures (Gandal 1969; Risser 1971; Wingfield and Farner 1976; Baker 1981). Such procedures may not affect the overall survival or reproductive potential of the subject (Ketterson and Nolan 1986; Westneat 1986; Westneat et al. 1986). Given the availability of local analgesics and the rapidity with which birds can recover from gaseous anesthetics such as isoflurane (Degernes et al. 2002), the practice of invasive techniques without the use of analgesics or anesthetics requires special justification. If anesthesia is used, the bird should not be released until the effects of anesthesia have completely disappeared (e.g., bird is alert and able to perch or stand without assistance). The decision to use or not to use analgesic and/or anesthetic agents must be based on the best interests of the animal and not on the convenience of the investigator.

### ***Access to controlled substances***

Non-veterinarians may be allowed to register with the Drug Enforcement Administration (DEA) to legally obtain substances on the Schedules (Class) II–V of the controlled substances list. Individual researchers and institutional departments can register with the DEA. “Mid-level practitioners,” defined as “other than a physician, dentist, veterinarian, or podiatrist” are permitted to conduct research using controlled substances if permitted by state law. Most states will issue licenses to researchers. Investigators outside the United States should consult with their own law enforcement agencies about access to these substances.

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 must be maintained (see [http://www.avma.org/reference/amduca/extralabel\\_brochure.pdf](http://www.avma.org/reference/amduca/extralabel_brochure.pdf)).

## ***Analgesia***

Analgesia is the reduction of pain. Analgesics are used to: alleviate pain in an animal prior to treatment or to ease pain in an individual recovering from an injury or surgery; sedate an animal prior to a minimally invasive but painful procedure; sedate an animal prior to induction of anesthesia for surgery. Injection is the most common delivery method of an analgesic. Chemical compounds commonly used as analgesics include opioids, steroidal and non-steroidal anti-inflammatory drugs, ketamine (discussed under Anesthesia), and  $\alpha_2$ -agonists (Machin 2005).

Opioids have been used effectively as analgesics in birds but the literature contains conflicting reports on species-specific efficacy and dose-specific responses (Machin 2005, Myers 2005). The primary concern surrounding the preemptive administration of an opioid analgesic prior to anesthesia is the potential for the opioid to critically depress cardiopulmonary function during the procedure. Klaphake et al. (2006) tested the effectiveness of butorphanol tartrate as an analgesic prior to anesthesia with sevoflurane on Hispaniolan Amazons. They reported no adverse effects in cardiopulmonary function of birds that received butorphanol prior to anesthesia compared to those who did not. However, as evidenced by the results of studies by Hoppes et al. (2003), Paul-Murphy et al. (2004), Sladky et al. (2006), and Riggs et al. (2008), great care must be taken (e.g., pilot studies or cage tests) to determine the appropriate dosage of an analgesic for a particular species..

The suppression of inflammatory responses can contribute to pain reduction (Hockin et al. 2001, Machin 2005). Although steroid-based anti-inflammatory compounds (e.g., corticosteroids) are attractive due to their strength and rapid action, they carry a risk of immunosuppression and potential antagonism with anesthetics. In contrast, non-steroidal anti-inflammatory drugs (NSAIDs) appear to be as effective as analgesics with fewer negative side effects. Preoperative administration of NSAIDs can reduce postoperative opioid requirements (Machin 2005); they can also be used effectively during surgical recovery. Commonly used NSAIDs include ibuprofen, carprofen, flunixin, and ketoprofen. Side effects of NSAIDs include gastric ulcers, regurgitation, tenesmus and nephrotoxicity and, with repeated and/or prolonged use, muscular necrosis at the injection site (Machin et al. 2001). Oxicams are a family of non-steroidal anti-

inflammatory agents (such as Meloxicam) that have been recommended for use in birds, including passerines.

Alpha<sub>2</sub>-agonists, such as xylazine and metatomidine, are useful as precursors to the administration of anesthetic compounds (see discussion below). However, they have limited utility as the sole analgesic or sedative agent as they can cause respiratory and cardiac depression, muscle tremors, and auditory sensitivity (see references in Machin 2005).

