

METHODOLOGY

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A pilot study on surgical implantation and efficacy of acoustic transmitters in fifteen loggerhead sea turtles (*Caretta caretta*), 2021–2022

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Abstract

Background The ability to detect the location of free-ranging sea turtles over time is desirable for ecological, conservation, and veterinary studies, but existing detection methods have limited sensitivity or longevity. Externally attached acoustic transmitters have variable, and sometimes short retention times for sea turtles. For several vertebrate taxa, surgically implanted acoustic transmitters have proven to be safe and effective for long-term detection; however, implanted transmitters have not yet been used for turtles.

Results In this pilot study, INNOVASEA acoustic transmitters were surgically implanted subcutaneously in the pre-femoral region of fifteen hospitalized loggerhead sea turtles (*Caretta caretta*) that had been rehabilitated after stranding due to cold-stunning. Model V16-4H transmitters (estimated battery longevity = 2435 days) were implanted in turtles measuring ≥ 50 cm straight carapace length (SCL), and model V13-1H transmitters (estimated battery longevity = 1113 days) were implanted in turtles measuring 30–49 cm SCL. Incision healing was monitored over several months prior to release. Twelve turtles' incisions healed without complication, on average, 55 days after surgery (median 47, range 41–100). Three turtles experienced incision complications, two of which healed after a second surgery, while the third required transmitter removal to promote healing. One of the fourteen implanted transmitters was confirmed to be dysfunctional prior to release, although it had been functional prior to implantation. To date, 100% of turtles released with functional acoustic transmitters ($n = 13$) have been detected a total of 915 times by 40 individual acoustic receivers off the coasts of Massachusetts, Rhode Island, New York, Virginia, North Carolina, and in southern New England offshore waters. Turtles with transmitters generated 5–235 detections (mean 70, median 43) on 1–13 individual acoustic receivers (mean 5, median 5) for periods of 3–400 days post-release (mean 118, median 87). Total detections and detection durations for these individuals are expected to increase over time due to anticipated transmitter battery longevity.

Conclusions This study demonstrates that surgically implanted acoustic transmitters are effective for the detection of free ranging sea turtles, but refinement of surgical methodology is needed in light of the observed complications. Monitoring of healing is critical when evaluating novel surgical techniques in wildlife.

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Keywords Acoustic transmitter, Surgery, Biotelemetry, Turtle, Loggerhead sea turtle, *Caretta caretta*, Anesthesia, Acoustic tag

Background

The seven species of sea turtles are of global conservation concern [1, 2] due largely to negative impacts of fisheries interactions, habitat loss, vessel collision, pollution, disease, and extreme weather events [3–10]. Conservation of sea turtles requires thorough understanding of their location in space and time, including their use of foraging, migratory, and breeding areas [11]. For example, knowledge of spatiotemporal habitat use is important for protection and restoration of nesting beaches, reduction of fisheries interactions and watercraft injury, and monitoring for interaction with other ocean industries. The ability to detect the location of a sea turtle over time is also desirable for monitoring the post-release outcome of turtles that have received medical care, which may inform future medical practices and locations of release [12]. Several methods have been used to identify and monitor the location of individual sea turtles, each with limitations. Methods such as flipper tags, photo-identification, passive integrated transponder tags, coded wire tags, and “living tags” (transplantation of distinctly colored tissue to an atypical location on the shell) require the presence of a human observer for detection, and may not be permanent [13]. The odds of human encounter are low, while the odds of external tag loss are high for such highly migratory oceanic species [12]. Even if physically detected by a motivated observer, determining the origin of the tag, and communicating the detection of that tag to interested parties may be challenging. Methods of remote detection, such as external tagging with satellite or acoustic transmitters, theoretically offer more consistent opportunities, but long-term monitoring can be hindered by premature tag detachment, tag damage, bio-fouling, and, for some tag models, limited battery life [11, 14–18]. Given their decades-long lifespan, methods for long-term remote detection of sea turtles are needed to understand these species’ habitat needs and movements.

For several vertebrate taxa, including fish, aquatic mammals, and crocodiles, surgically implanted acoustic transmitters have proven to be safe and effective for long-term monitoring [19–23]. For example, sand tiger sharks (*Carcharias taurus*) with implanted acoustic transmitters have been detected for over 10 years after release [21; J. Kneebone unpublished data]; and best-practice guidelines for surgical acoustic transmitter implantation have been described for pinnipeds [24]. The use of implanted acoustic transmitters for sea turtles has been discussed for many years but not yet executed. As a preliminary

study, Barco and Lockhart implanted an acoustic transmitter in a deceased loggerhead sea turtle (*Caretta caretta*) to study the sensitivity of detection and the possibility that the dense shell of the turtle could interfere with sound transmission [25]. Their results showed that detection of the internal transmitter was minimally reduced in comparison with an externally attached transmitter. Motivated by these results, and by recognition of the limitations of external transmitter attachment [11], we received permission to surgically implant acoustic transmitters into loggerhead sea turtles that had been rehabilitated after stranding due to cold-stunning. We sought to evaluate the surgical outcome for transmitters implanted subcutaneously in the pre-femoral region, evaluate the efficacy of two different sizes of transmitters, monitor the presence and movements of turtles with an array of acoustic receivers deployed in Nantucket Sound, and evaluate the success of detection in a subset of turtles using both internal acoustic transmitters and external satellite tags.

