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Anesthesia and Liver Biopsy Techniques for Pigeon Guillemots (*Cepphus columba*) Suspected of Exposure to Crude Oil in Marine Environments

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Abstract: This paper reports on the anesthesia and liver biopsy techniques used in adult and nestling pigeon guillemots (*Cepphus columba*) to test for continued exposure to residual crude oil in the marine environment. Populations of pigeon guillemots have declined significantly in Prince William Sound, Alaska, USA, possibly because of residual effects of crude oil in the environment after the *Exxon Valdez* oil spill in March 1989. Measurement of hepatic cytochrome P450 1A (CYP1A) is currently the best way to assess crude oil exposure from food sources; however, lethal sampling to obtain adequate liver tissue was not desirable in this declining population of birds. As part of a larger study to identify factors limiting the recovery of pigeon guillemots and other seabird populations, we surgically collected liver samples from adult and nestling guillemots to provide samples for measurement of hepatic CYP1A concentrations. Results from the larger study were reported elsewhere. Liver samples were taken from 26 nestling (1998) and 24 adult (1999) guillemots from a previously oiled site (Naked Island; 12 chicks, 13 adults) and from a nonoiled site (Jackpot Island/Icy Bay; 14 chicks, 11 adults). The birds were anesthetized with isoflurane. No surgical complications occurred with any of the birds and all adult and nestling birds survived after surgery to the point of release or return to the nest. Thirteen out of 14 chicks from the Jackpot Island/Icy Bay and 8 out of 12 chicks from Naked Island fledged. Four chicks at Naked Island were depredated before fledging. All adults abandoned their nests after surgery, so the study sites were revisited the following summer (2000) in an attempt to assess overwinter survival of the adults. All but 1 adult biopsied bird at the nonoiled site (Icy Bay) was found renesting, whereas only 2 birds at the previously oiled site (Naked Island) were similarly observed. The percent of 1999 breeders at Naked Island that returned to their nest sites to breed again in 2000 was low at nests of biopsied birds and nonbiopsied birds alike, suggesting that factors other than the surgical procedure were responsible for the low return rate among this group. These survival results provide strong support for using experienced veterinarians for nonlethal invasive sample collection from birds to document exposure to crude oil in the marine environment.
Key words: anesthesia, cytochrome P450 1A, liver biopsy, avian, pigeon guillemot, *Cepphus columba*

Introduction

The T/V *Exxon Valdez* oil spill in Prince William Sound, Alaska, USA, had acute and devastating effects on a wide range of habitats and species.[1] Although some injured populations recovered quickly after the spill, others took longer or have yet to show signs of recovery.[1] Given the delayed recovery of several species, an integrated study was launched to identify mechanisms limiting the recovery of a suite of 4 nearshore vertebrate predators such as the pigeon guillemot (*Cepphus columba*).[2] One proposed mechanism limiting the recovery of these species is continued exposure to residual oil, which can be assessed by assaying hepatic cytochrome P450 1A (CYP1A). This liver enzyme is rapidly, yet transiently, induced in many vertebrate species after exposure to hydrocarbons, such as polycyclic aromatic hydrocarbons found in petroleum products.[3] High CYP1A concentrations

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can result from either direct exposure to petroleum products from the environment or indirect exposure to these products via the food chain in invertebrate-feeding and piscivorous species such as pigeon guillemots.[2,3] Tissues for measurement of CYP1A usually are collected via lethal sampling; however, in this study, liver biopsies were surgically collected from anesthetized adult and nestling guillemots.[4] Concerns over the declining guillemot population warranted the use of antemortem techniques to collect liver samples. Techniques for inhalation anesthesia and surgical methods to collect liver biopsies have been described for many species of birds.[5,6] Most liver biopsy techniques involve a small abdominal wall incision caudal to the ventral keel; however, it is also possible to collect a liver biopsy by using endoscopic equipment or by fine-needle aspiration with ultrasound examination.[6,7] These techniques require a smaller incision (endoscopic procedure) and result in less soft tissue trauma, but it would be difficult to collect an adequate sample for CYP1A analysis. The use of these specialized pieces of equipment in a remote field setting would be difficult, if not impossible. The purpose of this paper is to describe the anesthesia and surgical techniques suitable for use in a remote field location such as Prince William Sound, including equipment, monitoring techniques, and complications. We provide a minimum estimate of overwinter survival for biopsied adult birds. Short-term survival of the biopsied chicks until fledging was evaluated, but estimating overwinter survival was not possible because guillemots do not return to their natal colonies until they are at least 2, and more likely, 3 years of age.[8]

