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RESEARCH ARTICLE

Biochemical and clinical responses of Common Eiders to implanted satellite transmitters

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ABSTRACT
Implanted biologging devices, such as satellite-linked platform transmitter terminals (PTTs), have been used widely to delineate populations and identify movement patterns of sea ducks. Although in some cases these ecological studies could reveal transmitter effects on behavior and mortality, experiments conducted under controlled conditions can provide valuable information to understand the influence of implanted tags on health and physiology. We report the clinical, mass, biochemical, and histological responses of captive Common Eiders (*Somateria mollissima*) implanted with PTTs with percutaneous antennas. We trained 6 individuals to dive 4.9 m for their food, allowed them to acclimate to this dive depth, and implanted them with PTTs. We collected data before surgery to establish baselines, and for 3.5 mo after surgery. The first feeding dive took place 22 hr after surgery, with 5 of 6 birds diving to the bottom within 35 hr of surgery. Plumage waterproofing around surgical sites was reduced $\leq$21 days after surgery. Mass; albumin; albumin:globulin ratio; aspartate aminotransferase; β_1-, β_2-, and γ-globulins; creatine kinase; fecal glucocorticoid metabolites; heterophil:lymphocyte ratio; and packed cell volume changed from baseline on one or more of the postsurgery sampling dates, and some changes were still evident 3.5 mo after surgery. Our findings show that Common Eiders physiologically responded for up to 3.5 mo after surgical implantation of a PTT, with the greatest response occurring within the first few weeks of implantation. These responses support the need for postsurgery censor periods for satellite telemetry data and should be considered when designing studies and analyzing information from PTTs in sea ducks.

Keywords: biomarker, platform transmitter terminal, radio telemetry, sea duck, *Somateria mollissima*, transmitter effect

Respuestas bioquímicas y clínicas de *Somateria mollisima* a los implantes de transmisores satelitales

RESUMEN
Los dispositivos de toma de datos biológicos, como los transmisores ligados a satélites (PTTs, por sus siglas en inglés), han sido ampliamente usados para delinear poblaciones e identificar patrones de movimiento en patos marinos. Aunque en algunos casos estos estudios ecológicos pueden revelar los efectos de los transmisores en el comportamiento y la mortalidad, conducir experimentos bajo condiciones controladas puede generar información valiosa para entender la influencia de los implantes en la salud y en la fisiología. En este trabajo reportamos las respuestas clínicas, en la masa, bioquímicas e histológicas de individuos en cautiverio de la especie *Somateria mollissima* con implantes de PTT con antenas bajo la piel. Entrenamos a 6 individuos para bucear a una profundidad de 4.9 m por su comida, permitiéndoles aclimatarse a dicha profundidad, y les pusimos implantes de PTT. Recolectamos datos antes de la cirugía para establecer referencias, y por 3.5 meses después de la cirugía. El primer buceo de alimentación tuvo lugar 22 horas después de la cirugía y 5 de las 6 aves bucearon hasta el fondo menos de 35 horas después de la cirugía. La impermeabilidad del plumaje alrededor de los sitios de la cirugía se redujo hasta 21 días luego de la cirugía. La masa, la albúmina, la relación albúmina:globulina, la aspartato aminotransferasa, las globulinas β_1-, β_2- y γ-, la creatina quinasa, los metabolitos glucocorticoides fecales, la relación anticuerpos:linfocitos, y el volumen celular cambiaron con respecto a la referencia en una o más de las fechas de muestreo posteriores a la cirugía. Algunos de estos cambios aún eran evidentes 3.5 meses luego de la cirugía. Nuestros resultados demuestran que los individuos respondieron fisiológicamente hasta 3.5 meses luego de la implantación quirúrgica de un PTT y que

las respuestas más fuertes ocurrieron en las primeras semanas de la implantación. Estas respuestas apoyan la necesidad de periodos post-quirúrgicos de habituación antes de recolectar datos de telemetría por satélite y deberían ser consideradas al diseñar estudios y analizar información obtenida mediante PTTs en patos marinos.

Palabras clave: marcador biológico, transmisores satelitales, telemetría de radio, pato marino, *Somateria mollissima*, efectos de los transmisores

INTRODUCTION

Understanding the movements of birds has been revolutionized by the use of attached devices such as radio transmitters, platform transmitter terminals (PTTs), and light-level and global positioning system (GPS) loggers. However, concerns remain about instrument effects on behavior, health, and even vital rates, and thus about the validity of information provided by these devices (White et al. 2013). Quantifying and reducing these effects has become increasingly important as technological advances and miniaturization allow for use to address an expanding suite of research questions, even for some of the smallest species (e.g., Ruby-throated Hummingbird [*Archilochus colubris*], <4 g; Zenzal et al. 2014). Yet, despite an increase in the number of studies using tags, there has been no corresponding increase in the documentation of their effects on the birds that carry them (Vandenabeele et al. 2011).

The use of satellite transmitters has provided important information for the conservation and management of many sea duck populations (e.g., Petersen et al. 1995, 2003, 2006, Oppel et al. 2009, Takekawa et al. 2010, Savard and Robert 2013). Assessing the effects of transmitters on sea ducks is more challenging than with many other avian species because of the often harsh and remote environments these birds inhabit. To fill this gap, we examined clinical and physiological metrics of Common Eiders (*Somateria mollissima*) implanted with PTTs with percutaneous antennas to determine whether there are detrimental effects of manipulation and transmitter implantation.

The utility of PTTs in birds has primarily been limited by device weight and attachment technique (Korschgen et al. 1984, Strikwerda et al. 1986, Benvenuti 1993). Transmitter weights have decreased substantially since first fitted on wild birds (Strikwerda et al. 1985), and attachment was generally considered a greater problem for diving birds with potential complications from disruption of insulating body feathers (Perry 1981) and underwater drag (Wilson et al. 1986). In an attempt to overcome harmful effects of external mounts, Korschgen et al. (1984, 1996) developed a surgical technique to implant transmitters into the abdominal cavity of waterfowl. Since its development, the technique has been used to implant PTTs in most North American sea duck species (e.g., Petersen et al. 1995, 2006, Dickson and Smith 2013, Savard and Robert 2013).