Methods

The federal permit for this study authorized surgery for 15 loggerhead sea turtles that were acquired through the New England Aquarium’s sea turtle rescue and rehabilitation program. Inclusion in the study required good health as determined by serial veterinary evaluation prior to surgery, and straight carapace length (SCL) of 30 cm or greater. Federal permission was granted after review of proposed methods for anesthesia and analgesia, aseptic technique, and subcutaneous implantation of the transmitter in the pre-femoral region. An allowance was granted to retain turtles for up to 8 weeks post-operatively to monitor incision healing prior to release (or longer if medically necessary), or until local environmental conditions were favorable for release. Requirements included notification of surgical complications, including infection, dehiscence, altered limb movement, swimming, or diving behavior.

Animals

Loggerhead turtles were admitted to the New England Aquarium’s sea turtle hospital in late autumn and early winter after stranding on beaches of Cape Cod, Massachusetts, USA due to cold-stunning. Details of the general triage and medical management of cold-stunned sea turtles and physiologic state of cold-stunned loggerhead turtles at New England Aquarium have been previously

described [26–28]. Turtles were gradually warmed, medically evaluated, and treated for common disorders, such as dehydration, acidosis, hyperkalemia, pneumonia, and physical injuries. Turtles were progressively provided with deeper water of greater volume, offered food, and serially examined physically and radiographically. Some were evaluated with point-of-care blood analysis (Stat Profile Prime Plus Vet Critical Care Analyzer, NOVA Biomedical, Waltham, MA) and, or by hematologic and plasma biochemical assessment at a commercial veterinary diagnostic laboratory (Idexx Diagnostics, North Grafton, MA). After several days of initial stabilization, turtles were maintained in natural sea water, with temperatures between 22 and 24° C (approximately 72–74 °F), and salinity of 31–32 ppt, with mechanical and biological filtration and ozone disinfection. Artificial overhead lighting and ambient window lighting provided a 10–16 h daylength that naturally varied seasonally. Turtles were selected as surgical candidates based on serial examinations after having completed antibiotic therapy at least 30 days prior to surgery, when applicable.

Procedures

All turtles received a single pre-operative antibiotic injection (oxytetracycline 42 mg/kg subcutaneously [SC] diluted in lactated ringer's solution [5 ml/kg]) on the day of surgery ($n=11$) or 1 day before surgery ($n=4$) [29]. Pre-operative analgesia was provided by ketoprofen (2 mg/kg intramuscularly [IM], [30]). Ketoprofen was initiated on the day before surgery, continuing once daily for four doses ($n=4$); or on the day of surgery as a single dose ($n=7$); or on the day of surgery and continuing once daily for five doses ($n=4$). Anesthesia was induced with dexmedetomidine and ketamine combined into a single syringe and given intravenously [31]. Dexmedetomidine and ketamine doses, respectively, were 0.05 mg/kg and 5 mg/kg for 11 cases; and 0.04 mg/kg and 4 mg/kg for four cases. Turtles were intubated with non-cuffed endotracheal tubes and manually ventilated with medical-grade compressed air at 10 cm H₂O maximal airway pressure. Respiratory rate was generally 2 per minute unless brief adjustments in rate were required to achieve adequate depth of anesthesia, or to aid anesthetic recovery. Sevoflurane was used to effect for seven cases [31] but was not needed for eight cases. Lidocaine (2 mg/kg) was infused intradermally and subcutaneously at the tag insertion site. Heart rate was monitored by Doppler positioned over the lateral base of the neck.

Acoustic transmitters included the V16-4H model for turtles ≥ 50 cm SCL (INNOVASEA Systems Inc., Halifax, Nova Scotia, Canada; 16 mm diameter, 68 mm length, 24 g in air, 10 g in water; estimated battery longevity = 2435 days), and INNOVASEA V13-1H for turtles

30–49 cm SCL (13 mm diameter, 30.5 mm length, 9 g in air, 5 g in water; estimated battery longevity = 1113 days; Fig. 1). These transmitters have individual identification numbers that are recorded by the receivers each time a transmission is detected. All transmitters were programmed to emit their 69 kHz signal over a nominal delay of 60 (± 20) sec; and their proper function was confirmed prior to insertion using an INNOVASEA VR-100 acoustic hydrophone. Briefly, each transmitter was activated in air by removing the external inhibitory magnet and the VR-100 hydrophone was held within 5 cm of the transmitter until at least two successful transmissions were recorded. Transmitters were disinfected prior to surgery by immersion in ortho-phthalaldehyde 0.55% solution (Cidex OPA, Advanced Sterilization Products, Irvine, CA) for at least 15 min; rinsed thoroughly with sterile saline solution, and external identification stickers were removed prior to insertion.