Materials and Methods

Birds and study sites

Twenty-six chicks (18–24 days of age; sex unknown) and 24 adult breeding pigeon guillemots (age and sex unknown) from 2 areas of Prince William Sound were anesthetized for liver biopsies July 20–26, 1998 (chicks), and June 15–23, 1999 (adults). Twelve chicks and 13 adult pigeon guillemots were biopsied from the Naked Island group in central Prince William Sound (60°40′N, 147°28′W). This group of islands, located 16.5 nautical miles from Bligh Reef, was the first land mass hit by the oil spill from the *Exxon Valdez.* Another 14 chicks and 11 adult pigeon guillemots were biopsied from the combined colonies of Jackpot Island (60°19′N, 148°11′W) and Icy Bay (60°14′N, 148°17′W) located in southwestern Prince William Sound, an area that was not directly impacted by the oil spill. Chicks were biopsied from both locations; however, adult birds were biopsied only from Icy Bay. Liver biopsies were collected for CYP1A analysis (reported in a larger study investigating direct and indirect effects of the oil spill on pigeon guillemot populations in Prince William Sound).[4]

Birds were captured at nest sites and transported individually in water resistant small-animal carrier boxes to a nearby field camp for surgery. To minimize disturbance at the nest sites, siblings (brood size equaled 1 or 2) were removed simultaneously. Adults were captured while incubating eggs. Most of the adult birds were caught with a small fishing net as they were flushed from the cavity entrance; however, some birds were captured by hand within the nest cavity. Whenever possible, both adults in a breeding pair were captured and biopsied to minimize the total number of nest disturbances. Three pairs and 7 single breeding birds were captured at Naked Island and 5 pairs and 1 single breeding bird were captured at Icy Bay for a total of 10 and 6 nest disturbances, respectively. In 7 of 8 pairs where we managed to capture both mates, the first bird was captured during the afternoon or evening and its mate was captured at the nest the following morning. All of the eggs from the biopsied birds' nests were collected and incubated and hatched in captivity. After surgery, adults were held for a minimum of 1 hour, and released from shore near the field camp. The distance from the field camp to an individual's nest ranged from approximately 4 to 27 km. Adult birds were released next to the shore by gently tipping the boxes toward the water and opening the top, allowing escape without further handling. General observations about the bird's behavior were noted, including whether the birds initially swam or flew.

Anesthesia

Birds were anesthetized with isoflurane (Aerrane, Anaquest, Madison, WI, USA) in oxygen, delivered from a precision vaporizer (Table Top/Research Small Animal Anesthesia Machine, AM-1001-D, Anesco, SurgiVet Inc, Waukesha, WI, USA) via a modified avian Jackson Rees anesthesia circuit (Anesco, SurgiVet). Oxygen was supplied from standard ''E'' cylinders, and waste gas was scavenged by an activated carbon disposable canister (F-Air, AM Bickford Inc, Wales Center, NY, USA). Anesthesia was induced via mask delivery of isoflurane, followed by endotracheal intubation. Isoflurane concentrations were varied as necessary to maintain a medium plane of anesthesia.[5] Endotracheal tubes for chicks were uncuffed 2.5-mm inner diameter and for adults were uncuffed 3.0-mm inner diameter (Cole Tracheal Tube, Rusch Ag, Kernen, Germany). Oxygen flow rate was 1 L/min.