Implantation of transmitters with or without a percutaneous antenna is generally preferable to external attachment (e.g., Dzus and Clark 1996, Iverson et al. 2006); however, harmful effects have been described with both attachment techniques. In a meta-analysis of studies comparing internal and external attachment for birds, White et al. (2013) reported that externally attached devices consistently affected body condition, reproduction, metabolism, and survival, whereas effects of internal devices were not consistent. Esler et al. (2000) and Iverson et al. (2006) suggested a postsurgery data censor period to reduce bias caused by short-term transmitter effects. Effects associated with devices attached externally to waterfowl include weight loss, abnormal behavior, infection, decreased return rates, and increased preening (Perry 1981, Pietz et al. 1993, Robert et al. 2006, Enstipp et al. 2015). Responses to implanted devices have been more varied. Korschgen et al. (1984) reported that 2 of 14 captive birds implanted with a radio transmitter without a percutaneous antenna showed signs of infection, whereas wild birds implanted with similar transmitters had no difference in foraging, preening, or resting time compared to controls. Two percent of Canvasbacks (*Aythya valisineria*) implanted with a radio transmitter without a percutaneous antenna died as a result of surgical complications, but postrelease mortality was similar to that expected for the local population (Olsen et al. 1992). Harlequin Ducks (*Histrionicus histrionicus*) implanted with radio transmitters with percutaneous antennas lost more mass than banded controls in the first few weeks after implant, but there was no difference in mass 1 yr later (Esler et al. 2000). No Canada Geese (*Branta canadensis*) implanted with radio transmitters with percutaneous antennas died during surgery, and 1-yr survival was similar to controls, but 2-yr and 3-yr survival were lower than controls (Hupp et al. 2006). Common Eiders implanted with PTTs with percutaneous antennas had lower 1-yr survival (67%) compared to controls (88%; Fast et. al. 2011), but King Eiders (*S. spectabilis*) implanted with simiar devices had high (94%) annual survival (Oppel and Powell 2010). Nest survival, nest-site fidelity, and breeding-site arrival of Black-tailed Godwits (*Limosa limosa*) implanted with PTTs with percutaneous antennas were similar to controls ($n = 41$); but apparent survival, nesting propensity, and egg viability were lower for PTT birds (Hooijmeijer et al. 2014). In sea ducks, presumed or known short-term (~2 wk) mortality ranged from relatively low (3%) for Harlequin Ducks implanted with radio transmitters with percutaneous antennas after anesthetic technique modifications (Mulcahy and Esler 1999) to relatively high (43%) for scoters implanted with PTTs with percutaneous antennas (Rosenberg and Petrula 2000).

Measuring biomarker response is a useful technique to investigate transmitter effects (Kenward 2001) and has been used to determine the responses of pigeons and passerines to transmitters (Gessaman and Nagy 1988, Suedkamp Wells et al. 2003, Schulz et al. 2005). In seabirds, biomarkers have been used to assess how carrying devices affects physiological stress. For example, Common Murres (*Uria aalge*) and Thick-billed Murres (*U. lomvia*) fitted with geologgers had higher baseline corticosterone compared to controls 1 yr after attachment, but survival was not affected (Elliott et al. 2012). Black-legged Kittiwakes (*Rissa tridactyla*) carrying GPS loggers increased their baseline corticosterone levels more than controls during chick rearing, but mass and breeding success were maintained (Heggoy et al. 2015). Also, Hollmén et al. (2001) and Wayland et al. (2002, 2003) found biomarkers a valuable method for assessing physiological condition in Common Eiders.

Our objective was to determine the physiological responses of Common Eiders to intra-abdominal PTTs for 3.5 mo after surgery. We addressed this objective by evaluating mass, biochemical, and histological biomarkers in captive birds before and after implantation of a 38–47 g PTT. We also provide a description of findings from clinical examinations and key behavioral observations during the first few days following surgery.

METHODS

Common Eider eggs salvaged from nests of wild birds from the Yukon–Kuskokwim Delta, Alaska, USA, were hatched at the Alaska SeaLife Center (ASLC) in Seward, Alaska, in 2003. Birds were housed in an outdoor aviary with shallow pools (<1 m deep) and fed Mazuri sea duck pellets (Purina Mills, St. Louis, Missouri, USA), blue mussels (*Mytilus edulis*), and krill (*Euphausia superba*) prior to the experiment.

We conducted our study at the ASLC between September 2005 and March 2006 in an outdoor seawater aquarium, in which we constructed a dive column (1.5 × 1.5 m wide, 4.9 m deep) with an attached terrestrial haul-out (Latty et al. 2010). We chose this time period to minimize seasonal influences of molt and breeding on biomarkers.

Experimental Design

We used 6 Common Eiders (3 male, 3 female; denoted herein as M1, M2, M3, F1, F2, and F3) in our experiment. We chose Common Eiders as our study species because (1) they have been implanted with similar PTTs in field studies (Petersen and Flint 2002), (2) the ratio of transmitter mass to body mass is within the recommended range (<5%; Kenward 2001), and (3) foraging depths in the wild (Guillemette et al. 2004) are consistent with the depth of the dive column in our aquarium. Also, their ecology is broadly similar to that of threatened eiders for which an understanding of transmitter effects is listed as a management goal (K. Laing, U.S. Fish and Wildlife Service, personal communication). In addition to the work described here, we also conducted a complementary study using the same cohort to determine whether implanted PTTs affect Common Eider dive behavior (Latty et al. 2010).

Prior to beginning the experiment, we trained birds to dive to the bottom of the dive column by providing food in a metal tray on the side of the dive column at progressively lower depths. This was necessary because these captive-raised birds had not previously foraged at depths >1 m. Once birds were diving to the bottom, we passed Mazuri sinking waterfowl pellets and blue mussels through a PVC pipe onto an acrylic feeding tray on the floor of the dive column 4 or 5 times daily, thus allowing only benthic foraging. We provided the birds fresh water, ad libitum in a water bowl, throughout the study.