After anesthetic induction, turtles were positioned in left, oblique, ventral recumbency at approximately a 60° angle, and the right hind leg was secured in extension to expose the right pre-femoral region (Fig. 2). The skin was disinfected with chlorhexidine and isopropyl alcohol-infused gauze pads, and the surgical site was draped. Surgery was completed by one of two veterinarians. An approximately 2 cm scalpel incision was made in the cranial 1/3 of the pre-femoral skin, approximately 1/2 the distance between the carapace and plastron. This location was chosen for its relatively deep subcutaneous



Fig. 1 INNOVASEA V13 (top) and V16 (bottom) acoustic transmitters. These models were surgically implanted subcutaneously in the pre-femoral region of fifteen loggerhead turtles (*Caretta caretta*), with the V13 used for turtles that had straight carapace length (SCL) between 30 and 49 cm, and the V16 used for turtles ≥ 50 cm SCL. In this photograph, the inhibitory magnet and adhesive stickers are still attached to the surface of each transmitter. These are removed to activate the transmitter prior to implantation. Scale shows 1 mm increments. Photograph courtesy of Deana Edmunds

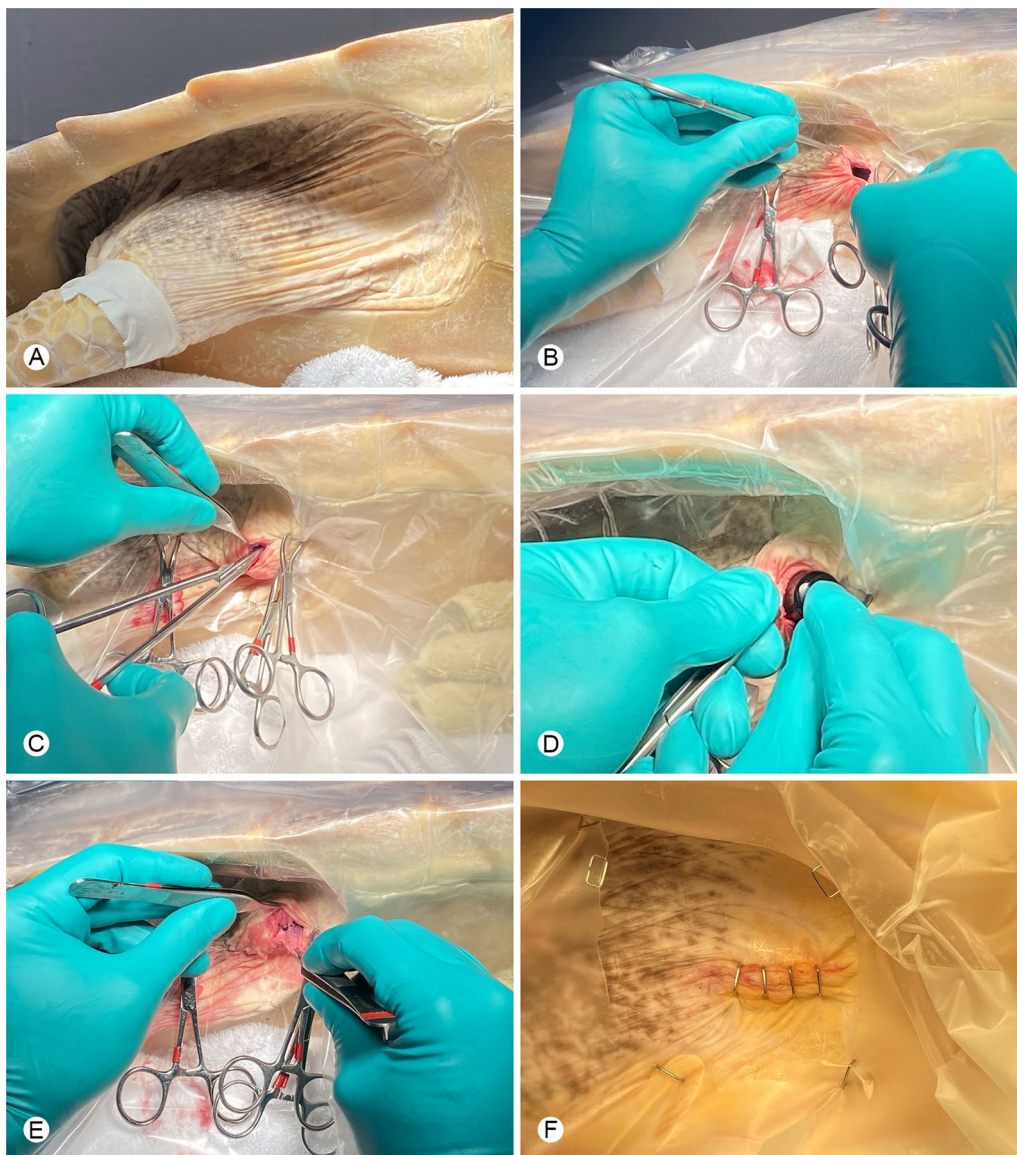


Fig. 2 Surgical method that was used for implantation of subcutaneous acoustic transmitters in the right pre-femoral region of 15 loggerhead sea turtles (*Caretta caretta*). In all images the turtle's head is to the right and its carapace is at the top. **A** Anesthetized turtle is placed in left lateral oblique ventral recumbency with the right hind leg secured in extension. **B** Approximately 2 cm skin incision is made. **C** Blunt and sharp subcutaneous dissection creates a tunnel to accept the transmitter. **D** Transmitter is inserted and toggled to orient it as parallel as possible to the long axis of the turtle. **E** Subcutaneous sutures are placed to close tissue over the transmitter. **F** Skin staples are placed at approximately 5 mm intervals

