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Abstract: Two pilot trials and one study in a closely related grebe species suggest that Western grebes (*Aechmophorus occidentalis*) will not tolerate intracoelomic transmitter implantation with percutaneous antennae and often die within days of surgery. Wild Western grebes ($n = 21$) were captured to evaluate a modified surgical technique. Seven birds were surgically implanted with intracoelomic transmitters with percutaneous antennae by using the modified technique (transmitter group), 7 received the same surgery without transmitter implantation (celiotomy group), and 7 served as controls (only undergoing anesthesia). Modifications included laterally offsetting the body wall incision from the skin incision, application of absorbable cyanoacrylate tissue glue to the subcutaneous space between the body wall and skin incisions, application of a waterproof sealant to the skin incision after suture closure, and application of a piece of porcine small intestine submucosa to the antenna egress. Survival did not differ among the 3 groups with 7 of 7 control, 6 of 7 celiotomy, and 6 of 7 transmitter birds surviving the 9-day study. Experimental birds were euthanized at the end of the study, and postmortem findings indicated normal healing. Significant differences in plasma chemistry or immune function were not detected among the 3 groups, and only minor differences were detected in red blood cell indices and plasma proteins. After surgery, the birds in the transmitter group spent more time preening tail feathers than those in the control and celiotomy groups. These results demonstrate that, in a captive situation, celiotomy and intracoelomic transmitter implantation caused minimal detectable homeostatic disturbance in this species and that Western grebes can survive implantation of intracoelomic transmitters with percutaneous antennae. It remains to be determined what potential this modified surgical procedure has to improve postoperative survival of Western grebes that are intracoelomically implanted with transmitters with percutaneous antennae and released into the wild.

Key words: *Aechmophorus occidentalis*, celiotomy, implantation, telemetry, transmitter, Western grebe.

INTRODUCTION

Western grebes (*Aechmophorus occidentalis*), one of the marine bird species most often affected by oil spills in California^{9,13,19} (Massey and Ziccardi, unpubl. data) are believed to be in decline on the West coast of the United States.¹⁴ A safe and effective technique for tracking this species would enable biologists to link important Western grebe

winter (marine) and summer (freshwater) habitats. In addition, it would provide a tool for long-term postrelease monitoring of birds after rehabilitation from oiling.

Attachment of external transmitters to some species of diving birds has been met with limited success. The use of harnesses to attach transmitters to Barrow's goldeneyes (*Bucephala islandica*) significantly decreased the time spent feeding in favor of increased preening, and no birds were subsequently resighted when compared with 66% of those without transmitters.²⁶ Adélie penguins (*Pygoscelis adeliae*) with externally attached devices range a shorter distance, swim slower, and dive less deeply and less efficiently than those without attached devices.²⁷ Harnesses and other external attachment techniques also have been reported to have adverse behavioral and demographic effects on implanted birds.^{6,33} Implanting transmitters into the coeloms of birds has circumvented some of the problems with external attachment techniques,^{17,23} however, the technique has not worked for every species.^{11,20} In addition, a percutaneous transmitter antenna is required for satellite trans-

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mitters and enhances signal output of VHF transmitters.

Unfortunately, although established techniques are used successfully in many seaduck species,²⁴ early field trials suggest that Western grebes do not tolerate implantation of intracoelomic transmitters with percutaneous antennas. In 2004, 2 of us (DN, JE) and a surgeon experienced with the technique implanted 10 Western grebes with satellite transmitters by using the procedure of Korschgen et al.¹⁷ with the following minor modifications: the transmitters were sterilized with hydrogen peroxide gas plasma, not chlorhexidine diacetate solution; the core body temperature was maintained with a heating pad, not a warm water bottle; no feathers were removed from either incision site; and the organs near the implant were not bathed with potassium penicillin. The birds were determined to be healthy before surgery based on physical examination and were released several hours after surgery. All died after release, with a median postoperative survival time of 4 days (range, 1–29 days). After an oil spill in Ventura, California (USA) in 2005, 3 of the authors (DM, JM, and MZ), all the surgeons experienced with the same technique, implanted intracoelomic satellite transmitters in oiled Western grebes that had been rehabilitated. Healthy birds were selected as surgical candidates based on their physical condition, behavior, complete blood cell count, and plasma chemistry. They were housed in captivity after surgery and monitored. Implanted grebes experienced problems with waterproofing at the incision and antenna egresses, developed coelomitis, and 3 of 4 died or had to be euthanized within 1 wk of implantation. In a project that evaluated the fall migration of closely related eared grebes (*Podiceps nigricollis*), Boyd and Schneider⁴ relocated only 1 of 17 eared grebes implanted with transmitters that contained an external percutaneous antenna; whereas, they relocated 12 of 25 birds implanted with transmitters that contained internal antennas. Boyd and Schneider⁴ hypothesized that low detection and return rates for external antennae transmitters were probably because eared grebes are sensitive to foreign objects that protruded from their bodies. The failure in these studies that involved apparently healthy wild grebes suggests that physiologic features unique to the Podicipedidae family could be the source of this problem.

Of the birds implanted and immediately released in the 2004 trial, none was recovered for postmortem evaluation. Postmortem examination of rehabilitated birds from the 2005 Ventura Oil

Spill implanted with intracoelomic transmitters had transmitters fully encased with fibrous scar tissue. This finding suggests that the grebe foreign-body response is adequate to accept implanted transmitters, because such a response is expected after intracoelomic transmitter implantation.^{17,21} Continuous preening, lack of diving, and inadequate waterproofing at the antennae exit site was evidenced by matted feathers and a lack of water beading on feathers in transmitter-implanted Western grebes in the 2005 surgeries. These observations suggested an inadequate skin-to-antennae seal and a lack of waterproofing as possible causes of implantation failure in this species. An alternative hypothesis to explain transmitter implantation failure could be stress-related immune response (cellular or humoral), which reduces wound healing or potentiates secondary bacterial infection.³¹

Because of the need for a successful means to track grebes and their apparent intolerance for implanted transmitters when using the standard methodology, this current study evaluated the short-term clinical and pathologic changes caused by a modification to the surgical technique of Korschgen et al.¹⁷ in an effort to improve immediate postoperative survival of Western grebes and to determine the factors associated with grebes' intolerance to implanted transmitters.