Chilling has been used for analgesia (Mueller 1982). Ethyl chloride can temporarily numb a small area for quick incision, such as in laparotomy (Risser 1971). Refrigerants such as dichlorodifluoromethane may also be used for cryosurgery. However, it is difficult to control the degree of chilling, and frozen tissue may be permanently damaged or rendered inoperable. As the relationship between hypothermically induced immobility and analgesia has not been clearly established, the use of chilling as an analgesic is strongly discouraged.

### ***General anesthesia***

An anesthetic is an agent that produces analgesia (loss of pain sensation) and, in the case of general anesthetics, immobilization and loss of consciousness so that the individual is unresponsive to stimulation. Anesthesia ideally minimizes stress and eliminates pain during a research procedure. It also provides adequate restraint during the procedure. Every anesthetic agent has specific advantages and disadvantages. The investigator must be fully knowledgeable about the physiologic and pharmacologic sensitivities of the avian species to be studied (Ludders 1998) as well as the pharmacologic characteristics of the drug(s) to be used in the research project. The investigator should be aware of the potential synergistic or antagonistic effects of the drugs to be used. Regulations that implement the Animal Welfare Act mandate the requirement for training and instruction of personnel who administer analgesic and anesthetics [9 CFR 2.32(c)(3)].

The most important message on the subject of anesthesia is that there are no easy answers and no single agent that is ideal for all situations. The investigator, in consultation with an avian veterinarian or veterinary anesthesiologist, must take the time to determine which agent or combination of agents is appropriate for the species being studied, the procedure to be performed and be able to justify that decision. When information concerning the effect of a drug on the species under consideration is unavailable, pre-experimental testing to assess dosages

is strongly advised (Mercado et al. 2008). Curro (1998), Machin (2004), and Gunkel and Lafortune (2005) provide excellent reviews on avian anesthesia. However, the state of knowledge about avian medicine and anesthesiology is developing rapidly, so even the most experienced investigator should take the time to review recent literature before selecting a specific drug.

General anesthetics are administered either as a gas or as an injection. Inhalation anesthetics have the advantage that dosage is easily adjusted during the procedure. In addition, due to the rapid clearing that occurs in the avian respiratory system, recovery usually is extremely rapid. If birds are to be released soon after a procedure, inhalant anesthetics may be the better choice. Injectable anesthetics have the advantages of rapid administration and require less equipment in the field. However, the injectable anesthetics usually result in prolonged recovery periods.

Some inhalants require little equipment. Methoxyflurane (Metofane) has been used successfully for recovery surgery in the field, by placing the drug on a cotton ball in a small jar, and placing this over the bird's face until anesthesia is induced (e.g., MacDougall-Shackleton et al. 2001, 2006). Unfortunately, methoxyflurane is no longer commercially available in many countries, including the United States. Isoflurane is a common choice of inhalant anesthetic for birds (e.g., Redig 1998). However, this compound is much more volatile than methoxyflurane and maintenance of proper anesthesia requires the use of a vaporizer. Light-weight portable systems are available for field use, and portability is primarily limited by the size of oxygen tank used (e.g., Lewis 2004, Small et al. 2004, Boedeker et al. 2005). Using isoflurane, anesthesia can be rapidly induced with a dose of 2 to 4% with a flow rate of 2 L/min oxygen. Recovery occurs very rapidly, within a minute or two of removing anesthesia. Placing birds in an induction chamber designed for rodents can lead to injury as birds flail about prior to becoming unconscious.

Injectable anesthetics may be administered in a muscle mass (IM), in a vein (IV), or intra-osseous (Heard 1997). Intravenous administration provides more predictable reactions, faster induction and usually results in a faster recovery. These methods of administration require some skill, even with large species and can be inappropriate for small species. The dosage for most injectable anesthetics varies inversely with weight (Boever and Wright 1975); that is, small birds may require relatively higher dosages. Hence, the weight of the subject must be accurately measured prior to administration of any drug. However, dosage information for specific anesthetics should be researched for each species prior to administration.

Machin and Caulkett (2000) compared the effects of the anesthetics isoflurane (an inhalant) and propofol (an injectable) on female Canvasbacks (*Aythya valisineria*) in preparation for implanting intraabdominal transmitters. Ducks that received propofol experienced a smoother, more rapid induction and recovery than those given isoflurane. While some females in both treatment groups abandoned their nests following the surgical implantation procedure, the abandonment rate of the group that received propofol was approximately half of the group that received isoflurane.