Before implanting transmitters, we allowed 53 days for the birds to acclimate to the greater dive depth, because waterfowl diving to greater depths acclimate to the increased physiological demands (Stephenson et al. 1989). Veterinarians deemed that birds were in good physiological health prior to the study. After surgery, we monitored the activity of birds using video cameras as part of the concurrent study described above. This allowed us to determine the timing of resumption of feeding dives, as well as behavior and kinematics during the dives.

Biomarkers

We used primary biomarkers (albumin:globulin ratio [A:G], creatine kinase [CK], packed cell volume [PCV], and fecal glucocorticoid metabolites [FGMs]) to assess responses of Common Eiders to implanted PTTs. We chose these a priori, on the basis of specific physiological responses we wished to test: A:G for immunological response, CK for muscle damage, PCV for general health, and FGMs for physiological stress. We included secondary biomarkers (aspartate aminotransferase [AST], glucose, heterophil:lymphocyte ratio [H:L], lactate dehydrogenase [LDH], and serum protein fractions [albumin, α_1-, β_1-, β_2-, and γ-globulins]) to further characterize responses and evaluate findings of primary parameters.

We employed A:G as a metric of inflammatory response (Hochleithner 1994, Harris 2000). Albumin is the portion of blood proteins primarily composed of nonimmunological constituents, whereas globulins mainly comprise proteins involved in the response to both specific and nonspecific immunological insults (Harris 2000). Globulin fractions were then used to help characterize the timing and duration of the inflammatory response (Hochleithner 1994). For example, an increase in α_1-globulins may indicate acute inflammatory reactions, whereas an increase in γ-globulins is generally associated with chronic infection or liver disease (Kaneko 1989).

We used CK to evaluate muscle condition because the enzyme is a sensitive and specific indicator of muscle damage in birds (Lumeij et al. 1988a). CK has been used to assess damage caused by a variety of conditions, including capture and handling, extreme exertion, and some medications (Bollinger et al. 1989, Dabbert and Powell 1993, Aktas et al. 1997, Guglielmo et al. 2001). We used AST and LDH to further assess the findings from CK because these parameters can provide supportive evidence of muscle damage (Hochleithner 1994) and respond on a different time scale (Lumeij et al. 1988a, 1988b). For example, in pigeons induced with muscle damage, CK responded most quickly (16 hr) but normalized within 66 hr, whereas AST remained elevated for 6 days (Lumeij et al. 1988b).

Because PCV can be affected by a host of factors (e.g., trauma, parasitism, septicemia, chronic disease, toxicity, nutritional deficiences, dehydration; Dein 1986, Campbell 1995), we used it as a general health index. Also, red blood cells provide significant oxygen storage for diving birds (Keijer and Butler 1982, Stephenson et al. 1989, Butler 1991); therefore, decreased PCV may decrease an eider's ability to keep metabolism primarily aerobic while submerged. We assessed albumin (Hochleithner 1994) and glucose (Harris 2009) to help interpret responses because these biomarkers tend to be less sensitive indicators than PCV and therefore less likely to change in response to minor insults. Albumin generally decreases with disease (Woerpel and Rosskopf 1984); increases are seen with dehydration (Hochleithner 1994). Glucose may increase in response to stress, diabetes mellitus, and renal disease (Woerpel and Rosskopf 1984, Harris 2009). Low blood glucose is rare in birds and usually results from hepatic disease, excessive utilization of glucose, prolonged starvation, and endocrine disease (Woerpel and Rosskopf 1984, Hochleithner 1994, Harris 2009).

We used FGMs to evaluate the stress response. Corticosterone is the primary stress hormone in birds (deRoos 1961), and its metabolites are measurable in feces (Wasser et al. 2000, Ludders et al. 2001, Washburn et al. 2003). FGMs have been shown to increase within 2 hr of an adrenocorticotropic hormone challenge in Harlequin Ducks (Nilsson 2004) and have been used to assess stress caused by externally attached transmitters (Suedkamp Wells et al. 2003). In birds, FGMs may vary with daily and annual rhythms, social status, mass, and condition; as well as with sample age and condition, assay selection, and storage (see Millspaugh and Washburn 2004). Consequently, we used repeated measures to analyze data, handled all samples in the same fashion, collected samples only between 1000 and 1500 hours, and did not include samples from physiologically demanding periods such as molt or breeding. Also, Nilsson (2004) found no change in captive Harlequin Duck blood corticosterone after normal handling associated with blood collection or intramuscular injections of saline. We used H:L to further assess stress responses (Gross and Siegel 1983) because it is an index of chronic stress (Vleck et al. 2000), and we compared patterns with FGMs.

Blood and Fecal Sampling

We captured birds in random order and collected a 2–3 mL blood sample from the right jugular vein of each individual 62 and 29 days prior to surgery, on the day of surgery, and at 2, 8, 14, 21, 28, 56, 91, and 105 days postsurgery between 0800 and 1200 hours. Sessions for capturing and sampling all birds in the aviary averaged 51 min (range: 26–77 min) from capture of the first bird to release of the last. Average time from capture to blood draw was 2 min (range: 1–8 min). We last fed birds at ~1600 hours the day before blood collection, though a small amount of residual food may have been available on the dive column floor from feedings that occurred the day prior (i.e. 16–20 hr before we collected blood). We analyzed blood samples the day of collection or froze serum at –80°C. During these captures, a veterinarian conducted a brief physical exam of the surgical sites.

We collected fecal samples at 3, 2, and 1 days prior to surgery, and 3, 6, 13, 20, 27, 55, and 104 days after implant. We collected all samples between 1000 and 1500 hours with a wooden tongue depressor within 20 min of deposit and froze samples at –20°C within 3 hr of collection. We did not collect fecal samples on days that birds were handled and bled.

To further characterize baseline variability, we also tested for differences using an extended baseline that included an additional 5 serum samples and hematology results obtained from each individual every ~2 mo during the year prior to collecting the baselines mentioned above.