MATERIALS AND METHODS

Capture

Western grebes ($n = 24$; 14 female, 10 male) wintering in Puget Sound, Washington (USA) were captured over a 2-day period in February 2007 by using a modified neutrally buoyant gill net technique.⁵ They were individually identified with a plastic leg band (TabBand, Phoenix, Arizona 85043, USA) and a passive transponder tag (AVID, Folsom, Louisiana 70437, USA) implanted in the left pectoral muscle. Body weights were obtained at the time of capture (average, 1,288 g; range, 1,000–1,604 g) and daily thereafter. All the birds were given an oral multivitamin supplement that contained thiamine (Sea Tabs, Pacific Research Laboratories, San Diego, California 92152, USA) at the time of capture and every other day thereafter. The captured birds were temporarily housed on elevated, netted platforms within plastic transport crates, and were provided oral fluids and nutritional supplementation per the guidelines for the care of oil-affected birds.²² To prevent the aspergillosis infection that is often associated with

Table 1. Analytes measured in different hematologic tests.

Test	Analytes measured
Complete blood cell count	Packed cell volume
	Total granulocyte count
	Total white blood cell count
	Basophil number and percentage of total
	Eosinophil number and percentage of total
	Heterophil number and percentage of total
	Lymphocyte number and percentage of total
Plasma chemistry	Monocyte number and percentage of total
	Alanine aminotransferase
	Amylase
	Aspartate aminotransferase
	Blood urea nitrogen
	Calcium
	Cholesterol
	Creatine phosphokinase
	Creatinine
	Fibrinogen (determined by using the heat precipitation method)
	γ -Glutamyl transpeptidase
	Glucose
	Lactate dehydrogenase
	Lipase
	Phosphorus
	Potassium
	Sodium
	Total carbon dioxide
	Triglycerides
	Uric acid
Plasma protein electrophoresis	Total protein
	Pre-albumin amount and percentage of total protein
	Albumin amount and percentage
	α -1 Globulin amount and percentage
	α -2 Globulin amount and percentage
	γ -Globulin amount and percentage

housing grebes in captivity, the grebes were administered prophylactic itraconazole (25 mg p.o., suspension, Janssen Pharmaceutica N.V., Beerse, B-2340, Belgium) at the time of capture and once daily for the duration of the study.

Care and housing

Within a day of capture, all the birds were flown by a commercial carrier to a research facility (School of Veterinary Medicine, Davis, California) where they were housed indoors in 3-m-diameter freshwater rehabilitation pools. The water was maintained at 16°C and depth at a minimum of 1.3 m to allow birds to swim, dive, and forage. Air temperature was maintained at 18°C. An ad libitum diet of whole, previously frozen, and thawed fish was provided in wire baskets mounted at the water surface on the edge of the pool. Twice daily, fish were tossed to birds to stimulate feeding, and twice daily the birds

were force-fed 3 to 4 whole fish. A veterinarian evaluated the birds for physical well-being daily.

Clinical pathology and immunology

Once, between 1.6 and 4.2 hr after capture, and again on postoperative days 1, 3, 5, 7, and 9, the birds were bled via jugular venipuncture (25-gauge, 5/8-inch needle; and 3-ml syringe) for evaluation of blood parameters. These analyses included complete blood cell counts (manually performed), plasma chemistries, plasma protein electrophoresis profiles, and corticosterone concentrations (Table 1). To evaluate the humoral immune response, the birds were inoculated with 0.1 ml i.v. of a 1% suspension of sheep red blood cells on the day of surgery, and antibody response was measured every other day thereafter.² The sheep red blood cell antibody titers were determined from each plasma sample according to the method described by Wegmann and Smithies.³²

Antibody titers were expressed as \log^2 of the reciprocal of the highest dilution that gives visible agglutination.

The T-cell-mediated immune response was assessed by phytohemagglutinin (PHA) skin test.³ One day after surgery, each bird was injected with 0.1 ml of 5 mg/ml of PHA (Sigma L8754, Sigma-Aldrich, St. Louis, Missouri 63103, USA) intradermally in the left wing web. A similar volume of phosphate buffered saline solution was injected into the right wing web. The cell-mediated immune response was calculated as the difference in wing web swelling between the mitogen-injected and control site on each bird 24 hr after injection.³

Surgery

Before surgery, the birds were assigned to 1 of 3 treatment groups (control, celiotomy, or transmitter group) by using block randomization. On the day of surgery (2–3 days after capture), all the birds were mask induced with isoflurane (Forane®, Baxter Healthcare Corporation, Deerfield, Illinois 60015, USA), intubated, and maintained on isoflurane with 100% oxygen. The birds in the celiotomy and transmitter groups were masked with isoflurane anesthesia, intubated, and maintained under anesthesia for the duration of the surgical procedure; whereas, control birds were anesthetized, intubated, and maintained under anesthesia for 15 min. The birds in the transmitter group were implanted with transmitters with external percutaneous whip antennas (26 g, PTT-100, Microwave Telemetry Inc., Columbia, Maryland 21045, USA) by using modifications to a previously described procedure, as follows.¹⁷ Feathers were parted over the incision site and held aside by using a gel liquid bandage (Facilitator®, Blue Ridge Pharmacy, Raleigh, North Carolina 27607, USA). The skin was disinfected with iodophor solution (Povidone-Iodine Swabstick, Dynarex Corporation, Orangeburg, New York 10962, USA). A ventral midline abdominal skin incision was made with a scalpel blade. The subcutaneous space between the skin and muscle was bluntly dissected and the abdominal musculature incised approximately 1-cm lateral to the midline skin incision. The transmitter was implanted intracoelomically caudal to the liver and adjacent to the right wall of the coelomic cavity after manually rupturing the abdominal and caudal thoracic air sacs.¹⁸ A 1-cm² piece of porcine small intestine submucosa (SIS) (Vet BioSIS, Smiths Medical North American, Waukesha, Wisconsin 53186, USA) was placed over the base