During anesthesia, heart rate, respiratory rate, and cloacal temperature were monitored. Heart rate was monitored by thoracic auscultation and with an ultrasonic Doppler flow detector (Model 811, Parks Medical Electronics Inc, Aloha, OR, USA) that was taped to the dorsal surface of the tarsal joint overlying the cranial tibial artery. A cloacal temperature probe (Electro-Therm TM99A, Cooper Instrument, Middlefield, CT, USA) was placed approximately 2 cm inside the cloaca. Different anesthetists monitored anesthesia each year, so different parameters were recorded. For chicks, anesthesia time (time from mask induction to discontinuation of isoflurane), surgery time, cloacal temperature, and isoflurane concentrations were recorded. For adults, time from capture to induction, anesthesia time (time from mask induction to extubation), surgery time, time from induction to excitement phase, time from extubation to release, total time from capture to release, respiratory rate, cloacal temperature, heart rate, and isoflurane concentrations were recorded. Ventilatory assistance was provided as necessary by manual compressions of a rebreathing bag. Adult birds and chicks considered hypothermic (less than approximately 38°C) were given warmed, lactated Ringer's solution (4–5 ml administered subcutaneously in the ventral inguinal web at a dosage of approximately 1–2% of the bird's body weight). Hypothermic birds were also supplied with warm water bottles during recovery in their restraint boxes.

Surgery

Surgeries were performed in summer field camps established at 2 locations in Prince William Sound. All anesthesia and surgical procedures were done in an enclosed shelter. Supplemental heat was provided with either propane or kerosene heaters when the inside temperature dropped below approximately 15°C. Sterile technique and equipment were used to prevent iatrogenic introduction of pathogenic bacteria into the coelomic cavity. Individual sterile surgery packs and transparent plastic drapes (Surgical Drape, Veterinary Specialty Products Inc, Boca Raton, FL, USA) were used with each bird. Sterile surgical gloves were worn by the surgeon for each procedure and surgical caps and masks were worn by the surgeon and anesthetist.

Once the birds were anesthetized, intubated with an endotracheal tube, and stabilized in a medium plane of anesthesia,[5] they were placed in dorsal recumbency on a piece of rigid foam insulation to minimize conductive heat loss from the patient to the table top. The bird's head and neck were positioned slightly lower than their bodies to prevent accidental tracheal aspiration of foregut contents during the procedure.

Monitoring devices were placed as described in the previous section. Just caudal to the xyphoid process of the keel, an area approximately 2 cm wide by 3 cm long was gently plucked to remove feathers. This plucked area was cranial to the brood patch that was present in all adults. A similar-sized area was plucked in the chicks. The feathered margins of the plucked area were taped with short strips of tape (Micropore Tape, 3M Co, St Paul, MN, USA) to prevent feather contamination in the surgical site. In adult birds, feathers at the caudal margin of the keel were not plucked, but were gently folded cranially and taped out of the way of the surgical field to minimize the amount of feather plucking, yet still have an adequate surgical field size. Once the tape was removed after surgery, these feathers were laid back down to cover the surgical site and to provide insulation from the cold water. We noted that these feathers covered the surgical site in a similar manner to the feathers cranial to the brood patch. The plucked surgical site was a smaller area than the brood patch in all cases.

The surgical site and taped margins were aseptically prepared with 1% povidone iodine solution (Betadine, Purdue Frederick Co, Norwalk, CT, USA). A sterile transparent surgical drape with an adhesive, fenestrated area in the center was placed over the bird and surrounding area. The transparent drape facilitated visual monitoring of the birds' respirations by the anesthetist during surgery and helped to retain body heat. A 1.5- to 2.0-cm skin incision was made starting just caudal to the xyphoid process of the keel. Subcutaneous fat and connective tissue was bluntly undermined to visualize the linea alba. The linea alba was incised to enter the coelomic cavity. In the chicks, the caudal margin of the liver extended caudal to the xyphoid process, and the liver was easy to visualize and biopsy. However, the liver was much more difficult to locate in the adult birds because of its position dorsal to the keel and cranial to the xyphoid process. Once the liver was visualized, a curved mosquito forceps was used to gently grasp an approximately 3×10-mm section (approximately 0.05–0.1 g) of the liver along the caudal edge of the right lobe. This piece of liver was incised with a scalpel blade and immediately transferred to the anesthetist who wrapped the tissue in aluminum foil, and placed it in a cryogenic vial. The vial was then frozen in liquid nitrogen. Hemostasis of the incised liver margin was not necessary with any of the surgeries, because hemorrhage was minimal. The linea alba and skin were sutured separately using simple continuous layers of 4-0 polyglactin 910 suture (4-0 Vicryl, Ethicon Inc, Somerville, NJ, USA). A small piece (3 × 3 cm) of a sterile, transparent, adhesive, waterproof dressing (Tegaderm, 3M Co) was placed over the incision and surrounding plucked skin to provide a temporary barrier to bacteria while the incision was healing.