Satellite Transmitters

After providing all food on the bottom of the dive column and collecting baseline samples for ~2 mo, birds were implanted with either a PTT 100 (45.7–47.4 g; Microwave Telemetry, Columbia, Maryland, USA) or a 5130 PTT (38.3–42.7 g; HABIT Research, Victoria, British Columbia), using procedures similar to those of Korschgen et al. (1984, 1996). Birds were 29 mo old at the time of surgery. Approximate transmitter dimensions were 70 × 35 × 15 mm; they were 22.5 mL in volume; and the antenna was 200 mm long and 1.7 mm in diameter. Transmitter mass was 2.1–2.9% of body mass. Surgeries took place at the ASLC under sterile conditions and were performed by veterinary surgeons experienced in the technique. We describe the surgical procedure in detail in Latty et al. (2010).

Biomarker Analysis

Unless stated otherwise, we followed manufacturers' recommendations for analyzing chemistries. We used an i-STAT PCA handheld analyzer and an EC8+ cartridge (Abbott Point-of-Care, East Windsor, New Jersey, USA) to determine glucose and an IDEXX VetTest chemistry analyzer and corresponding cartridge (IDEXX Laboratories, Westbrook, Maine, USA) to evaluate CK, AST, and LDH. For IDEXX tests, we diluted samples 1:2 serum to 0.9% saline, except a highly elevated CK and LDH sample, which we diluted to 1:8.

Total protein was analyzed at the Marshfield Clinic Laboratories (Marshfield, Wisconsin, USA) by the biuret method (Hochleithner 1994, Lumeij 1997) using a Roche Modular Analytics System. We determined protein fractions (pre-albumin, albumin, and α_1-, α_2-, β_1-, β_2-, and γ-globulins) using a Serum Protein Electrophoresis (SPE) Kit (Beckman Coulter, Fullerton, California, USA) to separate and stain fractions, and a Beckman Coulter densitometer to measure electrophoretic densities. We included pre-albumin and albumin in the albumin portion and all globulins in the globulin portion for A:G (Harris 2000). Because the α_2-globulin peak was not always distinguishable, we did not include it as a secondary parameter.

We determined white-blood-cell proportions by making $\geq$2 blood smears using fresh untreated blood directly from the collection syringe. After drying, we stained slides using a quick-dip stain system (Jorgensen Laboratories, Loveland, Colorado, USA). We then used a microscope at 100$\times$ to manually count heterophils and 1,000$\times$ to differentiate white cells. We determined PCV by centrifuging a capillary tube of heparinized blood for 3 min at 3,500 revolutions min^{-1}. We then used a Micro-Capillary Reader (IEC, Needham Heights, Massachusetts, USA) to measure the ratio of cells to the total sample.

We used a ^{125}I Radioimmunoassay Kit (MP Biomedicals, Orangeburg, New York, USA) for measuring FGMs, based on Nilsson's (2004) techniques for Harlequin Ducks. Pooled samples showed that the assay accurately measured fecal metabolites (males: slope = 1.01, r = 0.998; females: slope = 1.12, r = 0.997). Serial dilutions of fecal extracts with steroid diluent yielded values parallel to the standard curve for both males and females. Mean intra-assay (between duplicates) coefficient of variation (CV) was 2%, and inter-assay CVs were 21% and 6% for controls with mean values of 85 and 621 ng mL^{-1}, respectively.

Mass

We weighed birds with a 2,500 g Pesola spring scale (Pesola AG, Baar, Switzerland) 7 days prior to surgery. After implantation, we recorded mass to the nearest 2 g when birds stepped onto a waterproof bench scale (Rice Lake Weighing Systems, Rice Lake, Wisconsin, USA) to drink from a fresh-water bowl in the aviary. To ensure accuracy of measurements during subfreezing conditions, we placed a heating pad directly beneath the scale to keep temperatures within the manufacturer's recommended range. We recorded mass routinely after foraging bouts because birds generally drank from the water bowl postfeeding. Due to the opportunistic fashion of mass data collection, we did not obtain data for each individual at every weighing session.

Statistical Analyses

We compared primary parameters using SAS 9.1 (SAS Institute, Cary, North Carolina, USA) in a multistep approach using all 6 birds as their own controls. We assessed normality with a Shapiro-Wilk test and applied log or square-root transformations as needed. To account for changes that occurred as birds physiologically acclimated to deeper diving during the baseline collection period, we used a repeated-measures mixed-effects model (Littell et al. 1996) or Friedman test to examine for differences across the presurgery baselines (62 and 29 days prior to surgery and the day of surgery) for blood biomarkers. Only PCV (F = 10.76, df = 1 and 11, P = 0.007) changed in response to deeper diving during the acclimation period; therefore, for all follow-up paired tests, we used the day-of-surgery values as baseline for PCV and the mean of the 3 presurgery baselines for the other biomarkers. We used a repeated-measures mixed-effects model (Littell et al. 1996) or Friedman test to examine for changes in biomarkers related to PTT implantation and carrying the device. We included data from the 3 primary baseline and all post-implant sampling days in this overall mixed-effects model. We controlled for multiple comparisons across the 4 primary parameters with a Holm-Bonferroni procedure (Holm 1979). If the null hypothesis (i.e. no effect) was rejected for a primary biomarker, we conducted 2-tailed paired t-tests or Zimmerman procedures (Zimmerman 1996) between baseline and each of the postsurgery sampling dates and for the secondary parameters associated with that primary parameter. We conducted all paired tests both with and without bird M3 (described below). To assess how seasonality may have affected results, we also conducted paired tests using an extended baseline (mean for each bird from samples collected every ~2 mo for 1 yr prior to PTT implantation).

To test whether PTT implantation affected a bird's mass, we first calculated the change in mass after surgery by subtracting postsurgery weights and transmitter mass from presurgery weights. We examined 2 periods, 1–14 and 1–119 days postsurgery, using repeated-measures mixed-effects models. We included number of feedings prior to measurement and days since surgery as model effects. If mass changed during either period, we used one-tailed least-squares means to determine how long weight loss occurred.

RESULTS

Behavioral and Clinical

Surgeries took place on November 30, 2005, and averaged 19 min (range: 11–29 min) from incision to closure. Bird M3 required treatment for bradycardia during surgery. All birds showed signs of lethargy (drooping bill and wings) for 8–10 hr after surgery, and some were seen shivering within 24 hr of surgery (minimum recorded temperature for Seward, Alaska, on the 2 days postsurgery was –8°C). Lethargy was not noted after day 2, except for M1 (further described below). Although the effect was not quantified, we noted that birds spent substantial time preening surgical sites (especially the antenna exit site) for the first few weeks after surgery.