of the transmitter antenna, with the antennae puncturing the SIS to help seal the antenna exit site. The body wall was closed with simple interrupted absorbable sutures (3-0 Vicryl) (Ethicon, Inc., Piscataway, New Jersey 08854, USA). Absorbable methoxypropyl cyanoacrylate (Tissuebond II®, Veterinary Products Laboratory, Phoenix, Arizona 85013, USA) was placed between the body wall and the skin incision, and the skin was closed with the same absorbable suture material by using a simple interrupted pattern. Finally, a liquid bandage (New-Skin Liquid Bandage, Medtech, Jackson, Wyoming 83001, USA) was painted over the skin incision site. Additional modifications to the Korschgen et al.¹⁷ implantation procedure included the following: transmitters were sterilized with hydrogen peroxide gas plasma (STERRAD®, Advanced Sterilization Products, Irvine, California 92618, USA), not chlorhexidine diacetate solution; core body temperature was maintained with a heating pad, not a warm water bottle; no feathers were removed from either incision site; and organs near the implant were not bathed with potassium penicillin. The celiotomy group underwent the same procedure as the transmitter group, including manual rupture of the air sacs, except the transmitter was not implanted. The birds in all 3 treatment groups received meloxicam (0.75 mg p.o., s.i.d.; range, 0.5–0.8 mg/kg) (Boehringer Ingelheim, St. Joseph, Missouri 64506, USA) on days 1 and 2 after surgery. The study terminated on postoperative day 9 after the birds were examined and blood samples collected. Except for 5 control birds that were released, the birds were euthanized, and a complete necropsy with histopathology was performed.

Behavioral observations

To facilitate identification, while the birds were anesthetized, a uniquely colored and patterned ribbon was attached to the skin of each bird's crown by using a single simple interrupted suture (3-0 Prolene) (Ethicon, Inc., Cincinnati, Ohio 54242, USA). In addition, the birds in the celiotomy and transmitter groups were marked on the white feathers of the neck with permanent colored ink (Sharpie®, Sanford, LP, Oakbrook, Illinois 60523, USA). Control birds, scheduled to be released at the end of the study, were not marked with permanent ink.

The birds were videotaped daily for 30 min between the hours of 1030 and 1140, then again for another 30-min session between the hours of 1420 and 1550 by using mounted digital video

Table 2. Behavioral ethogram used to categorize behaviors of Western grebes while in captivity.

Behavior	Description
Awake float	Floating without performing any of the other behaviors
Breech	Lift body off water surface and ruffle feathers or flap wings
Bump	Make contact with another bird; either bird may initiate contact
Charge	Elongate neck and quickly swim toward another bird
Dive	Elongate neck under water and submerge body completely
Drink	Put bill beneath water surface or scoop water into bill
Feed float	Float with fish visible in bill
Out of sight	Bird not visible on video recording
Preen other	Run bill through feathers or rub head on feathers anywhere except tail
Preen tail	Run bill through feathers at base of tail
Rest float	Float with neck flexed and head resting on back
Swim forward	Swim more than a body length without being charged

cameras. The first 5 min of each recording session was discarded because of the disruption caused by the experimenter leaving the room after manually turning on the cameras. Behaviors were analyzed from video recordings by using focal animal sampling. The behavior of each individual was observed daily for 5 min, both in the morning and the afternoon. Behavioral occurrences (Table 2) during each 5-min session were recorded by using an event recorder.¹ Behaviors were mutually exclusive and coded as either events or states. The observer (LG) was not masked during coding because transmitter antennae were clearly visible in the treatment group and the control group lacked ink marks on their necks.

Thermography

By using a FLIR ThermaCAM S65 infrared camera (FLIR Systems, Inc., Wilsonville, Oregon 97070, USA), each bird's ventral body was photographed on the day of surgery after recovery from anesthesia and after having spent time in a conditioning pool. This procedure was repeated on day 9 after surgery. The birds were netted from the pool, wrapped in a towel, and walked to the camera station. The handler uncovered the ventral body and presented it to the camera so that both infrared and visual spectrum digital images could be recorded. Associated software (ThermaCAM™ Researcher Version 2.8, FLIR Systems AB, Danderyd, 182 36, Sweden) was used to measure the highest visible temperature in each thermogram. Temperature was measured 3 times for each image, and the mean was used for statistical analysis. Core body temperature measured via cloacal probe was taken just after thermography on the day of surgery and on days 3 and 9 after surgery.

Statistical analyses

Descriptive statistics (means and standard deviations [SD]) were calculated for total bird weight, cloacal temperature, thermographic temperature readings at the incision site, hematologic and biochemical parameters, serum electrophoresis, plasma corticosterone concentrations, and sheep red blood cell antibody response by group and by day of collection (when applicable). Statistically significant differences among the groups for the different sampling times were evaluated by using a variety of methods. Individual differences among preoperative blood analytes, sheep red blood cell response, PHA reaction, and the thermographic readings for the 2 sampling days were determined by using 1-way analysis of variance for those traits that satisfied the appropriate assumptions (normality tested by Shapiro-Wilk *W*, and homogeneity of variance as assessed by the Levene Statistic) or nonparametric Kruskal-Wallis analysis of variance methods for those that did not. Subsequent pairwise comparisons were made by using Tukey analyses or Mann-Whitney *U* methods, respectively. Repeated measures analysis of variance techniques were used to determine within-factor (time) and between-factor (treatment group) changes in all other parameters after surgery (postoperative days 1, 3, 5, 7, and 9) for those analytes in which data sets were sufficiently robust to allow for assessment. The assumption of sphericity was tested for each repeated measures analyte, and, for those that failed to meet this assumption, the Greenhouse-Geisser epsilon factor was used to adjust the subsequent *P*-value. Pairwise comparisons were made by using the Bonferroni adjustment for multiple comparisons. The results were considered statistically significant at a *P*-value of 0.05 or less. All statistical analyses were accom-