Immediately after surgery, birds were weighed (with a spring balance) and measured (tarsometatarsus length, head-bill length, and wing-chord length), and 2 ml of blood was collected from the basilic vein (blood was collected postoperatively from adult birds only). All birds were individually marked with a US Fish and Wildlife Service metal band and unique combination of 3 colored plastic leg bands. In adults, these procedures were all performed while the bird was still under anesthesia to minimize the stress of handling, whereas in chicks the measurements were obtained from awake birds. After recovery from anesthesia, the birds were extubated and placed in their transport boxes with supplemental heat (provided by water warmed to 40°C and placed in sealed plastic bags). Chicks were taken back to their nest site as soon as possible, whereas adult birds were held in a quiet, dark area for approximately 1 hour before being released.

Postbiopsy fledging (nestling) and adult overwinter resighting rates

Chicks were monitored after biopsy until they fledged, or until the chick was lost to predation. Chicks were weighed at least once before fledging (''prefledging weights''). Not enough data were collected to distinguish between peak and fledging weights, so only 1 set of weights is reported here. After fledging, no further field resighting data or survival data were collected. In June 2000, the 2 study sites were revisited to determine survival rates of adult guillemots that had been biopsied the previous year. All nest sites from which adults had been caught and biopsied the previous year were checked for nesting activity. Whenever possible, adults were caught, checked for bands, and weighed and measured for body mass and body condition assessments.

Statistics

Data were evaluated by the Shapiro-Wilk *W* test for normalcy and determined to be suitable for parametric analyses. Initial and final cloacal temperatures were compared by a 1-tailed paired *t* test for both chicks and adults. Requirement for assisted respiration was compared between chicks and adults by a chi-square test. The potential effect of postcapture transportation time on adult physiologic values (temperature, heart rate, and respiratory rate) at the initiation of anesthesia was tested by 1-way analysis of variance. Student's *t* test was used to assess whether birds that flew versus those that swam upon release differed with respect to their transportation and presurgical holding time, total time from capture to release, anesthesia time, surgery time, or time between extubation and release. A 2-tailed paired *t* test was used to compare 1999 body weights of adult guillemots that were recaptured and weighed in 2000. All analyses were performed on a commercial statistics program (JMP, SAS Institute, Cary, NC, USA).[9] Statistical significance was assigned at $P < .05$.

Results

Chicks weighed 311 ± 72 g (mean ± SD; reference range 180–407 g, n = 26) at the time of surgery. Anesthesia was induced at 3.5–4.0% isoflurane, and maintained between 1.0 and 4.0%, with most (89%, 16 of 18 recorded) between 3.0 and 4.0%. Anesthesia time was 24.7 ± 3.8 minutes (reference range, 19–36 minutes), and surgery time was 9.4 ± 3.0 minutes (reference range, 5–16 minutes). Initial cloacal temperature (the first-recorded temperature obtained after anesthesia induction) was 39.1 ± 1.3°C (reference range, 36.1–41.9°C) and final cloacal temperature was 38.7 ± 1.4°C (reference range, 34.9–41.9°C). The mean temperature decrease of 0.4°C was not statistically significant ($Z = 1.59$; $P = .062$). Ventilatory assistance was required for only 2 chicks, and 5 chicks regurgitated during anesthesia.