Investigators cannot assume that what works for one species will work with another (Samour et al. 1984; Kabat et al. 2008). Langlois et al. (2003) tested the same two anesthetics as Machin and Caulkett (2000) on Hispaniolan Amazons but used a lower dose of propofol (5 mg/kg). Propofol recovery times, even at this lower dosage, were prolonged relative to recovery from isoflurane. In addition, six of 10 propofol-tested birds had agitated recoveries. The contrasting results of these two studies highlight the often dosage- and taxon-specific effects of anesthetic agents.

### ***Drug combinations***

Anesthetics and analgesics may be combined with each other or other drugs for synergistic or antagonistic effects (e.g., Vesal and Eskandari 2006, Vesal and Zare 2006). Muscle relaxants such as diazepam or midazolam may be used in birds, but only in conjunction with an analgesic agent. Currently, ketamine, which was once the injectable anesthetic of choice, is rarely used as the sole chemical anesthetic agent because muscle relaxation is poor, analgesia is inadequate, and recovery is often violent. Due to issues with its activity and effectiveness, ketamine is commonly mixed with other pharmaceuticals (Kilander and Williams 1992; Muir et al. 1995; Heard 1997; Mostachio et al. 2008; Rahal et al. 2008). Understanding the complexities of drug interactions requires specialized knowledge. Investigators wishing to use pharmaceuticals should consult, and practice with, a veterinarian experienced in working with avian species.

*Example 1* - Durrani et al. (2008) tested the relative utility of detomidine and ketamine on the induction, maintenance, and recovery from anesthesia in Rock Pigeons (*Columba livia*). Birds given either detomidine or ketamine exhibited light anesthesia and superficial analgesia; birds given both drugs exhibited deep anesthesia and analgesia (assessed by testing of body reflexes). During recovery, birds given detomidine or both drugs exhibited hypothermia,

depressed respiration, and bradycardia, but a generally smooth recovery; birds given only ketamine exhibited hyperthermia, rapid respiration, tachycardia, and a rough recovery. The authors concluded that detomidine is safe for use in bird handling and minimally invasive procedures whereas a combination of detomidine and ketamine is suitable for major surgery; ketamine alone is not a suitable option as an anesthetic agent.

*Example 2* - Atalan et al. (2002) assessed the sedative-anesthetic effects of a combination of medetomidine, butorphanol, and ketamine on domestic pigeons. The authors concluded that this combination was a reliable option. The authors applied atipamezole as an antagonist following ketamine and were somewhat dissatisfied with the length of time that atipamezole took to reverse the sedative effects. In this particular study, the pigeons tended to wildly flap their wings during recovery and had to be restrained to prevent self-injury. Other experiments using medetomidine on pigeons and parrots have met with mixed success (Sandmeier 2000; Pollock et al. 2001; Lumeij and Deenik 2003). In contrast, Langan et al. (2000) used a combination of medetomidine and ketamine followed by propofol on captive Ostriches (*Struthio camelus*) and found the combination to be very effective in achieving profound sedation and anesthesia. Administration of atipamezole resulted in smooth anesthetic reversal. Taken together, these studies illustrate: the taxon-specific nature of anesthetic effectiveness; the importance of background research and consultation with veterinary anesthesiologists; and, the importance of recognizing the fundamental differences between working with birds and with mammals.

*Example 3* - Teare (1987) examined the effectiveness of yohimbine hydrochloride (an  $\alpha_2$ -antagonist) in easing the recovery of Helmeted Guineafowl (*Numida meleagris*) from anesthesia induced by a xylazine-ketamine combination. Administration of yohimbine 40 min after induction of anesthesia shortened all facets of the recovery process relative to a saline control with no apparent side effects.

*Example 4* - Thil and Groscolas (2002) tested the efficacy of tiletamine-zolazepam on King Penguins (*Aptenodytes patagonicus*) under field conditions. Tiletamine is a dissociative anesthetic and zolazepam is a muscle relaxant with anti-convulsive properties. All treated individuals were immobilized within 5 min and remained so for approximately 1 hr. While three of the eight incubating adults did not resume incubation following treatment, the authors reported no adverse physiological effects. They did state, however, that future experiments with this treatment should examine the utility of various antagonists to reduce the recovery time.