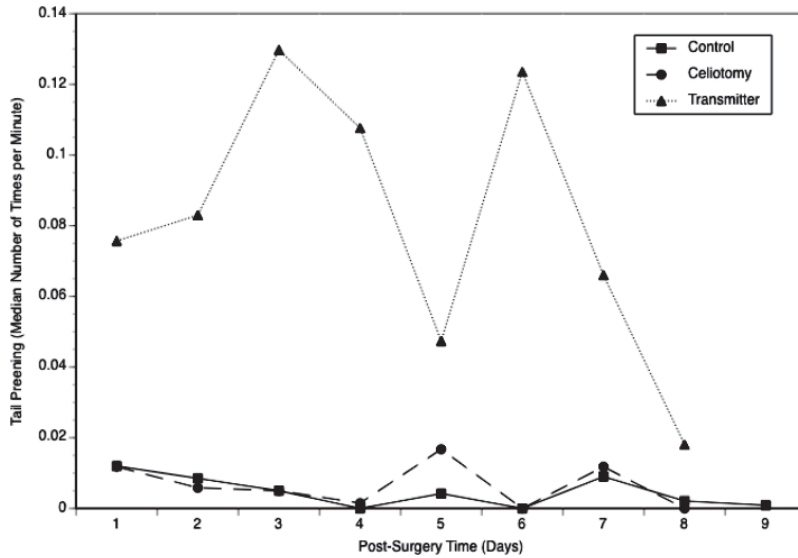


Figure 1. The median number of times per minute that Western grebes were seen preening tail features on days 1, 3, 5, 6, and 9 after intracoelomic implantation of radiotransmitters.

published by using appropriate software (SPSS 17.0, SPSS, Chicago, Illinois 60606, USA).

RESULTS

Three of the 24 birds transported from Washington State to California died within 48 hr of transport and were necropsied; they did not have signs of infectious diseases or other problems that were relevant for the other birds or the study. The remaining 21 birds were assigned treatment groups by using block randomization ($n = 7$ per group). One bird from the transmitter group did not recover from anesthesia. Necropsy revealed that, although considered healthy on physical examination, it was a poor surgical candidate because of renal tubular necrosis and hepatitis. One bird from the celiotomy group died 4 days after surgery, and necropsy revealed a severe bacterial pneumonia and air sacculitis considered likely related to surgery. All of the other birds ($n = 19$) survived until the study was terminated 9 days after surgery. The 5 control birds that were not euthanized were returned to the capture site in Puget Sound, permanently banded, and released. The control birds were anesthetized for an average of 18 min. The birds in the celiotomy and transmitter groups were maintained under isoflurane anesthesia for an average of 32 min and 42 min, respectively.

During the 9 days during which the birds were observed after surgery, the median proportion of time that the control birds were awake and

floating was greater than for the transmitter group ($P = 0.026$). The median number of tail preening events per minute was greater for transmitter than celiotomy and control birds, which did not differ ($P < 0.001$) (Fig. 1). Although no other statistically significant differences were found among the groups, overall differences among postoperative days was noted in the median times per minute that the birds were seen bumping other birds ($P = 0.004$), the median number of breeches seen per minute ($P = 0.002$), and the median proportion of time seen swimming ($P = 0.026$).

Complete necropsies performed on day 9 after surgery (6 transmitter birds, 6 celiotomy birds, and 2 control birds) revealed no significant findings or lesions suggestive of disease caused by anesthesia, surgery, or transmitter, or being housed in captivity. Infiltrates of heterophils, macrophages, multinucleated giant cells, and colonies of bacilli were visualized microscopically in the incision site of 4 of 7 celiotomy birds and 5 of 6 surviving birds in the transmitter group. Aerobic bacterial culture of the coelom yielded light growth of bacteria from 1 of 2 control birds, 5 of 7 celiotomy birds, and 4 of 7 transmitter birds. Bacterial species recovered included light growth of pure or mixed populations of *Edwardsiella hoshinae*, *Enterococcus faecium*, *Escherichia coli*, and *Klebsiella pneumoniae*. Histopathology did not reveal evidence of coelomitis because of bacterial infection in any of these birds. On gross examination, all the transmitters were encapsu-

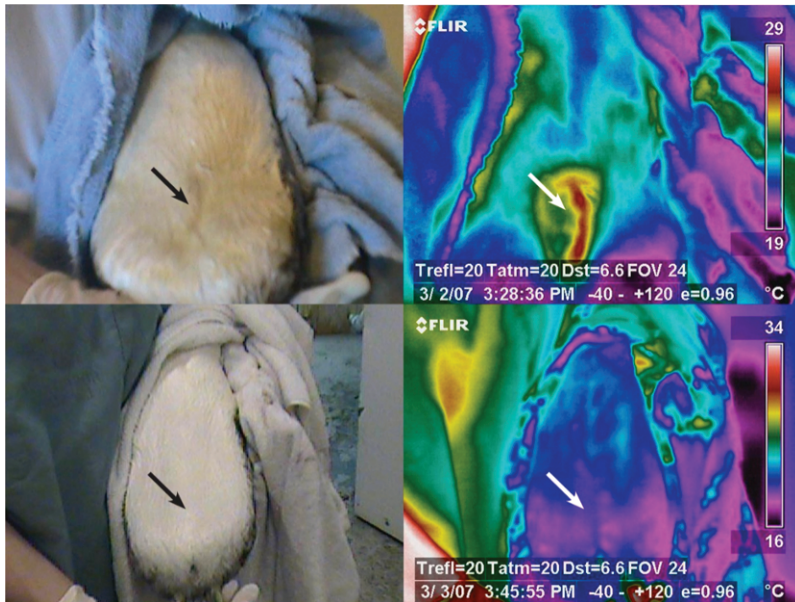


Figure 2. Paired digital photograph (black arrow) and FLIR (white arrow) images of the ventral midline incision on a Western grebe on the day of surgery and on postoperative day 9.

lated in fibrous connective tissue. Fifteen of the 19 birds necropsied (79%) had mild-to-marked lymphoplasmacytic enteritis, often with unidentified cestodes present.