M3 was the first to dive to the bottom of the dive column 22 hr postsurgery. All but one bird dove to the bottom for food within 35 hr of surgery. The remaining bird (M1) showed signs of severe lethargy and appeared to be unable to climb a ~15 cm ramp between the water and dry aviary. This bird remained in the water until we manually removed it and placed it on the haul-out. Once removed, it had difficulty standing and holding up its bill. The bird was not performing foraging dives, and we force-fed it to minimize the chance of permanent health problems. The bird's condition improved and it began performing foraging dives 50 hr after implant.

We found that F2 had loss of waterproofing and subsequent water intrusion into the plumage at the abdominal incision site, and 5 of 6 birds had water intrusion at the antenna exit site on day 2. The only bird without water intrusion at the antenna exit site on day 2 was M1, which had not yet begun diving. On day 8, we found no gross wetness of feathers at abdominal incision sites, but 4 birds had water intrusion into the plumage at the antenna exit site. Also, all birds had varying degrees of matting of contour and down feathers at the antenna exit site. We also found that F2 had swelling without further signs of infection at the exit site on day 8. We did not find wet plumage at either surgical site on day 14, but all birds had matting of contour and/or down feathers at antenna exit sites, suggesting some loss of insulation and possibly waterproofing. We also noted that several of the birds plucked feathers from around antenna exit sites, exposing bare skin, with M3 having a 1.5 cm^2 plucked area. By 21 days postsurgery, all birds maintained waterproofing at both surgical sites, and those with bare skin 1 wk prior were regrowing feathers.

M3 developed a limp after surgery, and on day 17 we found a wound on its foot that advanced to involve the webbing between the third and fourth toes and complete necrosis with disassociation of the distal 2 phalanges of the fourth toe. A veterinarian amputated the affected toe 7 wk postsurgery to minimize further health risks. Histopathology showed severe granulomatous necrotizing pododermatitis consistent with bacterial embolization and subsequent osteomyelitis. Although the etiology of this condition is uncertain, limping has been reported in wild Common Eiders following PTT implantation (Fast et al. 2011). Because we cannot rule out the possibility that the condition was related to surgery, we included this individual in our primary analyses but also conducted separate tests with this bird removed. The bird dove and successfully maintained condition despite being visibly irritated by the affected leg and sometimes not using it for propulsion during dives.

Biomarkers

The A:G ratio, CK, PCV, and FGMs varied across sampling dates ($F = 15.9$, df = 10 and 50, $P < 0.001$; $Q = 24.1$, df = 10, $P = 0.01$; $F = 16.3$, df = 10 and 50, $P < 0.001$; and $F = 2.2$, df = 9 and 40, $P = 0.04$, respectively). The A:G ratio was lower than baseline on days 2, 8, 14, 28, and 56 after surgery (Figure 1). Creatine kinase was higher on day 2 and was lower on days 8, 14, and 91 (Figure 1) compared to presurgical values. Packed cell volume was lower on all days (Figure 1) postsurgery compared to the presurgery baselines. Fecal glucocorticoid metabolites were higher on day 3 (Figure 1) compared to baseline.

Excluding M3 from the analyses did not affect the significance of changes in A:G. For CK, day 8 was no longer lower than baseline ($t = 2.6$, df = 4, $P = 0.06$) when M3 was excluded. For PCV, day 105 was no longer lower than baseline ($t = 2.2$, df = 4, $P = 0.10$) when M3 was excluded. For FGMs, days 27 ($t = –4.8$, df = 4, $P = 0.01$) and 55 ($t = –3.2$, df = 4, $P = 0.03$) were higher than baseline when M3 was removed.

Replacing the general baseline (mean from 62 and 29 days prior to and the day of surgery) with the mean of the extended 1-yr baseline (general baseline plus 5 additional samples obtained every ~2 mo prior to collecting the general baselines) did not change the results of A:G, but CK was no longer lower on days 8 ($t = 2.1$, df = 5, $P = 0.09$) and 91 ($t = 2.5$, df = 5, $P = 0.06$). We did not conduct tests using the extended baseline for PCV because it changed during the acclimation period. No extended baseline was available for FGMs.

Secondary biomarker AST was elevated on days 2 and 14, and LDH did not change (Table 1). All globulin fractions except α_1 were elevated after surgery: β_1 on days