*Example 5* - Mulcahy et al. (2003) treated 20 Spectacled Eiders (*Somateria fischeri*), 11 King Eiders (*S. spectabilis*), and 20 Common Eiders (*S. mollissima*) with propofol, bupivacaine, and ketoprofen for the surgical implantation of transmitters. Propofol was administered to maintain anesthesia, bupivacaine was injected subcutaneously along the incision inducing local analgesia, and ketoprofen was injected IM at the time of surgery for analgesia during recovery. Of the 16 males operated upon, nine (56%) died within 4 days; one of the 35 (2%) females died. Necropsy revealed severe renal damage and visceral gout. The authors concluded that the ketoprofen caused the lethal renal damage and that male eiders may be more susceptible to renal damage than females due to the relatively short period of time they spent on land. Mulcahy et al. (2003) expanded their conclusions to suggest that non-steroidal anti-inflammatory drugs should not be used on any species prone to renal insufficiency. Alternative options for analgesia should be considered.

### **Local anesthesia**

Given the difficulties of administering some of the common general anesthetics and the potential adverse effects, the use of local anesthetics is attractive, especially if the procedure is simple and the bird is to be released quickly. As with general anesthesia, however, the dosages for local anesthesia are uncertain and the effects may be prolonged and unpredictable (Graham-Jones 1965). To some extent, the problem is one of size (Gandal 1969; Klide 1973), with small species more susceptible to overdose. Extreme care must be taken in calculating dosages. A dose as small as 0.1 ml of 2% lidocaine is a lethal overdose for a 30-g bird. Studies on mammals indicate that several common local anesthetics, including 1% procaine, 0.2% tetracaine, 0.5% lidocaine (with and without epinephrine), 2% chlorprocaine, 0.25% dibucaine, 2% mepivacaine, and 2% pirocaine, have temporary but severe myotoxic effects (Basson and Carlson 1980; Foster and Carlson 1980). Diluting local anesthetics (with sterile, preservative-free normal saline solution or sterile water) would increase their margin of safety. The intramuscular use of local anesthetics should be undertaken with caution. Currently, the preferred short-acting drug is lidocaine and the long-acting drug is bupivacaine (Machin 2005). However, the most appropriate route of administration should be investigated for each species and drug.

Anesthetic compounds have also been employed in two areas unrelated to use in surgical procedures. Anesthesia as an agent for capture technique is discussed in the section on

capture and marking. It is also used to minimize nest desertion by adults following capture and handling. Smith et al. (1980) developed an anesthetic technique using methoxyflurane (an inhalant anesthetic) to reduce nest abandonment by Gray Partridge (*Perdix perdix*) following handling and transmitter attachment. Of the six birds anesthetized with methoxyflurane following handling but prior to replacement on the nest, none abandoned and all but one successfully fledged young. No mortality was reported. Rotella and Ratti (1990) used the same technique to reduce nest abandonment in Mallards (*Anas platyrhynchos*). Following handling, transmitter attachment, and marking, female Mallards were anesthetized and placed back on their nest to recover. Only two of 80 treated females abandoned their nest following treatment, a rate significantly lower than previous studies. Individuals should always be monitored until fully recovered. The authors caution against placing anesthetized individuals back on nests in areas with predators or during inclement weather.

## **E. Surgery**

### ***Positioning***

The subject should be positioned in as natural a position as possible but appropriate for exposure of the surgery site. If the procedure is relatively long, consider regularly rotating the animal to avoid blood pooling.

### ***Monitoring***

Navarez (2005) provides an excellent review of considerations for monitoring animals while under anesthesia. Navarez (2005) stresses two critical points at the start of the review. First, planning how vital signs will be monitored is a critical component of pre-operative decision-making. Second, equipment cannot replace knowledge of and experience with the anesthetic agents and the species involved. Basic vital signs, such as reflexes or respiration, can be monitored without equipment and an investigator must be ready to perform continuous observations should equipment fail. Second, planning how vital signs will be monitored is a critical component of pre-operative decision-making.