All the birds lost between 5% and 20% (on average, 14%) of their initial body weight between the time of capture and study termination, with average weight loss similar among treatment and control groups. On the day of surgery, the mean (SD) load ratio (transmitter weight (g)/body weight (g) \times 100%) was $2.47 \pm 0.34\%$. Cloacal temperatures decreased over time over the course of the study, with no statistically significant differences among groups. Significant differences ($P = 0.031$) in the FLIR readings were noted immediately after surgery; the control group had a significantly lower visible temperature, which indicated less heat loss in the thermogram ($P = 0.034$) than the transmitter and celiotomy groups ($P = 0.077$). These differences were indistinct by day 9 after surgery ($P = 0.177$). The feather structure that surrounds the incision in the celiotomy and transmitter birds was not waterproof immediately after surgery, and the FLIR images revealed heat loss at the incision site in birds from both groups. This heat loss was not evident on day 9 after surgery (Fig. 2).

Cloacal and FLIR temperatures at the incision site were taken on a subset of 11 birds (3 control, 3 celiotomy, 5 transmitter birds) on the day of surgery after they had been in the freshwater

pool, for a median time of 44 min after recovery and again on day 9 after surgery. A significant negative correlation was present between infrared and cloacal temperatures on the day of surgery ($P = 0.033$) (Fig. 3) but not on day 9 after surgery ($P = 0.11$) (Fig. 3). However, there was no significant difference between cloacal body temperatures for individual birds between the 2 sampling periods ($P = 0.095$).

Retrospectively, mean analyte values from blood taken at the time of capture were analyzed to determine if there were differences among the control, celiotomy, and transmitter groups. Values were similar except for triglycerides ($P = 0.001$), with values significantly higher for the transmitter group than for the celiotomy group ($P < 0.001$).

After surgery, significant differences in mean values throughout the study were detected in only 4 analytes: heterophil percentage ($P = 0.003$), with transmitter birds having lower values than controls ($P = 0.005$); monocyte percentage ($P = 0.004$), with transmitter birds having higher values than controls ($P = 0.004$) and celiotomy birds ($P = 0.028$); albumin percentage of total protein ($P = 0.012$), with controls differing from transmitter birds ($P = 0.012$); and β globulin percentage of total protein ($P = 0.032$), with controls differing from transmitter birds ($P = 0.043$). Differences were not detected among the 3 groups in mean antibody response to sheep red blood cells ($P = 0.698$) or mean PHA response ($P = 0.21$). Further

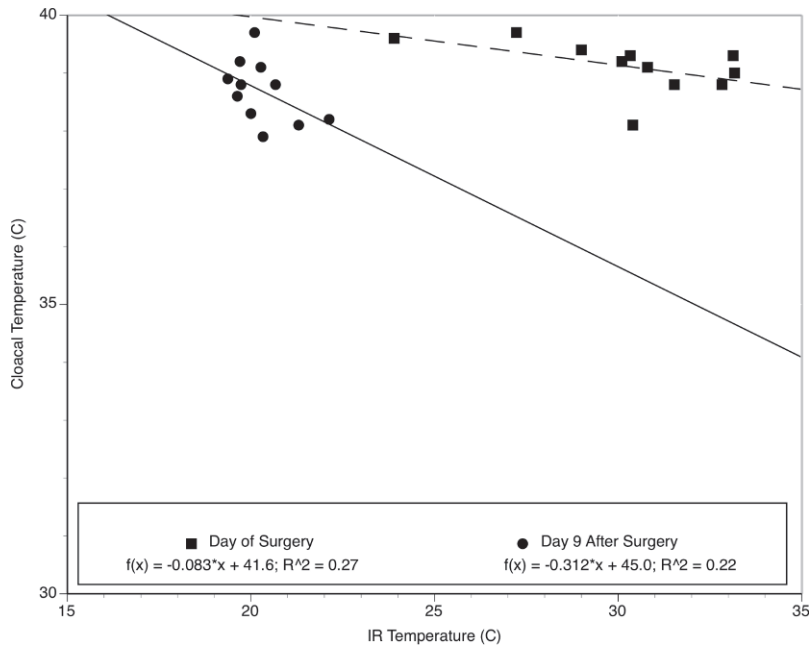


Figure 3. Scatter plot correlation between infrared temperature taken at the incision site and the cloacal temperature taken on a subset of 11 Western grebes (3 control, 3 celiotomy, and 5 transmitter birds) on the day of surgery after birds had been in the freshwater pool for a median time of 44 min after recovery and on day 9 after surgery.

details on blood parameter changes over time will be reported elsewhere.

DISCUSSION

Six of 7 Western grebes implanted with intracoelomic transmitters with percutaneous antennae by using a technique modified from Korschgen et al.¹⁷ survived to 9 days after surgery, by which time most birds had died in the 2 prior pilot trials. These data demonstrate that, at least in a captive situation, Western grebes are capable of being implanted with intracoelomic transmitters with percutaneous antennae and survive. The lack of differences in survival, cellular immunity, and humoral immunity among the 3 treatment groups as well as the lack of differences among groups and over time in 40 of the 44 clinical chemistry analytes measured suggests that celiotomy and intracoelomic transmitter implantation caused minimal detectable homeostatic disturbance in this species.