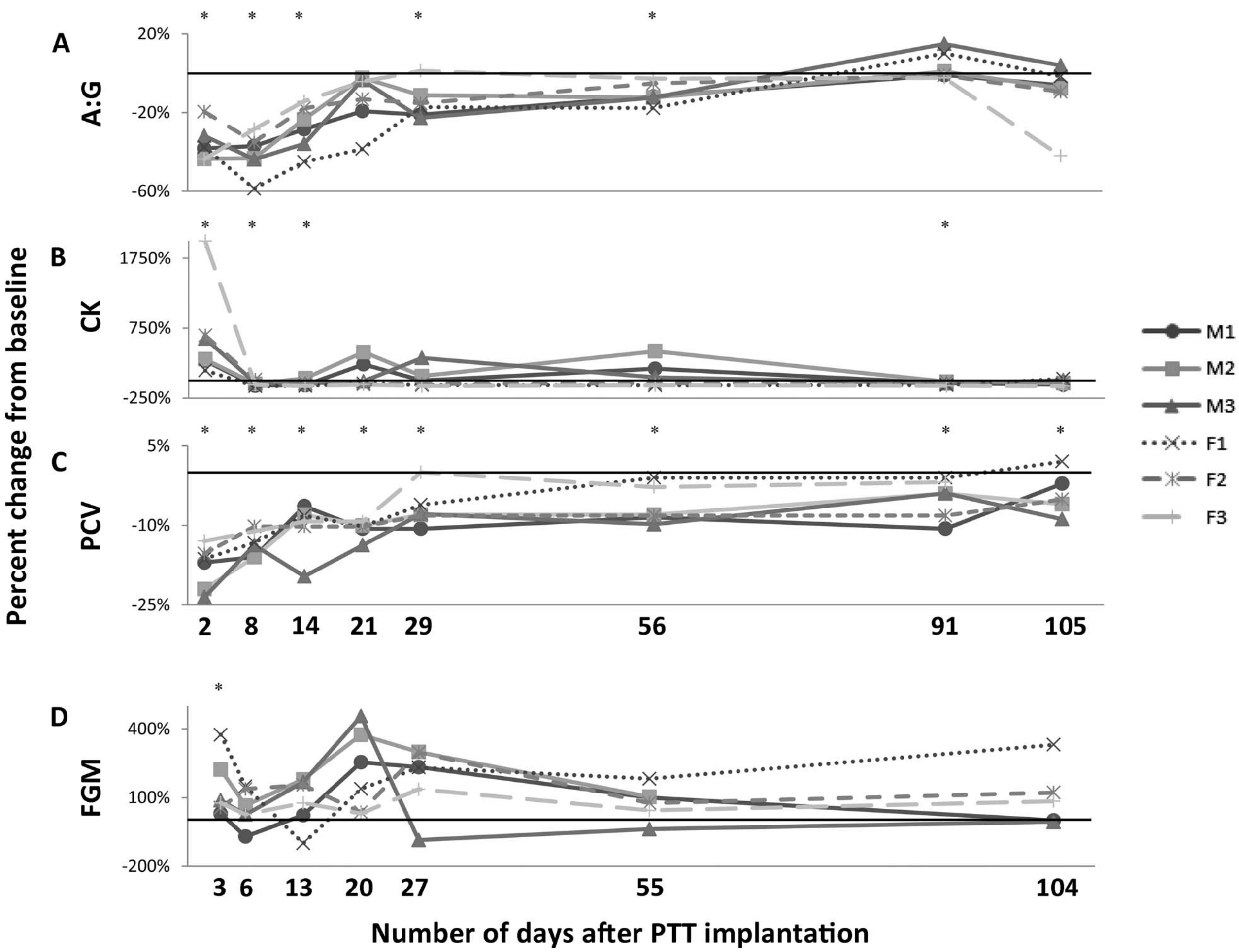

FIGURE 1. (**A**) Percent change from baseline of albumin:globulin ratio (A:G), (**B**) creatine kinase (CK), (**C**) packed cell volume (PCV), and (**D**) fecal glucocorticoid metabolites (FGMs) for individual Common Eider after implantation of satellite transmitters with percutaneous antennas ($n = 6$). Solid black line indicates no change. *Postsurgery value is different from baseline using a 2-tailed paired *t*-test.

8, 14, 21, and 56; β_2 on days 2–56; and γ on days 8, 14, 21, 28, and 91 (Table 1). Secondary biomarkers indicative of general health included albumin, which was higher on day 21, and glucose, which did not change (Table 1). The additional stress parameter, H:L, was elevated on days 2, 8, 21, and 28 (Table 1).

Mass

Birds weighed 1,633–1,873 g before surgery. We recorded >700 individual mass measurements postsurgery with $\geq$1 measurement on 98 of the 119 postsurgery days. The number of times we fed birds on the day of measurement ($F = 129.1$, df $= 4$ and 20, $P \leq 0.001$) and the number of days since surgery (time: $F = 89.3$, df $= 1$ and 121, $P \leq 0.001$; time^2: $F = 8.9$, df $= 1$ and 121, $P = 0.004$) were predictors of mass for the 2-wk period after surgery, but not for the full 3.5-mo term. Mass for each bird, adjusted for the number of feedings, decreased by 4–12% between the baseline measurement 1 wk before surgery and 2 days after surgery (the first day all birds began drinking fresh water and standing on the scale). Birds regained mass quickly, weighing less than baseline only 1–5 and 8 days after surgery (Figure 2).

DISCUSSION

Our data suggest that captive Common Eiders physiologically responded to abdominally implanted PTTs with percutaneous antennas primarily for the first few weeks, but also for up to 3.5 mo after surgery. In addition to changes in all primary and most secondary biomarkers, we observed clinical complications that may have been related to surgery in some birds. Although we did not include a control group because of size limitations of the

TABLE 1. Biomarker responses of 6 Common Eiders surgically implanted with 38–47 g satellite transmitters with percutaneous antennas.

			Number of days after PTT implantation							
Parameter		Baseline	2	8	14	21	28	56	91	105
Alpha 1 globulin (g dL^{-1})	Mean	0.93	0.84	0.99	0.97	0.92	0.93	1.01	0.81	1.02
	SD	0.06	0.12	0.09	0.10	0.1	0.09	0.06	0.10	0.11
	t		1.6	−1.3[b]	−0.9	0.2	0	−2.2	1.9	−1.3
Beta 1 globulin (g dL^{-1})	Mean	0.22	0.25	0.38	0.32	0.3	0.27	0.3	0.24	0.29
	SD	0.04	0.03	0.05	0.02	0.04	0.04	0.03	0.03	0.06
	t		−1.2	−5.2[a,b]	−7.5[a]	−3.6[a]	−1.9	−4.7[a]	−0.7	−1.9
Beta 2 globulin (g dL^{-1})	Mean	0.45	0.8	0.98	0.98	0.84	0.87	0.67	0.59	0.72
	SD	0.12	0.3	0.23	0.25	0.18	0.14	0.12	0.1	0.34
	t		−3.0[a]	−5.8[a,b]	−7.0[a]	−4.1[a]	−12.9[a]	−3.0[a]	−1.8	−1.7
Gamma globulin (g dL^{-1})	Mean	0.14	0.12	0.19	0.23	0.24	0.21	0.17	0.19	0.17
	SD	0.03	0.03	0.06	0.08	0.08	0.08	0.04	0.02	0.04
	t		1.2	−3.2[a,b]	−2.8[a]	−5.1[a]	−2.9[a]	−2.5	−3.5[a]	−1.8
Aspartate aminotransferase (AST; U L^{-1})	Mean	0.6	17.5	8.8	10.3	26.3	0	0	0	0
	SD	1.4	19.5	14.7	12.8	19.3	0	0	0	0
	t		−3.4[a]	−1.9	−2.8[a]	−2.5	1	1	1	1
Lactate dehydrogenase (LDH; U L^{-1})	Mean	686	1356	546	463	558	507	693	595	590
	SD	221	1517	107	144	129	106	160	182	223
	t		−0.9	0.9	2.2	0.8	1.9	0	1	1.1
Albumin (g dL^{-1})	Mean	1.72	1.34	1.6	1.88	2.1	2.06	2.04	2.03	2.01
	SD	0.22	0.21	0.17	0.12	0.28	0.29	0.24	0.25	0.21
	t		2.4	0.5[b]	−1.3	−2.7[a]	−1.8	−1.8	−1.7	−1.8
Glucose (mg dL^{-1})	Mean	216	204	194	216	218	207	209		207
	SD	18	17	22	10	11	18	24		18
	t		0.9	2.2	0	−0.3	0.8	0.5		0.8
Heterophil-to-lymphocyte ratio (H:L)	Mean	0.58	3.15	1.47	1.31	1.55	0.91	0.97	0.58	0.67
	SD	0.33	2.09	0.8	0.86	0.66	0.3	0.71	0.15	0.42
	t		−7.0[a]	−3.1[a]	−1.7	−4.7[a]	−2.9[a]	−1.2	−0.7	−0.4