Adults weighed 478 ± 26 g (reference range, 435–520 g, n = 24). Anesthesia was induced at 3.5% isoflurane for all but 1 bird, which was induced at 3.5–4.0%. Maintenance concentrations of isoflurane varied more widely for adults than for juveniles, ranging from 0.5 to 4.5%, with most (67%, 16 of 24) between 2.0 and 4.0%. Anesthesia time was 52.2 ± 8.3 minutes (reference range, 41–72 minutes), and surgery time was 14.9 ± 5.4 minutes (reference range, 9–35 minutes). Times from capture to anesthesia induction, extubation to release, and total time from capture to release were 81.5 ± 48.7 minutes (reference range, 11–174 minutes), 68.4 ± 17.7 minutes (reference range, 25–104 minutes), and 196 ± 58.9 minutes (reference range, 105–300 minutes), respectively. No adult birds regurgitated during or after surgery. Twenty (83%) of 24 adults experienced an excitement phase characterized by attempted flapping of restrained wings during anesthesia induction just before achieving a plane of anesthesia sufficient for intubation. This excitement phase occurred at 4.1 ± 1.5 minutes (reference range, 2–7 minutes). Initial cloacal temperature was 40.0 ± 0.8°C (reference range, 38.6–42.1°C) and final cloacal temperature was 39.3 ± 1.4°C (reference range, 36.8–41.8°C). The mean temperature decrease of 0.6 °C was statistically significant ($Z = 2.43$; $P = .011$). The chicks were significantly cooler than the adults at the start ($Z = 3.06$; $P = .004$), but not at the end ($Z = 1.20$; $P = .237$); mean temperature difference between adults and chicks was 0.9°C at the start and 0.6°C at the end.

Initial heart rate for adults was 233 ± 45 beats per minute (bpm; reference range, 140–300 bpm), and final heart rate was 219 ± 40 bpm (reference range, 140–300 bpm). The highest recorded heart rate was 325 bpm and the lowest was 70 bpm. Nine (38%) adults experienced mild bradycardia (subjectively defined as heart rate ≤ 140 bpm), and 1 (4%) experienced severe bradycardia (70 bpm). The bird with severe bradycardia had a partial obstruction of the endotracheal tube because of a kinked neck, and its heart rate returned to the reference range after postural corrections were made. One bird experienced an undetermined cardiac arrhythmia, based on auscultation, which resolved with reduction of isoflurane concentration. That bird was noted to have an abnormally pale-colored liver, consistent with possible hepatic disease,[6] but the etiology was not determined histologically. The abnormality apparently was not life threatening, because this bird was recaptured on an active nest the following year.

Initial respiratory rate for adults was 37.3 ± 8.8 respirations per minute (rpm; reference range, 21–54 rpm) and final respiratory rate, recorded for only 10 birds, was 28.5 ± 9.6 rpm (reference range, 15–42 rpm). The highest recorded respiratory rate was 54 rpm and the lowest was 0 rpm. Nineteen (79%) of 24 adults required ventilatory assistance for periods ranging from 4 to 39 minutes. Respiratory depression followed by respiratory arrest was initially addressed by reducing or eliminating isoflurane delivery and initiation of manual ventilation, but because birds typically became responsive to surgical stimuli before restoration of spontaneous breathing, assisted ventilation while maintaining isoflurane delivery became standard practice. Depth of anesthesia was then determined primarily by heart rate and response to stimuli, with isoflurane concentrations being adjusted accordingly. Time from anesthesia induction to requirement for assisted respiration was 18.7 ± 5.0 minutes (reference range, 10–29 minutes). Spontaneous ventilation resumed within minutes after isoflurane concentration was reduced or eliminated. Adult birds were more likely than chicks to require assisted respiration ($\chi^2 = 29.3$; $P < .0001$). Initial temperature, heart rate, and respiratory rate did not correlate with transportation and presurgical holding time after capture ($F = 3.75$, 0.689, and 0.0919, respectively; $P > .05$).