Constraints of sample size did not permit testing surgical modifications separately nor did they allow inclusion of a treatment group by using the traditional implantation method. Consequently, this captive experiment did not identify which, if any, of the surgical modifications are responsi-

ble for improved postoperative survival. Furthermore, potential differences in environmental conditions, such as food availability and adverse weather conditions, or even oiling and rehabilitation as in the case of 2005, could not be accounted, also making it difficult to compare new surgical modifications with the traditional procedure. These results, however, do demonstrate that surgery and transmitter implantation did not diminish Western grebe's humoral and cellular immunity when compared with controls and that the immune response was adequate to survive anesthesia and surgical implantation of a sterile foreign body. After surgery, no detectable differences in humoral or cellular immunity were detected among the 3 treatment groups, and postmortem necropsies revealed that all transmitters were fully encapsulated in a fibrous connective tissue. Meloxicam was not used as a postoperative analgesic and anti-inflammatory in either prior attempt to implant transmitters in Western grebes, and it is not known if this could somehow have improved the immune systems ability to respond to surgery.

Bacteria were visualized in the incision site from several birds and cultured from the coelomic cavity of others. The presence of inflammatory cells noted microscopically in the healing celiot-

omy incisions and the presence of normal wound healing, however, suggests that, under the conditions provided, this species is able to adequately respond to the small number of bacteria introduced, even when sterile techniques are used or that entered after surgery before wound healing prevented bacterial ingress. Given that the transmitters were placed into the respiratory system (abdominal air sac), it is not surprising that intracoelomic bacteria were present in low numbers. However, in previous work, no bacteria could be cultured from the coeloms of lesser Canada geese (*Branta canadensis parvipes*) sampled 1–2 years after implantations with transmitters and regrowth of air sac membranes (Mulcahy, unpubl. data). Based on long-term survival measured by tracking of implanted birds, disruption of the abdominal air sac has not been problematic in the numerous other species where the Korschgen et al.¹⁷ procedure has been used successfully. The high prevalence of Western grebes (79%) in this study that presented with cestodes and mild-to-marked lymphoplasmacytic enteritis at necropsy is likely not clinically important, because Western grebes are known to host 11 cestode species that are thought to cause little clinical disease.²⁹

In reviewing the results of the 2005 pilot trial, analysis of the data suggests that an inadequate skin-to-antennae seal and a lack of waterproofing are possible reasons why implantation failed. The Korschgen et al.¹⁷ surgical technique was modified in an effort to reduce the loss of waterproofing and to decrease preening. Modifications also were focused on minimizing potential leakage into and out of the coelomic cavity at the incision and antennae sites. Alterations included laterally offsetting the body-wall incision from the skin incision, application of absorbable cyanoacrylate tissue glue to the subcutaneous space between the body wall and skin incisions, application of a waterproof sealant to the skin incision after suture closure, and application of a piece of porcine SIS to the antenna so as to interpose it between the antenna collar and the internal body wall. Porcine SIS has been used in other cases as a xenograft to speed wound healing and form an effective tissue seal at burn sites.¹² By using the modified surgical technique, leakage into the coelomic cavity at the incision and antennae sites was not observed; however, analysis of the behavioral data suggests that rewaterproofing was not rapid at the antennae site and that subsequent increased preening was not eliminated. The birds with transmitters tail preened significantly more frequently than the

control or celiotomy birds, up to a median of 15 times per minute on some days. Conversely, because they were not spending as much time tail preening, the control and celiotomy birds spent a greater proportion of time awake and floating. The lack of increased preening at the abdominal incision site with the possible exception of an increase in breaching immediately after surgery, suggested that the surgical modifications made there were sufficient to enable waterproofing and prevent excessive preening. The 2005 pilot trial demonstrated increased preening of both surgical sites because of seepage of serous fluid from the incision areas. This contaminating fluid caused birds' preening efforts to fail at realigning feathers to form a waterproof barrier and resulted in excessive preening.

Prior failed attempts to implant Western grebes with transmitters could have been because of the surgical technique used or surgically induced behavioral changes such as decreased feeding ability or diving ability that could have increased susceptibility to predation and resulted in mortality. No decrease in feeding or diving behavior was noted in the transmitter group in the current study, although this hypothesis was likely not well tested because of the shallow depth of the pools and the availability of food. In a natural setting, greater attention to preening the tail and the antennae site could increase the risk of predation in transmitter birds. However, during the initial 5 min of the recordings, while the experimenter was manipulating the cameras, it was noted that all the birds moved and dove in unison when observers were present.

Before surgery, only triglycerides were found to be statistically significantly different among the groups. After surgery, heterophil and monocyte percentage of white blood cells and levels of albumin and β globulin were the only statistically significant clinical pathologic differences detected among the transmitter, celiotomy, and control groups. The presurgical triglyceride difference is likely because of a type I error, because a large number of traits were measured and the 3 groups were effectively randomized. Complete white blood cell counts for all 3 groups increased on days 3 and 5 after surgery, as would be expected with a stress leukocytosis.⁷ Compared with controls, the mean heterophil percentage in the transmitter group was significantly depressed on days 3 and 5 after surgery, a trait also observed in the total numbers of heterophils detected, although this was not statistically significant. This pattern is consistent with sequestration of heter-

ophils from an inflammatory response¹⁶ because heterophils form the first line of cellular defense against invading microbial pathogens in the lungs and air sacs, which lack resident macrophages.¹⁰ Conversely, the mean percentage of monocytes was elevated in the transmitter group on days 3 and 5 after surgery compared with celiotomy and control groups, with a similar, though not statistically significant, increase in total number of monocytes seen. The increase in the percentage of monocytes probably reflects inflammation. Monocytosis is thought to occur early in the inflammatory process and was one of the most consistent findings associated with inflammation or trauma in 2 species of black cockatoos, *Calyptorhynchus magnificus* and *Calyptorhynchus funereus*.¹⁵ Based on heterophil and monocyte percentages of white blood cells, a foreign-body response to the transmitter likely peaked between days 3 and 5 after surgery.