[a] Significant difference ($P < 0.05$) between baseline and number of days after implantation using a 2-tailed paired *t*-test.
[b] $n = 5$.

aviary and dive column, the inclusion of extended baseline data from the full year before surgery allowed us to use each bird as its own seasonal and presurgery control.

Behavioral and Clinical

Some mortality in the first few weeks after surgery is not uncommon in studies using transmitters (e.g., Cox and Afton 1998, Rosenberg and Petrula 2000), and mortality in

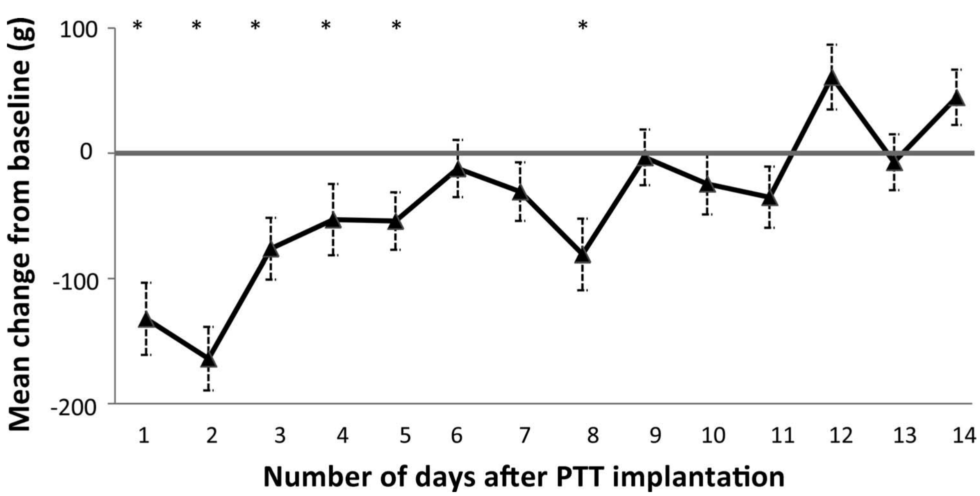

FIGURE 2. Mass change (least squared mean ± SE) of 6 Common Eiders after surgical implantation of a PTT with percutaneous antenna. Mass adjusted for transmitter weight and model solutions for the number of feedings prior to measurement. *Postsurgery value is less than baseline using a one-tailed paired *t*-test.

sea ducks fitted with transmitters has been attributed to avian predators in previous field studies (Rosenberg and Petrula 2000, Iverson et al. 2006). Based on our observations, behavioral responses such as lethargy, increased preening, and dive cessation may help explain increased mortality, given that free-ranging eiders exhibiting these symptoms could be at an increased risk of predation.

Limping after PTT implantation was reported in 3 of 5 Common Eiders reobserved within 38 days of surgery in Arctic Canada (Fast et al. 2011). In our study, limping in one bird shortly after implantation progressed to require surgical intervention 17 days after surgery. This bird remained visibly irritated by the leg throughout the study. If similar foot conditions occur in wild eiders, which use feet during propulsion to the bottom and while foraging on the bottom, detrimental effects of PTTs on dive behavior previously reported (Latty et al. 2010) could be exacerbated.

Metabolic rates could also be affected by implanted transmitters. While several of our observations could affect energy use, plumage wetting and increased preening probably have the greatest potential to increase energetic demands (Enstipp et al. 2015). Hartung (1967) demonstrated that even minor loss of waterproofing at 0°C increased the metabolic rate of Mallards (*Anas platyrhynchos*) by ~30%. Although we observed shivering only in the first day postsurgery, thermoregulatory consequences may have continued longer because plumage at surgical sites was affected (wet and matted) until 21 days after surgery. Additionally, wild Common Eiders may dive 20–40 m (Elliot 1898, Dickson and Smith 2013), Spectacled Eiders (*S. fischeri*) dive 40–70 m during winter (Petersen et al. 2000, Lovvorn et al. 2003), and King Eiders have been recorded diving to 43 m (Mosbech et al. 2006). Increased water pressure at depth may exacerbate plumage wetting. By contrast, marking birds on breeding grounds where prey are often found at shallower depths could reduce the effects of loss of waterproofing.