space and its distance from the hind leg to minimize effects of the transmitter on hind limb movement. Blunt and sharp dissection with scissors created a subcutaneous tunnel cranially and caudally from the skin incision, extending into the subcutaneous tissue and fat, but not penetrating the coelomic muscle wall. We sought to place the transmitter parallel to the long axis of the turtle, at least 2 cm deep to the skin surface, with the mid-point of the tag directly underlying the incision. The transmitter

was initially placed into the incision along its longitudinal axis, and the incision was only widened enough to accept its diameter. The transmitter was then toggled into the subcutaneous tunnel to achieve the desired, more parallel orientation. Surgical methods are shown in Fig. 2.

Subcutaneous tissue closure was achieved using polyglactone suture (e.g., Monoweb, Patterson Veterinary, Devens, MA) in one ($n=8$) or 2 ($n=7$) layers, in a simple continuous ($n=13$) or simple interrupted pattern ($n=2$).

All subcutaneous suture material was size 2–0 except for one case in which the superficial, simple interrupted subcutaneous closure used size 3–0 suture. Skin closure was achieved with surgical staples ($n=14$), or 2–0 poligle-caprone suture in an interrupted cruciate pattern ($n=1$). Cyanoacrylate tissue adhesive was used as a final layer on the incision in eight cases. Selection of suture size, pattern, number of layers, and use of cyanoacrylate was at the discretion of the surgeon based on assessment of the surgical site during closure.

To maintain acceptable heart rate and to facilitate anesthetic recovery, additional methods that were used at the discretion of the attending veterinarians included epinephrine (0.05–0.1 mg/kg IM; [32]), atropine (0.05 mg/kg IM; [33]), doxapram (5 mg/kg IM; [34, 35]), or GV-26 acupuncture [36].

Turtles were maintained out of water until animal care staff members judged them to be fully recovered from anesthesia, and then they were returned to their rehabilitation pool on the same day as surgery. Food was offered post-operatively on the day of surgery ($n=11$) or the following day ($n=4$). Turtles were monitored for incision healing and general convalescence by daily behavioral and feeding observations, weekly physical examinations, and monthly radiographs (Fig. 3). Functionality and detection of the transmitters were

assessed in the rehabilitation pools prior to release using an INNOVASEA VR-100 acoustic hydrophone.

In addition to acoustic transmitters, six turtles were also equipped with ARGOS-linked satellite transmitting tags (Wildlife Computers Inc., Redmond, WA, USA) just prior to their release to the wild. Three tag models were used: one SPLASH10-283 (109 mm length, 32 mm width, 26 mm height, 99 g in air), two SPOT-287 (70 mm length, 41 mm width, 23 mm height, 72 g in air), and three SPOT-395 (75 mm length, 40 mm width, 19 mm height, 86 g in air). We attached satellite tags to the turtles' carapace using materials and methods developed at the New England Aquarium and described in the Wildlife Computers tag attachment manual [37]. All satellite tags were treated with Micron66 antifouling paint. Turtles were tagged on the day of release to reduce the probability of tag displacement in the captive setting. We double-tagged this subset of turtles to compare detection duration of externally attached satellite tags with internally implanted acoustic tags, and to assess whether loggerhead turtles in known locations (derived from satellite tag geolocation data) were detected by co-located acoustic receivers in Nantucket Sound.

Upon full healing of the surgical sites, turtles were released from West Dennis Beach, MA (41.65°N,