No mortality of nestling or adult guillemots occurred during anesthesia or surgery and all birds were returned to their nest cavity or released. Upon release, 6 (25%) adults swam away and 16 (67%) flew (release events not recorded for 2 birds). Whether a bird swam or flew upon release bore no relation to transportation and presurgical holding time, total time from capture to release, anesthesia time, surgery time, or time between extubation and release ($Z = 0.308$, 0.366, 0.516, 0.258, and 1.87, respectively; $P > .05$). Those that swam typically dove for extended periods while distancing themselves from the shore. After a substantial distance was obtained, both swimmers and fliers would rest on the water and preen. All releases were judged satisfactory with the exception of 1 bird that swam away with a head tilt and frequent head shaking. This bird had a slow recovery from anesthesia, and during surgery was noted to have very little subcutaneous and coelomic fat and a prominent keel, indicating poor body condition. All adults that underwent surgery abandoned their nests.

Nest sites of biopsied chicks were revisited after surgery and peak body weights and fledging body weights were determined for as many chicks as possible. Some chicks were killed by mink (*Mustela vison*) or fledged before the nest sites were revisited, preventing additional weight measurements. At Jackpot Island/Icy Bay, the prefledging weight (last recorded weight) for the biopsied chicks was 490 ± 44 g (n = 12). The prefledging weight for the Naked Island biopsied chicks was 403 ± 29 g (n = 6). Thirteen (93%) of 14 biopsied chicks from Jackpot Island/Icy Bay and 8 (67%) of 12 biopsied chicks from Naked Island were confirmed to fledge. The fledging fate of 1 Jackpot Island chick was not confirmed; however, its biopsied older sibling successfully fledged from this nest. Four Naked Island chicks from 3 nest cavities were removed by predators before fledging. After fledging, no biopsied chicks were resighted.

Two adult birds in the Naked Island group were resighted in June–July 1999 after the liver biopsy surgery, including 1 on the nest the day after surgery (this nest was abandoned on the following day). During the breeding season the following year (June 2000), a marked difference was found between the numbers of biopsied adult birds resighted at Icy Bay compared to Naked Island. Ten (91%) of 11 biopsied adults at Icy Bay were confirmed to be alive and nesting the following year, compared to only 2 (15%) of 13 biopsied adults from the Naked Island group. At Icy Bay, 5 (83%) of 6 nests from which adults were captured for surgeries in 1999 were active again in 2000. At all of these nests, both adults had been caught and biopsied the previous year and all 10 birds were nesting with the same mates again in 2000. Seven of 10 birds were recaptured and weighed, whereas the remaining 3 birds were simply resighted and identified from their leg markers. No significant difference was found in the birds' body weights between years (1999: 473 ± 24 g; 2000: 476 ± 25 g). All 7 nests of the biopsied birds (5 at Icy Bay and 2 at Naked Island) contained 2 eggs, the maximum clutch size. At Naked Island, only 3 (30%) of 10 nests from which adults were captured for biopsies in 1999 were active in 2000. At 2 nest sites in which both adults had been biopsied in 1999, single adults were recaptured and identified in 2000. At 1 of these active nests, the mate of the biopsied bird also was captured, but was unbanded, indicating that the surviving biopsied bird had paired with a different adult. The mate at the second active nest was not captured or observed. An unbanded dead adult (killed by mink) was found at a third nest site from which an adult biopsied bird had been captured the previous year, but its mate was not observed. A cold egg was present and the nest had been abandoned. The overall pigeon guillemot nesting activity at Naked Island was markedly reduced in 2000, including previously active nest sites in which no adults had been captured for surgery in 1999.