Testing an animal's reflexes is perhaps the simplest method for assessing the depth of anesthesia. In birds, testing corneal reflexes using a lubricated cotton swab can be an effective reflex test. Another simple test is the toe pinch; foot withdrawal following the pinch can be interpreted as a pain reflex, which indicates an insufficient depth of anesthesia (Nevarez 2005). In addition to monitoring reflexes, investigators also need to monitor heart rate and respiration, both as indicators of depth of anesthesia and adequate tissue perfusion. Methods for monitoring heart rate include stethoscopes, Doppler monitoring, electrocardiograms (ECG), and direct blood pressure monitoring using arterial catheters and pressure transducers. Methods for monitoring respiration include respiratory rate monitors, pulse oximetry, capnography, and blood gas analysis. Stethoscopes can also be used to monitor respiration. Of these monitoring options, only respiratory rate monitors are suitable for deployment in most field situations. Some anesthetics may not induce eye closure. In such cases, the open eyes should be bathed with an optical wetting agent every few minutes or should be protected by application of an ophthalmic ointment which should be placed on to the eye as a strip across the eye along the upper or lower lid. Drops and ointments containing steroids should be avoided unless specifically prescribed by a veterinarian.

Hypothermia is one of the most common complications of anesthesia (Boedecker et al.2005, Nevarez 2005). Normal bird body temperature ranges from 40 to 44°C. Heat loss can occur via convection (i.e., air exchange at the body surface), radiation (i.e., heat loss due to heat differential between body and surroundings), conduction (i.e., heat loss due to contact with a colder surface), and evaporation from lungs and skin. All four forms of heat loss must be accounted for during a surgical procedure and subsequent recovery. Heating pads and forced air warmers are commonly used to maintain both the body temperature of the subject and the temperature of the subject's surroundings. Even if steps have been taken to maintain temperatures during the procedure, investigators should also monitor their subject's core temperature. Heating pads should be avoided or used with extreme caution, as they have been known to cause severe skin burns even if used at low temperatures.

Other potential complications with anesthesia include cardiac arrhythmias or arrest, and severe respiratory depression leading to respiratory arrest. A detailed discussion of these issues with recommendations for their avoidance and management can be found in Gunkel and Lafortune (2005).

### ***Incision closure and treatment***

Closure of surgical incisions can be difficult and proper closure techniques require appropriate skills and materials. Consultation with a veterinarian is recommended to learn an appropriate closure technique for the incision and surgical procedure to be used, particularly if sutures are necessary. Older cyanoacrylic tissue glues (e.g., Tissu-Glu<sup>®</sup>, Ellman International, or Vetbond<sup>®</sup>, 3M Corp.; N.B. household super glues are toxic to tissues) took several minutes to dry, which markedly increased handling time. New, fast-drying versions of cyanoacrylate surgical glues such as Dermabond<sup>®</sup>, LiquiBand<sup>®</sup>, SurgiSeal<sup>®</sup>, and Nexaband<sup>®</sup> take under a minute to set and are far less toxic than earlier surgical glues. The need to close incisions to avoid infection justifies the cost (~\$20/vial). It may be advisable to flush the incision with sterile saline solution as well as place triple (or other) antibiotic ointment on the incision. Inadvertent wounds caused during the procedure should also be treated. Surgical staples are an effective and rapid means of closing large incisions in medium-sized and large birds that are maintained in captivity or that can be held in captivity until the incisions heal and the staples are removed. Staples are not advised for field use. If multiple tissue layers have been incised, it is important to use the appropriate incision closure technique for each. Some small incisions heal faster if no surgical glue is used and in some cases of small skin incisions, the best decision may be to not use sutures or glue. Repeated surgeries on a single subject are discouraged unless they are part of a single experiment and have scientific justification.

### ***Specific field surgeries***

Laparotomy: Laparotomy penetrates a body cavity and, thus, is considered a major surgical procedure. Exploratory laparotomy has several uses. Since sex cannot be determined by external appearance in juvenile birds and adults of many species, laparotomy provide information on sex in monomorphic species (e.g., Lawson and Kittle 1973) and stage of gonadal development (e.g., Wingfield and Farner 1976; Schwab 1978), it can indicate presence of parasites, respiratory disease, gross condition, and activity of other organs, and it can be used for placement of transmitters into the body cavity.

Many experts perform laparotomies with only a topical anesthetic or no anesthetic at all (Risser 1971; Piper and Wiley 1991), especially in the field where speed of operation is important so that the bird can be released quickly and in a condition to avoid predators. Such usage is not

recommended for anyone lacking adequate instruction and abundant practice on anesthetized or recently deceased birds. Even skilled practitioners should practice following any significant hiatus in performance.