Although not well understood, the decreased plasma albumin percentage seen in the transmitter birds could be because of decreased hepatic production, decreased dietary intake, or loss of plasma albumin. More likely, however, the decrease in plasma albumin percentage was from an increase in other proteins, such as the β globulin, which causes a relative decrease in the proportion of albumin in total proteins. The increased β globulin seen in this group, which includes the acute phase proteins fibrinogen and transferrin,⁸ could be because of increased production and is likely in response to inflammation.²⁸

Immediately after surgery, there was a significant negative correlation between cloacal and the FLIR temperatures at the incision site, which show that birds that lost more heat at the incision site had a lower core body temperature. By day 9 after surgery, this correlation was no longer present. No significant group effect in cloacal temperatures in individual birds was measured immediately after surgery, which suggests that the birds were able to maintain their core body temperature, despite losing heat at the incision site. Hypothetically, if the birds were losing heat at the incision site, yet maintaining core body temperature, they were doing this at the expense of increased energy use. The longer that birds lose heat at the incision site and expend energy to maintain core body temperature, the greater potential this has to be a significant contributing factor that impacts postoperative survival, especially when combined with potential decreases in foraging and other synergistic factors. The physiologic cost of compensating for heat loss at the

surgery site could not be calculated from these data but should be considered when determining postoperative release criteria for birds implanted with transmitters. It was not surprising that, with similar survival in all 3 treatment groups, few differences were detected in the plasma chemistry and complete blood cell counts, and none was seen in immune function. Although the birds in all the groups were obviously in a negative energy balance, there were no indications noted that this prevented or reduced wound healing or postsurgical recovery.

The few statistically significant differences that were found among the control, celiotomy and transmitter groups suggest that the actual surgical implantation of transmitters is just one of multiple stressors that wild birds undergo when captured and surgically implanted with transmitters. Consequently, when attempting to maximize survival and minimize stress and the negative impacts of surgery, biologists and veterinarians must work to minimize stressors at every step of the procedure. Minimizing the consequences associated with the capture, handling, and transportation of wild birds is just as important as improving the surgical technique. Although not well described in birds, an interactive effect is likely between stress, thermoregulation, behavior, nutrition, and immunity that conspires against survival after capture, handling, and surgery. Whereas parting of the feathers is an improvement over the plucking of feathers to prepare a surgical site, infrared data demonstrated considerable heat loss at the incision site after surgery because of poor waterproofing. Postoperative preening can increase the attention of predators and possibly increase predation. Although the degree of predation versus scavenging in the 2004 Nysewander and Evenson trial could not be determined, they found transmitters from most of the 10 Western grebes that died after surgery in or near Bald eagle (*Haliaeetus leucocephalus*) perches (snags, pilings, etc.) (Nysewander and Evenson, unpublished data). In addition to being a predation risk, postoperative preening also comes at a metabolic cost. In a captive study on white-winged scoters (*Melanitta fusca*), the metabolic costs of preening were 1.5 to 2 times greater than the costs of resting or swimming.²⁵ Metabolic needs would be exacerbated further by increased heat loss at the incision site because of impaired waterproofing. After surgery, birds also will have higher metabolic needs from healing, which could occur concurrently with decreased alimentation because of decreased foraging. Although not seen

in the current experiment, at some point, the negative energy costs associated with surgical recovery, increased metabolic needs, increased preening, increased thermoregulation, and decreased feeding could reduce immune responses and impair healing. In addition to the surgical modifications described, Western grebes in this study were housed in 16°C water compared with the 8°C water where they were captured. In addition, they were fed and were not required to react to predators. Even so, the birds lost a mean of 14% in body weight over the 12 to 13 days of captivity. This finding could reflect the difficulty of acclimating any wild-caught species to captivity, and Western grebes are reported to be notoriously difficult to keep in captivity.³⁰ Post-mortem and immunologic findings suggest that, even though the birds were in negative energy balance, being handled daily and in a captive situation, these factors were insufficient to impede postsurgical healing or cellular or humoral immune response.

CONCLUSIONS

The traditional Korschgen et al.¹⁷ procedure remains useful in species that tolerate it; however, it is recommended that the modifications presented here be used when implanting intracoelomic transmitters with percutaneous antennae in birds in the Podicipedidae family and birds in other families where difficulties have been demonstrated with implanted transmitters. This captive study was an initial step for evaluating the long-term survival of Western grebes released into the wild after transmitter implantation. Future studies should determine long-term postrelease survival of Western grebes implanted with satellite transmitters with percutaneous antennae by using these modifications.

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LITERATURE CITED

1. Blumstein, D. T., J. C. Daniel, and C. S. Evans. 2006. JWatcher 1.0: An Introductory User's Guide. www.jwatcher.ucla.edu. Accessed 14 December 2009.
2. Boa-Amponsem, K., M. Picard, M. E. Blair, B. Meldrum, and P. B. Siegel. 2006. Memory antibody responses of broiler and leghorn chickens as influenced by dietary vitamin E and route of sheep red blood cell administration. *Poult. Sci.* 85: 173–177.
3. Bourgeon, S., R. Criscuolo, Y. Le Maho, and T. Raclot. 2006. Phytohemagglutinin response and immunoglobulin index decrease during incubation fasting in female common eiders. *Physiol. Biochem. Zool.* 79: 793–800.
4. Boyd, W. S., and S. D. Schneider. 2000. Using radio telemetry to describe the fall migration of eared grebes. *J. Field Ornith.* 71: 702–707.
5. Breault, A. M., and K. M. Cheng. 1990. Use of submerged mist nets to capture diving birds. *J. Field Ornith.* 61: 328–330.
6. Calvo, B., and R. W. Furness. 1992. A review of the use and the effects of marks and devices on birds. *Ring. Migr.* 13: 129–151.
7. Campbell, T. W. 1994. Hematology. *In:* Ritchie B. W., G. J. Harrison, and L. R. Harrison (eds.). *Avian Medicine: Principles and Application*. Wingers Publishing, Lake Worth, Florida. Pp. 176–198.
8. Cray, C., and L. M. Tatum. 1998. Applications of protein electrophoresis in avian diagnostics. *J. Avian Med. Surg.* 12: 4–10.
9. Hampton, S., R. G. Ford, H. R. Carter, C. Abraham, and D. Humple. 2003. Chronic oiling and seabird mortality from the sunken vessel S.S. *Jacob Luckenbach* in Central California. *Mar. Ornith.* 31: 35–41.
10. Harmon, B. 1998. Avian heterophils in inflammation and disease resistance. *Poult. Sci.* 77: 972–977.
11. Hatch, S. A., P. M. Meyers, D. M. Mulcahy, and D. C. Douglas. 2000. Performance of implantable