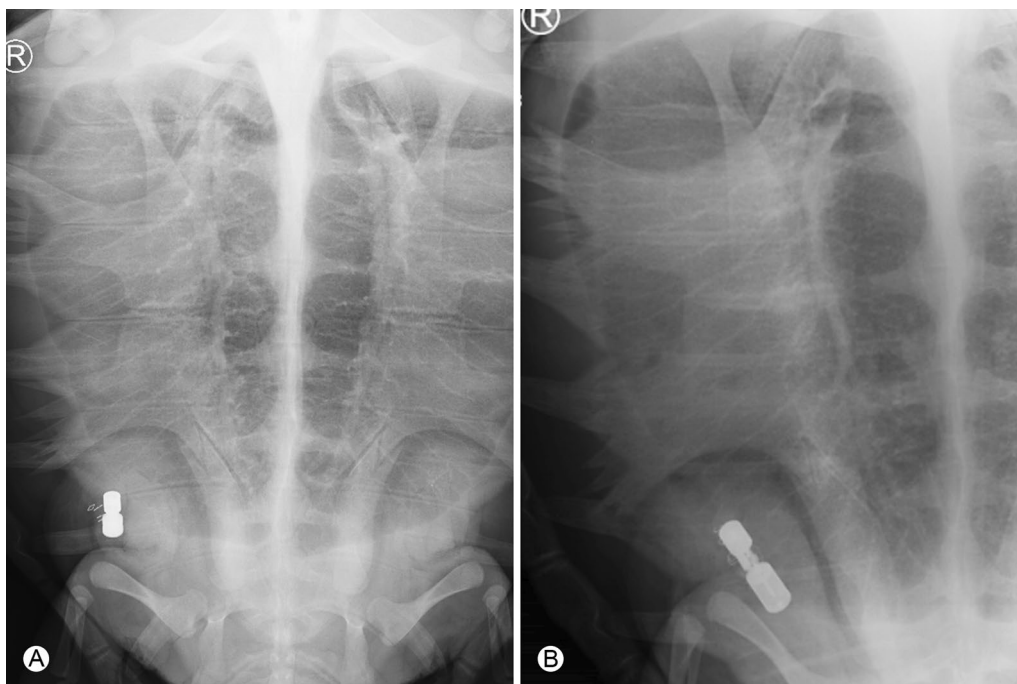


Fig. 3 Dorsoventral radiographs of two loggerhead turtles (*Caretta caretta*) with acoustic transmitters implanted subcutaneously in the right pre-femoral region. In both images the head is oriented to the top; the right hind leg is on the left side of the image (mirror image following radiologic convention). The right femur is just posterior to the tag insertion site. **A** Turtle 20–1145 with V13 transmitter, straight carapace length 45 cm; **B** Turtle 21–0839 with V16 transmitter, straight carapace length 50 cm

-70.17°W) into Nantucket Sound in mid-to-late summer of the year following their stranding.

Post-release monitoring

Turtles with implanted acoustic transmitters were tracked within Nantucket Sound by a fixed acoustic receiver array maintained by the New England Aquarium. This receiver array was deployed seasonally in 2021 (10 receivers, July–November) and 2022 (17 receivers, June–October), and included two receiver models (VR2-W and VR2-Tx, INNOVASEA Systems Inc., Halifax, Nova Scotia, Canada) (Table 1, Fig. 4). Authorized through a Memorandum of Agreement with the United States Coast Guard, acoustic receivers were attached to pre-selected Aids to Navigation (ATON), such that the receivers were submerged approximately 3–3.5 m (10–12 ft) below the surface. We chose ATONs based on known

loggerhead turtle habitat use from previously collected satellite tag data, and to maximize our spatial coverage of Nantucket Sound. Detections of acoustic-tagged loggerheads outside of Nantucket Sound were obtained through collaboration with the Mid-Atlantic Acoustic Telemetry Observation System (MATOS [38]). MATOS is a database for researchers to manage and share acoustic transmitter and receiver data from projects throughout the Northwest Atlantic. Loggerhead acoustic transmitter IDs were uploaded to MATOS during summer and fall of 2021 and 2022. Loggerheads with satellite tags transmitted ARGOS-derived locations ($n=6$) and dive information ($n=1$; depth resolution ± 0.5 m and temperature resolution $\pm 0.05^\circ$ C) via Service ARGOS (Toulouse, France). For any satellite tags with shorter-than-expected tracks, we examined tag records to determine the cause of early cessation of transmissions. Specifically, we looked

Table 1 Acoustic receivers deployed by New England aquarium in nantucket sound in 2021 and 2022

2021				
Station identification	Latitude	Longitude	Deploy Date	Recover Date
NS1	41.540817	-70.398467	8/3/21	11/2/21
NS2	41.449017	-70.292	8/3/21	11/2/21
NS3	41.415533	-70.215117	7/27/21	11/2/21
NS4	41.436867	-70.086217	7/27/21	11/2/21
NS5	41.626412	-70.19174	8/3/21	11/2/21
NS6	41.556167	-70.243833	8/3/21	11/2/21
NS7	41.599279	-70.289466	8/3/21	11/2/21
NS8	41.4485	-70.42	8/3/21	Lost
NS9	41.541513	-70.541745	8/3/21	11/2/21
NS10	41.551441	-70.346793	8/3/21	11/2/21
2022				
Station identification	Latitude	Longitude	Deploy Date	Recover Date
NS1	41.540817	-70.398467	6/14/22	9/29/22
NS2	41.449017	-70.292	6/14/22	10/31/22
NS3	41.415533	-70.215117	6/14/22	10/31/22
NS4	41.436867	-70.086217	6/14/22	10/31/22
NS5	41.626412	-70.19174	6/14/22	10/31/22
NS6	41.556167	-70.243833	6/14/22	10/31/22
NS7	41.599279	-70.289466	6/14/22	10/31/22
NS8	41.4485	-70.42	6/14/22	Lost
NS9	41.541513	-70.541745	6/14/22	10/31/22
NS10	41.551441	-70.346793	6/14/22	10/31/22
NS11	41.432035	-69.981778	6/14/22	10/31/22
NS12	41.5865	-70.405025	6/14/22	10/31/22
NS13	41.382609	-70.417907	6/14/22	9/28/22
NS14	41.500728	-70.565341	6/14/22	10/31/22
NS15	41.505384	-70.645291	6/14/22	10/31/22
NS16	41.521171	-70.574401	6/14/22	10/31/22
NS17	41.638381	-70.047928	8/24/22	10/31/22