Isoflurane and methoxyflurane are both used as anesthetics for this procedure. Recently, MacDougall-Shackleton et al. (2001, 2006) used methoxyflurane prior to field laparotomies for their studies on gonadal development in sparrows and cardueline finches. Unfortunately, methoxyflurane is no longer commercially available. Isoflurane is a superior choice, but requires a portable vaporizer for field use. For the studies cited above, McDougall-Shackleton et al. (2001,2006) placed a few mLs of metofane (methoxyflurane) on a cotton ball in a jar and held that over the birds face until it didn't respond to toe pinch, then remove the jar. The laparotomies took about 2 minutes, and the birds recovered within 5 minutes. Recovery from isoflurane is even more rapid. Topical application of xylocaine cream or ethyl chloride to the incision site may reduce discomfort of laparotomized birds (Risser 1971; Ritchie et al. 1994).

Several reports have shown that laparotomy has no effect on survival and does not disrupt breeding activity or winter foraging (Bailey 1953; Miller 1958, 1968 cited in Risser 1971; Wingfield and Farner 1976; Marion and Myers 1984; Ketterson and Nolan 1986; Piper and Wiley 1991). Piper and Wiley (1991) examined the effects of laparotomies on White-throated Sparrows. They used no anesthetic, restrained birds using a modified bander's grip (see Bailey 1953 for diagram), and allowed incisions to close naturally. Birds were held in cages post-procedure for 5 to 30 min before being checked for alertness and then released. Laparotomies appeared to have no significant effects on short-term fat storage, long-term survival, dominance status, or range size. The only significant effect reported was the observation that laparotomized birds were more likely to remain on the study site as winter residents. The consequences for this last observation are unclear. One explanation is that the occasional rupturing of an air sac during the operation might have hindered an individual's ability to fly long distances. Another possibility is that becoming more sedentary might be a generalized response to injury, as previously suggested by Westneat (1986).

Laparotomy has been used successfully on hatchlings (Fiala 1979). Fiala (1979) performed laparotomies on nestling Red-winged Blackbirds (*Agelaius phoeniceus*) using methoxyflurane (Metofane) vapors as an anesthetic, restraining birds on plexiglass sheets with adhesive tape, and closing the incisions with liquid skin adhesive. The only serious problem reported was occasional renal hemorrhage. Fiala (1979) reported minimal long-term effects. Plastic bands are

an excellent alternative to adhesive tape. This study highlights the fact that the most appropriate surgical approach should be researched and selected. It is important to avoid vital structures (like kidneys) and air sacs to the extent possible. Repeated laparotomies on birds at intervals of about a month to trace gonadal growth and regression can be done with the caveat that scar tissue at the incision site can make successive surgeries more difficult.

Any unsealed wound can be a route for infection or for herniation of abdominal tissues and organs. Therefore, laparotomy wounds should be sealed. Surgical glues serve this purpose well. Wounds in waterbirds should be sutured to reduce the potential for infection. In diving species, the wound must be sealed to avoid penetration of water into the body cavity as pressure increases with depth.

Over the last decade, several less invasive techniques have become available for sexing birds, including flow cytometry (Tiersch et al. 1991), fecal sex steroid (Goymann 2005; Palme 2005), and polymerase chain reaction-based techniques using feathers, blood, or tissue (Han et al. 2009; Griffiths and Tiwari 1993; Ellegren 1996; Griffiths et al. 1998; Fridolfsson and Ellegren 1999, Underwood et al. 2002). A major limitation to using PCR to sex birds has been resolved by the development of a multiplexed PCR that works in all avian species, even the many species for which there is no difference in between-sex intron length, which is the basis of traditional DNA-based sex determination (Han et al. 2009). All of these less invasive techniques require the return of samples to the laboratory and some delay during processing. Nevertheless, investigators are encouraged to explore the appropriateness of these techniques to their studies. Unless the research question requires immediate knowledge of an individual's sex or gonadal development, the less invasive techniques should be given primacy over laparotomies.