Biomarkers

The A:G ratio was lower than baseline for 2 mo after surgery, and γ-globulins were elevated for 3 mo. Elevations of β_1 and β_2 globulins through day 56 may be due to chronic liver or kidney effects or to other chronic inflammatory reactions during the first 2 mo of the study (Harris 2000). Normalized AST on day 21 and LDH on all postsurgery sampling days suggests that if liver or kidneys were affected, the effects did not lead to significant tissue damage since both enzymes are found in both tissues (Itoh et al. 1993). Elevation of γ-globulins (generally consisting of antibodies and complement) for 3 mo supports the finding of a sustained chronic inflammatory response and may be related to infection (Kaneko 1989). Although these elevations show that implanted eiders were able to mount an immunological response, such responses have a metabolic cost. For example, Eurasian Collared-Doves (*Streptopelia decaocto*) that were immune challenged with sheep red blood cells had an 8.5% increase in basal metabolic rate compared to controls (Eraud et al. 2005), and the resting metabolic rate of Eurasian Great Tits (*Parus major*) increased 4.5% in response to phytohaemagglutinin (Nilsson et al. 2007). While the magnitude of these increases was small, such changes could be relevant for individuals suffering from other effects.

All birds showed >100% increase from baseline in CK 2 days after surgery, and 4 had levels associated with myopathy (>1,000 U L^{-1}; Bollinger et al. 1989). Although serum CK decreased by day 8, blood CK is a result of muscle cell deterioration; therefore, the effects of muscle damage may persist well beyond normalization of blood CK (Wobeser 1997). Also, the peak activity and half-life of serum CK of Budgerigars (*Melopsittacus undulatus*) injected intramuscularly with muscle extract was 4 hr and 7.7 hr, respectively (Itoh et al. 1993). If we assume even moderately similar pharmacokinetics, 48-hr CK activities in the blood probably do not represent maximum postsurgery levels. While we cannot be certain of the etiology, lethargy and droopy wings, inability to move between the water and roost, and decreased dive performance (Latty et al. 2010) after PTT implant are consistent with clinical myopathy (Wobeser 1997). Although myopathy may reduce muscle performance and affect flight, diving, and movement, we caution that the use of muscle enzymes as a myopathy index has not been experimentally evaluated for Common Eiders. Also, we cannot differentiate between the causes of elevated CK; elevated blood CK may have been derived predominantly from surgical injury, although this would not necessarily negate the importance of this finding for understanding the potential impact of the PTT implantation process.

Elevation of AST in the first 14 days after surgery supports our suggestion of muscle damage. Although AST can be elevated by damage to other organs, such as the liver, this is probably unlikely because LDH, which also occurs at high levels in the liver (Itoh et al. 1993), was not elevated. The extended elevation of AST compared to CK is consistent with its longer half-life (Itoh et al. 1993).

PCV declined by 13–24% directly after surgery and remained an average of 4% lower than baseline 3.5 mo later, suggesting chronic anemia. Reduced PCV could indicate lower oxygen storage capacity (Keijer and Butler 1982, Stephenson et al. 1989). This would likely reduce calculated aerobic dive limits (Hawkins et al. 2000) and could therefore affect foraging. We included albumin and glucose as robust (i.e. more resistant to change from minor insults) metrics of health status. Albumin increased compared to baseline 21 days after surgery, and glucose did not change after surgery.

We found that FGMs were elevated compared to baseline only on day 3 post-surgery, with increases ranging from 30% to 374%. We used FGM as our primary stress metric, although H:L has been shown as a valid measure of stress in birds (Gross and Siegel 1983). In Adélie Penguins (*Pygoscelis adeliae*) with obvious injuries, elevations occurred in H:L but not serum corticosterone, leading Vleck et al. (2000) to suggest that H:L may be a better index of persistent stress than blood corticosterone. However, immunological responses to surgery, the implanted device, and infection could affect H:L. This may explain why H:L was elevated longer than FGMs.

Mass

Body mass can serve as a useful metric for assessing the effects of carrying a device. For example, Common and Thick-billed murres fitted with leg-borne geolocators weighed less than controls 1 yr after attachment, but return rates were similar (Elliott et al. 2012). We found that body mass returned to baseline by 9 days postsurgery. This shows that while biochemical changes were still occurring in response to surgery and carrying the transmitter, body mass was quickly restored. In contrast, wild Harlequin Ducks implanted with 15–17.5 g transmitters had a greater mass loss in the first few weeks after surgery than banded controls (Esler et al. 2000). Our birds may have recovered faster because of the disparities between captivity and the wild; in our study, birds were fed ad libitum in a feeding tray at a single depth and did not have to contend with the additional rigors or energetic demands of life in the wild.

Conclusions

While the optimal method to determine whether implanted PTTs affect the parameters they measure would be to follow an implanted and control group throughout the year, such studies are logistically challenging. Findings from other studies that have measured the effects of implanted transmitters in sea ducks are somewhat variable. For example, Iverson et al. (2006) reported no difference in survival among 4 attachment types for scoters (intra-abdominal with and without percutaneous antennas, subcutaneous, and externally attached) and Esler et al. (2000) found that 1-yr survival was similar to banded controls for Harlequin Ducks, but Fast et al. (2011) reported that Common Eiders implanted with PTTs with percutaneous antennas had lower 1-yr survival compared to controls. Despite appearing normal just days after surgery and engaging in behaviors such as diving and feeding, we found physiological responses to implanted PTTs for up to 3.5 mo after surgery. The range of behavioral and clinical responses we observed implies variation in how individuals cope with surgery and carrying PTTs. Researchers using implanted PTTs need to consider both the suite of responses that occur and this variability among individuals.

Although we cannot predict with certainty how the responses we found would ultimately affect implanted wild Common Eiders, they suggest potential health and behavioral effects for at least the first few weeks after surgery. Further study is needed to determine long-term responses and whether responses can be reduced by using smaller PTTs or improved surgical procedures. In addition, because our results are specific to Common Eiders diving to 4.9 m during the winter, future research should examine whether other sea duck species are similarly affected and whether responses vary with dive depth and/or season.

In our study, Common Eiders implanted with PTTs weighing 1.9–2.6% of body mass showed physiological responses for up to 3.5 mo postsurgery, well beyond censor periods suggested in other implanted transmitter studies. Our results support the need for data censor periods, but additional studies on a wider range of physiological effects and species are needed before an appropriate censor duration can be determined. Until then, scientists should use the responses described here and findings of other applicable studies to assess the suitability of implanted transmitters with percutaneous antennas for their particular project, study species, and research questions.

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R.D.A. wrote the paper. T.E.H., M.R.P., A.N.P., and R.D.A. contributed substantial materials, resources, or funding.

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