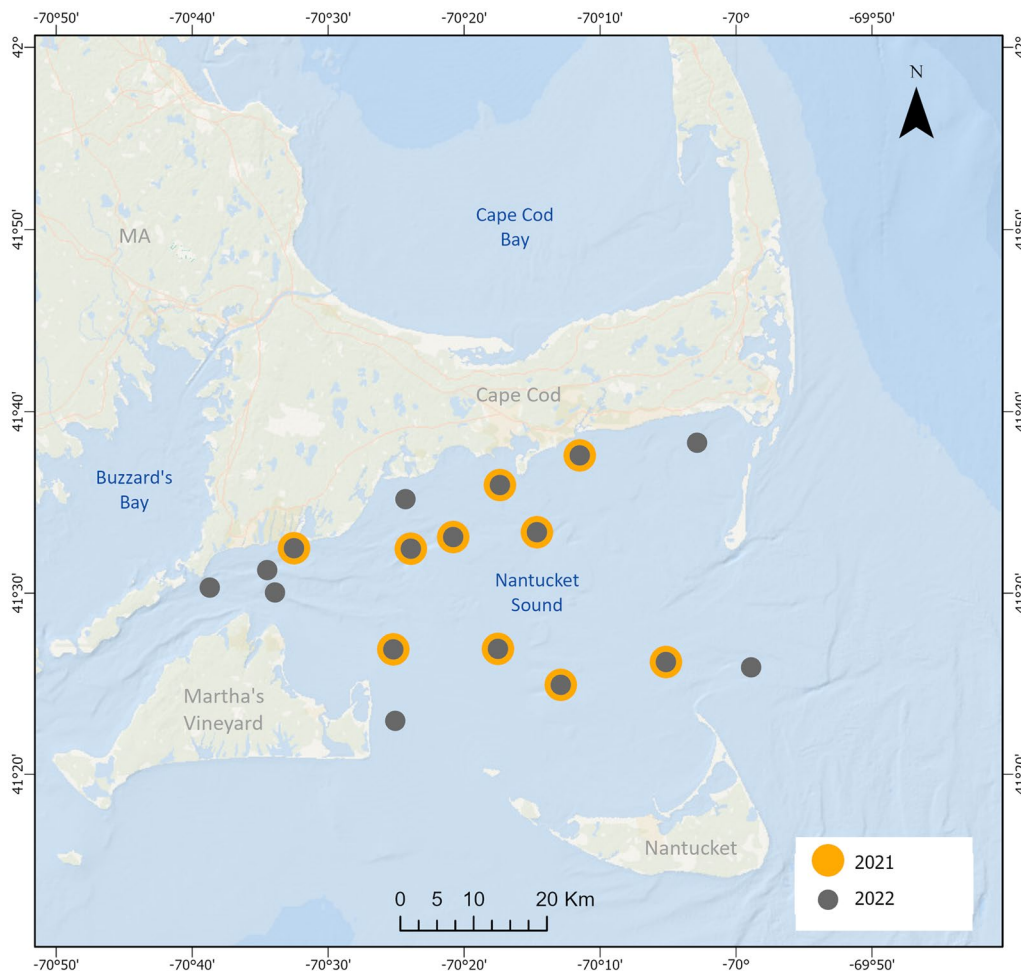


Fig. 4 Locations of New England Aquarium acoustic receivers in Nantucket Sound during 2021 ($n=10$) and 2022 ($n=17$)

at battery voltage and wet/dry sensor values in the status files to discern if the tags experienced drops in voltage or had evidence of biofouling, respectively [39]. We also examined location, temperature, and depth data (where available) to assess evidence of human interaction/mortality.

Post-hoc analyses

After release of all turtles, the following data were gathered from medical records for analysis: date admitted to the hospital, clinical history prior to surgery (i.e., morbidities, if applicable), date of surgery, SCL and body mass at time of surgery, model of acoustic transmitter, number of hospitalization days prior to surgery, number of days between discontinuing therapeutic systemic antibiotics and surgery, number of days between most recent hematologic and plasma biochemical analysis at the commercial laboratory and surgery, number of days between the most recent point-of-care blood analysis and surgery, number of days between the most recent radiographs and

surgery, surgery duration (incision to completion of closure), surgeon, day of food acceptance after surgery, date of surgical complication (if applicable), date of incision healing (final sutures or staples removed), and results of microbial and histologic evaluations (if applicable). Anesthetic data were gathered, including time between intubation and extubation, and time between extubation and return of the turtle to water.

We explored statistical analysis of variables that may have influenced surgical healing, categorizing turtles into two groups: (1) those that healed without complication, and (2) those that had surgical site complications. Binary logistic regression was performed using both a standard logistic regression model as well as Firth logistic regression model, which is an approach for rare events with small sample sizes. All variables were tested independently and in combination. Variables of interest included the duration between admission to the hospital and the date of surgery, body mass, SCL, ratio of body mass to SCL, use of cyanoacrylate

skin adhesive, number of layers in which the surgical site was closed, model of transmitter, surgery duration (min), and surgeon. Non-parametric correlation analysis was performed to examine the relationship between different continuous variables measured for turtles. Significance was assigned at $p \leq 0.05$. These analyses were conducted using the statistical program SPSS (version 28.0 for Macintosh, IBM SPSS Statistics, Armonk, NY).