Discussion

Adult birds were more likely than chicks to require assisted ventilation, even though they were generally maintained at lower concentrations of isoflurane. Anesthesia times were longer for adults than for chicks. This difference was explained by longer surgery times and additional processing under anesthesia for adults, and by different anesthesia end points used each year (eg, anesthesia termination was recorded at the time of extubation in adults compared to the time at which isoflurane was discontinued, but before extubation in chicks). The mean time from anesthesia induction to respiratory depression or arrest (18.7 ± 5.0 minutes) for adults was shorter than even the shortest isoflurane time of the chicks (19 minutes), indicating that if chicks were similarly affected by isoflurane they would have required assisted ventilation more frequently. The respiratory depression or arrest and bradycardia observed in some isoflurane-anesthetized adults may be related to dive physiology. Apnea is one of the most common complications of anesthesia in phocid seals, and this has been attributed to their ability to sustain prolonged periods of apnea with associated cardiovasculature changes during diving.[10] Bradycardia is a component of the classical dive response and has been documented in diving ducks,[11,12] although this may be more a feature of forced submergence than of voluntary diving.[13] Adult Pekin ducks anesthetized with halothane (3.5% induction, 2.0–2.5% maintenance) or isoflurane (3.5% induction, 2.0–3.0% maintenance) for 75 minutes experienced markedly increased heart rates, in contrast to our results for pigeon guillemots, although respiratory depression (but not apnea) was a consistent feature.[14]

Five (19%) of the chicks regurgitated whole or partial fish meals during or after surgery. We could not determine if the chicks had been recently fed and had food in their proventriculus before anesthesia and surgery. We chose not to delay the start of anesthesia to allow for potential emptying of their proventriculus, because we were concerned about keeping them from their nests any longer than was necessary. The potential risks of regurgitation during anesthesia and surgery included tracheal aspiration; however, the endotracheal tube within the trachea and head position lower than the body helped reduce this risk.[15] We were not aware of any aspiration complications in these chicks. The adults were all captured while incubating eggs, and were less likely to have eaten recently unless there had been a mate switch at the nest before capture.

The shorter surgery times for chicks compared to adults was related to the ease of locating and accessing the liver in the chicks, which usually extended caudal to the xyphoid process of the sternum. The distance between the xyphoid process and pubic bones was longer in chicks than in adults, as the sternum was still developing and the coelomic organs were comparatively larger in the young, rapidly developing birds.

The adult birds, but not the chicks, had a significant decrease in cloacal temperature from the beginning to completion of anesthesia. However, the chicks' cloacal temperatures were lower than the adults at the start of anesthesia. The adult birds' mean cloacal temperature at the start of anesthesia (40.0 ± 0.8°C) was comparable to published incubating adult guillemot body temperatures of

40.6°C.[8] Guillemot chicks are able to regulate their body temperature within a week after hatching.[8] However, once the chicks were removed from the nest cavity and transported via boat to the field camp, the chicks may have lost more heat compared to the adults because of their underdeveloped plumage. Once anesthesia was induced, it is likely that the chicks lost less heat compared to the adults because of the shorter anesthesia and surgery times. Adult surgeries were performed approximately 3 weeks earlier in the summer than chick surgeries and it is possible that the cooler ambient temperatures in mid-June versus mid-July could have influenced intraoperative body temperature loss in the adults. Environmental temperatures were not recorded during the surgeries to document this possibility.

We were concerned that the adults would abandon their nests if the biopsied chicks were gone for an extended period of time, so every effort was made to minimize the total time away from the nests for the chicks. We expected the adult birds to abandon the nest after adult guillemot surgery, so the elapsed time from capture to surgery was less critical, but still kept as short as possible to minimize the stress of captivity on the birds. Both chicks and adults were kept in a quiet, dark transport carrier before and after surgery to minimize stress.

No antibiotics were used in these birds. The surgeries were done in a sterile manner to prevent iatrogenic introduction of pathogenic bacteria into the coelomic cavity. Because the birds were returned to the nest cavity or released within an hour of surgery, there would not have been an opportunity to give more than a single dose of antibiotics, which would not confer adequate antimicrobial protection.[16] Likewise, no analgesic agents were used in these birds. Very little avian research has been conducted to determine efficacy and safety of postsurgical analgesics.[17,18] We were concerned about the safety of using analgesics with seabirds such as guillemots (particularly chicks) because no research has been conducted to assess their effects on these birds. We were also concerned about possible sedative effects of analgesics in adults that could have made them more vulnerable to injury or predation after release.