Implantation of transmitters: One of the most common field surgical techniques involves the implantation of a transmitter or biomonitor (e.g., Korschgen et al. 1984, 1996; Olsen et al. 1992; Harms et al. 1997; Hatch et al. 2000; Machin and Caulkett 2000). Most current studies follow the techniques of Korschgen et al. (1984, 1996) and Olsen et al. (1992). Korschgen et al. (1984) were among the first to explore the feasibility and utility of placing radio transmitters inside the body cavities of birds. Using several species of captive and wild ducks, the authors first evaluated the effectiveness of several anesthetics, including pentobarbital, ketamine, and xylazine, and discovered that the use of local anesthetic (2% lidocaine hydrochloride) was sufficient for the procedure. Although conditions were not aseptic, the authors wore gloves and kept surgical equipment in a cold sterilization tray containing zepharin chloride prior to use. The

authors made 2 cm incisions, inserted the transmitters, closed the incisions with surgical sutures, and treated all birds with antibiotics. Including time for anesthesia, the operations took 25 min per subject. Of the 31 subjects, the only complications reported were a systemic infection in a single female Canvasback and a local infection around the internally coiled antenna in a male Mallard. Later, Korschgen et al. (1996) modified their earlier technique by switching to isoflurane. They reported minor physiological responses to the surgery but no major adverse effects.

Olsen et al. (1992) used a similar technique to implant transmitters in Canvasbacks but used isoflurane as the anesthetic. Investigators wore gloves and equipment and transmitters were maintained in cold sterilization; incisions were closed with absorbable sutures. Over the three years of the study, 253 birds received implanted transmitters. Five individuals (2%) died during or immediately following surgery: one from hemorrhage in the airways, one from lung congestion, and three from respiratory complications due to lung flukes. The authors reported no post-operative complications for the remaining 248 individuals. Korschgen et al. (1996) modified their earlier technique by switching to isoflurane. They reported minor physiological responses to the surgery but no major adverse effects.

Mulcahy and Esler (1999) implanted transmitters into 307 Harlequin Ducks (*Histrionicus histrionicus*). Initially, birds were anesthetized using isoflurane. Surgeries were performed in a covered but unheated workspace on a motor vessel and birds were allowed to recover for a least one hour before release. In the first year of the study, 10.7% (11 of 103) of individuals died during surgery or within 14 days of release. As a result, the authors made the following alterations to their surgical procedures: birds were intubated, birds were placed on foam pads so that their heads rested below their body during the surgical procedures, and researchers monitored and maintained patient body temperature using temperature sensors, electrocardiograms, and hot water pads. Following the implementation of these alterations in procedures, mortality dropped to 2.9% (six of 204). Additional examples of surgical procedures for transmitter implantation can be found in Schultz et al. (1998, 2001).

## **F. Post-surgery**

Most birds will experience emergence delirium during recovery from general anesthesia, regardless of the anesthetic agent used (Curro 1998). If a subject was intubated during surgery,

extubation should occur when bird is awake enough to exhibit signs of objecting to the tube's presence (e.g., has swallowing reflex); the glottis should be examined for damage or obstructions following extubation (Curro 1998). During emergence delirium, subjects should be restrained with sufficient force to prevent self-injury (e.g., due to uncontrolled wing flapping) but the restraint should not interfere with ventilation and thermoregulation. It was once thought that rocking a bird from side to side during recovery from general anesthesia minimized emergence delirium; however, this procedure is now discouraged due to potential interference with normal blood flow and respiration during recovery (Gunkel and Lafortune 2005). Subjects should be provided with a warm and dimly lit (or dark) place in which to recover and should not be released into the wild until fully alert and are able to perch or stand on their own. One technique for passerines is to **loosely** roll them in a clean paper towel following surgery, and place them on their side in a recovery cage. This gently restrains them until they are sufficiently recovered to wriggle out.

## **G. Euthanasia**

The question of euthanasia primarily arises when an animal experiences pain and suffering that cannot be relieved or that will not abate over time or has an impairment that will affect its probability of survival. Euthanasia is also a necessary component of scientific collection (see Scientific Collecting). Irrespective of the purpose of the euthanasia, the technique used first and foremost, should produce unconsciousness swiftly and painlessly. In addition, the euthanasia technique should not interfere with post-mortem analysis and will be considerably influenced by what an investigator wishes to do with the cadaver (i.e., use it for a museum specimen or for tissue chemistry). Many techniques for euthanasia have been reviewed by the American Veterinary Medical Association (2007); these euthanasia guidelines were under review in 2009 and the American Veterinary Medical Association expects to issue an update in 2010. The American Veterinary Medical Association recognizes that recommended modes of euthanasia for captive animals are not always feasible in field situations. However, the challenges presented by field conditions do not release investigators from the responsibility of minimizing the pain and distress of animals to be euthanized.