In addition, we retrospectively analyzed total coliform (TC) data [most probable number (MPN)] for water samples that had been serially collected from each turtle's environment during hospitalization. For purposes of analysis, for days on which testing was not completed, data from the most recent prior result were used. Analyses were performed to compare coliform data for uncomplicated vs. complicated cases for two temporal scenarios: (1) evaluating coliform data from the date of transmitter implantation through the date of healing; and (2) evaluating coliform data from the date of transmitter implantation through the date of healing for uncomplicated cases, and the date of surgical repair for complicated cases. A *T* test using Microsoft Excel software was run under both scenarios for comparison of average TC MPN/100 ml, number of days of >1000 MPN/100 ml, number of days >10,000 MPN/100 ml, presence or absence of an increase to >1000 MPN/100 ml, presence or absence of an increase to >10,000 MPN/100 ml, number of increases to >1000 MPN/100 ml, and number of increases to >10,000 MPN/100 ml, with an increase defined by a TC concentration greater than the most recent prior sample. We also evaluated the number of times that each turtle was relocated among different enclosures under both of the above time scenarios.

Results

The fifteen turtles that had acoustic transmitters implanted were admitted to hospital between 12/18/2020 and 12/27/2020 ($n=4$), and 12/14/2021 and 1/10/2022 ($n=11$). Average SCL was 47 cm (median 45, range 37–61) and average body mass was 20 kg (median 18, range 8–39). Nine turtles were <50 cm SCL and were implanted with the V13 transmitter, while six turtles ≥ 50 cm SCL were implanted with the V16 transmitter. Four turtles were considered to have had uncomplicated rehabilitation prior to surgery, requiring only routine rehabilitative care, and no antibiotic therapy. Eleven turtles had some degree of illness that required management prior to surgery, including radiographic evidence of pneumonia that prompted the use of systemic antibiotics ($n=9$), one of which also showed chronic anorexia; and two turtles that had ophthalmic disorders.

Procedures

Surgery duration (incision to completion of closure) averaged 19 min (median 15, range 10–47). Thirteen implantations were completed by one surgeon, while two were completed by another surgeon. The time between intubation and extubation averaged 56 min (median 72, range 41–134), and the time between extubation and return of the turtle to water averaged 24 min (median 21, range 10–67). Two turtles were removed from the water for an additional period of recovery due to uncoordinated swimming. The final return-to-water time was not recorded for these two turtles, but it was later that same day. Methods to facilitate recovery included epinephrine ($n=7$), atropine ($n=7$), doxapram ($n=5$), and GV-26 acupuncture ($n=4$). These methods were often used concurrently (one or more used, $n=8$) or not at all (none used, $n=7$). Post-operatively, nine turtles began eating on the first day that food was offered [same day as surgery ($n=6$), the day after surgery ($n=3$)], and six began eating on the second day that food was offered [one day after surgery ($n=5$), 2 days after surgery ($n=1$)].

For all turtles, surgical sites were considered healed (all skin staples and sutures removed), on average, 66 days after surgery (median 50, range 41–141). Twelve turtles' surgical sites healed without complication, on average, 55 days after surgery (median 47, range 41–100). Among these 12 turtles, incision healing was considered excellent for six cases (minimal scarring, smooth surface, normal pigmentation, Fig. 5), and good for six cases (residual superficial eschar, irregular surface, and/or mild to moderate black pigmentation) (Fig. 6). For all cases for which healing was considered to be excellent, and for three cases for which healing was considered to be good, skin staples remained fully intact until the time of removal. For three other cases for which healing was considered to be good, one to two staples (out of 4–5 initial staples) prematurely sloughed between 3 and 5 weeks post-operatively.

Three turtles required surgical site repair 26, 48, and 55 days after initial surgery because the incision began to open, exposing the transmitter (Figs. 7, 8, 9). These turtles had straight carapace lengths of 42, 46, and 52 cm, thus had been implanted with V13, V13, and V16 transmitters, respectively. Their original surgery sites had been closed with one, one, and two subcutaneous suture layers, respectively; and skin closure had been completed with suture, staples, and staples, respectively. Loosening and loss of individual sutures and staples were noted for these cases beginning approximately 3 weeks post-operatively. Surgical repair was performed using similar methods to the initial procedure, including intravenous and local anesthesia ($n=3$), and inhalant anesthesia ($n=1$). The sites were explored, debrided, and lavaged,