- satellite transmitters in diving seabirds. *Waterbirds* 23: 84–94.
12. Hernandez-Divers, S. J., and S. M. Hernandez-Divers. 2003. Xenogeneic grafts using porcine small intestinal submucosa in the repair of skin defects in birds. *J. Avian Med. Surg.* 17: 224–234.
 13. Ivey, G. L. 2004. Conservation assessment and management plan for breeding Clark's and Western grebes in California. American Trader Trustee Council Report. 80 pp.
 14. Ivey, G. L. 2005. Conservation assessment of breeding western and Clark's grebes. *Northwestern Naturalist* 86: 101.
 15. Jaensch, S., and P. Clark. 2004. Haematological characteristics of response to inflammation or traumatic injury in two species of black cockatoos: *Calyptorhynchus magnificus* and *C. funereus*. *Comp. Clin. Pathol.* 13: 9–13.
 16. Klasing, K. C. 1991. Avian inflammatory response: mediation by macrophages. *Poult. Sci.* 70: 1176–1186.
 17. Korschgen, C. E., K. P. Kenow, A. Gendron-Fitzpatrick, W. L. Green, and F. J. Dein. 1996. Implanting intra-abdominal radiotransmitters with external whip antennas in ducks. *J. Wildl. Mgmt.* 60: 132–137.
 18. Kurtul, I., K. Aslan, G. Aksoy, and S. Ozcan. 2004. Morphology of the air sacs (*Sacci pneumatici*) in the rock partridge (*Alectoris graeca*). *Vet. Res. Comm.* 28: 553–559.
 19. Luckenbach Trustee Council. 2006. S.S. *Jacob Luckenbach* and associated mystery oil spills final damage assessment and restoration plan/environmental assessment. Prepared by California Department of Fish and Game, National Oceanic and Atmospheric Administration, United States Fish and Wildlife Service, National Park Service, Sacramento, California.
 20. Meyers, P. M., S. A. Hatch, and D. M. Mulcahy. 1998. Effect of implanted satellite transmitters on the nesting behavior of murre. *Condor* 100: 172–174.
 21. Mulcahy, D. M., K. A. Burek, and D. Esler. 2007. Inflammatory reaction to fabric collars from percutaneous antennas attached to intracoelomic radio transmitters implanted in harlequin ducks (*Histrionicus histrionicus*). *J. Avian Med. Surg.* 21: 13–21.
 22. Oiled Wildlife Care Network. 2000. Protocols for the Care of Oil-affected Birds. Davis, CA: Univ. of California, Wildlife Health Center. 80 pp.
 23. Olsen, G. H., F. J. Dein, G. M. Haramis, and D. G. Jorde. 1992. Implanting radio transmitters in wintering canvasbacks. *J. Wildl. Mgmt.* 56: 325–328.
 24. Peterson, M. R., W. W. Larned, and D. C. Douglas. 1999. At-sea distribution of spectacled eiders: a 120-year-old mystery resolved. *Auk* 116: 1009–1020.
 25. Richman, S. E., and J. R. Lovvorn. 2008. Costs of diving by wing and foot propulsion in a sea duck, the white-winged scoter. *J. Comp. Phys. B* 178: 321–332.
 26. Robert, M., B. Drolet, and J.-P. L. Savard. 2006. Effects of backpack radio-transmitters on female Barrow's goldeneyes. *Waterbirds* 29: 115–120.
 27. Ropert-Coudert, Y., R. P. Wilson, K. Yoda, and A. Kato. 2007. Assessing performance constraints in penguins with externally-attached devices. *Mar. Ecol. Progr. Ser.* 33: 281–289.
 28. Rosenthal, K. L. 2000. Avian protein disorders. *In: Fudge, A. M. (ed.). Laboratory Medicine Avian and Exotic Pets.* W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 171–175.
 29. Storer, R. W. 2000. The metazoan parasite fauna of grebes (Aves: Podicipediformes) and its relationship to the bird's biology. *Miscellaneous Publications*, no. 188. Museum of Zoology, Univ. of Michigan, Ann Arbor, Michigan. 90 pp.
 30. Stoskopf, M. K. 2003. Gaviiformes (loons), Podicipediformes (grebes), and Procellariiformes (albatrosses, fulmars, petrels, storm petrels, and shearwaters). *In: Fowler M. E., and R. E. Miller (eds.). Zoo and Wild Animal Medicine*, 5th ed. Saunders Publishing Company, St. Louis, Missouri. Pp. 110–117.
 31. Walburn, J., K. Vedhara, M. Hankins, L. Rixon, and J. Weinman. 2009. Psychological stress and wound healing in humans: a systematic review and meta-analysis. *J. Psychos. Res.* 67: 253–271.
 32. Wegmann, T. G., and O. Smithies. 1966. A simple hemagglutination system requiring small amounts of red blood cells and antibodies. *Transfusion* 6: 67–73.
 33. Withey, J. C., T. D. Bloxton, and J. M. Marzluff. 2001. Effects of tagging and location error in wildlife radiotelemetry studies. *In: Millsaugh, J. J., and J. M. Marzluff (eds.). Radio Tracking and Animal Populations.* Academic Press, San Diego, California. Pp. 43–47.

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