Because it was not possible to relocate biopsied chicks after fledging, postfledging survival comparisons could not be made between the biopsied chicks at the 2 locations, or between chicks and adults. The lower chick survival to fledging at the Naked Island site was the result of predation and we assumed it was not caused by any postsurgical complications. The biopsied chick survival to fledging could not be fairly compared to the overall chick survival of the nonbiopsied birds, because only chicks that were at least 18 days of age were included in the surgery groups. A high rate of predation on guillemot nests occurred at Naked Island during the summer of 1998, and many chicks were killed before starting the surgery study. The biopsied chicks' prefledging weights at each site were comparable to the nonbiopsied chicks' prefledging weights at each site in 1998.[4] The higher body weights for Jackpot Island/Icy Bay chicks compared to Naked Island chicks was noted in the nonbiopsied chicks as well.[4] It is unlikely that the small sample of liver that was removed (approximately 0.05–0.1 g) compromised the health of the biopsied chicks or adults. One study involving partial hepatectomies in galahs (*Elophus roseicapillus*) showed that complete regeneration of hepatic tissue occurred within 7 days of surgical removal of 6% of the liver.[19]

The confirmed 91% overwinter resighting rate in adult biopsied birds at the nonoiled site (Icy Bay) was excellent, compared to only 15% confirmed overwinter resighting rate at the oiled site (Naked Island). The 2-egg clutch size (maximal) in the 7 observed nests at the 2 locations suggested that the adult biopsied birds were in good condition the year after surgery. Clutch size for pigeon guillemots can vary with the location and year, but averages 1.8 eggs per nest.[20] The specific cause for the lower number of biopsied birds resighted at Naked Island in 2000 was not determined. High hepatic CYP1A enzyme activities in the Naked Island adults possibly indicated hepatic impairment that was associated with reduced survival immediately after the surgical procedure, or that CYP1A-associated hepatic impairment had an adverse effect on long-term survival.[3] Although the CYP1A enzyme activities were relatively low, it is not known what effects chronic or repeated exposure to low-level hydrocarbons has on long-term survival. If the surgical procedure was associated with postsurgical complications (eg, anorexia and weight loss, infections, hemorrhage, increased susceptibility to predation, and so on), one would expect equally poor survival at both study sites, which was not observed. No studies have been done in this guillemot population to assess adult survival rates in the oiled versus nonoiled areas.

Adult nesting guillemots typically reuse the same nest cavity year after year, but there can be competition for limited nest sites.[8] Unless the biopsied birds renested in the same nest cavity or general area, it would have been difficult to confirm 1-year survival in these birds, because only active nest sites from the previous year were checked in 2000. The use of leg bands to mark the biopsied birds made it difficult to reidentify them unless they were caught at a nest site or observed flying to or from a nest. Identifying these marked birds while they were on the water or roosting on rocks is difficult and was not attempted. The reason(s) for the low nesting rates on Naked Island in 2000 were not determined in the short field visit that year. The low return rate of adult biopsied birds at Naked Island may have been caused by higher instances of lack of breeding at this site. Nesting activity was reduced at most of the sites historically used, including those active sites that did not have adults captured the previous year. Possible explanations could include long-term oil effects, increased mink predation, weather or other environmental factors, reduced prey fish availability, or a combination of these.[4]

Conclusions

The use of inhalation anesthesia in these diving birds presented many challenges to experienced veterinarians. The 100% survival of nestling and adult pigeon guillemots during anesthesia and surgery, combined with very high chick fledging success and high adult overwinter resighting rates, demonstrated that these field techniques can be effectively used to collect important samples from birds with minimal long-term impact on their health. The field anesthesia and surgery techniques used in this study have applicability to a wide variety of aquatic and other avian species, particularly for projects involving nonlethal hepatic tissue sampling for assessing crude oil exposure. We demonstrated that this abdominal surgery can be performed in deep-diving birds (up to 45 m)[21] without apparent adverse effects. These results provide a strong argument for surgeries to be performed by experienced veterinarians to permit collection of liver samples without sacrificing avian species potentially affected by crude oil spills.

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