The American Veterinary Medical Association (2007) considers the following techniques to be acceptable for use with wild birds: barbiturates (injected, ideally intravenously), inhalant anesthetics, carbon dioxide inhalation, carbon monoxide inhalation. Gunshot to the head is

considered conditionally acceptable when other methods cannot be used. However, the carcass will have no value as a specimen if the head is destroyed by gunshot and so this method is inappropriate unless there is no intent to retain the carcass as a specimen. The following techniques are considered conditionally acceptable: nitrogen and argon inhalation, cervical dislocation, and thoracic compression. The use of injectable agents, such as barbiturates, is a rapid and dependable method of euthanasia. The use of barbiturates as a euthanasia agent in the field may influence carcass disposal due to possible detrimental effects on scavengers. The fastest and most humane result occurs if the drug is injected directly into the vein. Therefore, the needle gauge should be carefully considered so that it is not so large that it will damage the vein upon entry and make injection into the vein difficult. When it is believed that the needle has entered the vein, prior to injecting the drug into the vein, the plunger of the syringe should be gently and slightly pulled back to confirm venipuncture by a red flash of blood into the syringe. Generally acceptable techniques involve overdose with an anesthetic, either injected or inhaled, followed by the administration of a specific euthanasia compound. Such procedures pose little problem for laboratory studies, but may be impractical in the field. Field investigators who normally include hypodermic syringes as part of their equipment (e.g., for tissue sampling) will probably find that small bottles of anesthetic or euthanasia compound add little burden. Investigators who wish to consider this option must consult with a veterinarian to determine the appropriateness – especially the required dosage - and legality of the use of certain chemicals in the field. If pre-euthanasia chemicals are unavailable, the American Veterinary Medical Association (2007) states that an intraperitoneal injection of sodium pentobarbital is acceptable if an intravenous injection is impractical or impossible. Although intraperitoneal injections are slower to produce loss of consciousness, they do not require the same level of handling and restraint of subjects and can minimize an animal's distress. Intramuscular, subcutaneous, intrathoracic, intrapulmonary, intrahepatic, intrarenal, intrasplenic, intrathecal, and other nonvascular injections are not acceptable methods of administering injectable euthanasia agents. Euthanasia via inhalant (e.g., carbon dioxide or monoxide) is also a generally acceptable method but it can be cumbersome for field use and deployment. A portable solution is to carry a small tightly sealed vial with a cotton ball in it that has been soaked in isoflurane. Euthanasia is very rapid and is done by placing the bird's face in the jar. The realities of field situations often require mechanical means of dispatch. This technique, known as thoracic compression, results in a very rapid loss of consciousness, with death following soon after. It is easy to learn, thereby minimizing the probability of mistakes and the potential for subject distress. The technique also maximizes the scientific utility of

specimens, thereby minimizing the number of individuals collected for scientific research. The AVMA (2007) considers thoracic compression to be conditionally acceptable. The Ornithological Council has prepared a peer-reviewed fact sheet about thoracic compression that is available upon request.

The American Veterinary Medical Association (2007) considers the following chemical methods to be unacceptable under any conditions: chloroform, cyanide, formalin, household solvents (e.g., acetone), neuromuscular blocking agents, and strychnine. Chloroform, cyanide, and formalin are unacceptable both for the danger they pose to personnel and for the distressing aspects of their chemical actions in the body of research subjects. Neuromuscular blocking agents (e.g., nicotine, magnesium and potassium salts, all curariform agents) produce muscle paralysis in conscious animals, causing death through asphyxiation. Potassium salts stop the heart from contracting in conscious animals, causing distress until unconsciousness occurs. Potassium salts are acceptable for euthanasia only when administered to deeply anesthetized animals. Strychnine causes painful and protracted convulsions, prior to death by asphyxiation. The American Veterinary Medical Association (2007) considers the following physical methods to be unacceptable under any conditions: air embolism, blow to the head, burning, decompression, drowning, exsanguination, hypothermia, rapid freezing, and smothering.

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