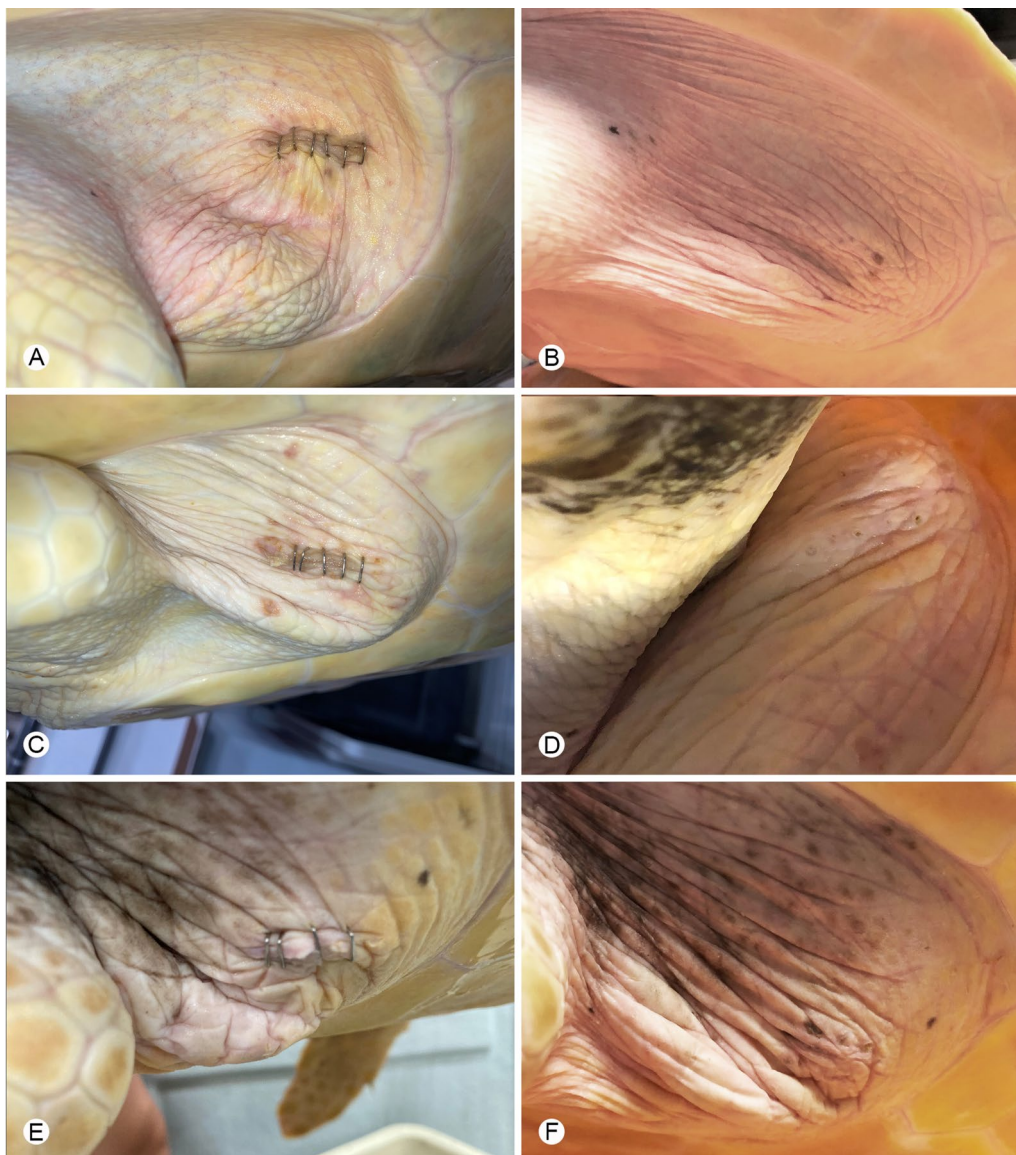


Fig. 5 Examples of excellent healing of uncomplicated surgical sites for three loggerhead turtles (*Caretta caretta*) in which subcutaneous acoustic transmitters were implanted in the right pre-femoral region. These cases have minimal scarring, smooth surface, and normal pigmentation. In all images the turtle's head is to the right and its carapace is at the top. **A, B** Turtle 20-1141 1 day post-operatively and 7 weeks post-operatively; **C, D** Turtle 20-1083 2 day post-operatively and 7 weeks post-operatively; **E, F** Turtle 20-1128 1 day post-operatively and 7 weeks post-operatively

leaving the transmitter in place, and attempting to create a deeper insertion site while freeing additional adjacent soft tissue to allow for more thorough closure over the surface of the tag. Closure was achieved in three layers. Two cases had aerobic bacterial cultures collected from the surgical site at the time of repair, which grew *Morganella*, *Proteus*, and non-hemolytic *Streptococcus*; and *Morganella*, respectively. Based on culture results and serial examinations, these two cases were treated with ceftazidime (22 mg/kg IM 3qd x 7wk [40]) and enrofloxacin (20 mg/kg PO q7d x 4wk [41]), respectively. Culture

was not performed during initial repair for the third case, because the turtle was already being treated with enrofloxacin, ceftazidime, and terbinafine due to radiographic and microbiologic evidence of pneumonia that had developed 3 weeks after initial surgery. These medications were being administered at the time of repair, and were continued for 1 month after repair.

Two of the cases that required repair were considered to be healed 34 and 98 days later, respectively. In the third case, the site began to open again 28 days after repair, and a decision was made to remove the transmitter. Removal

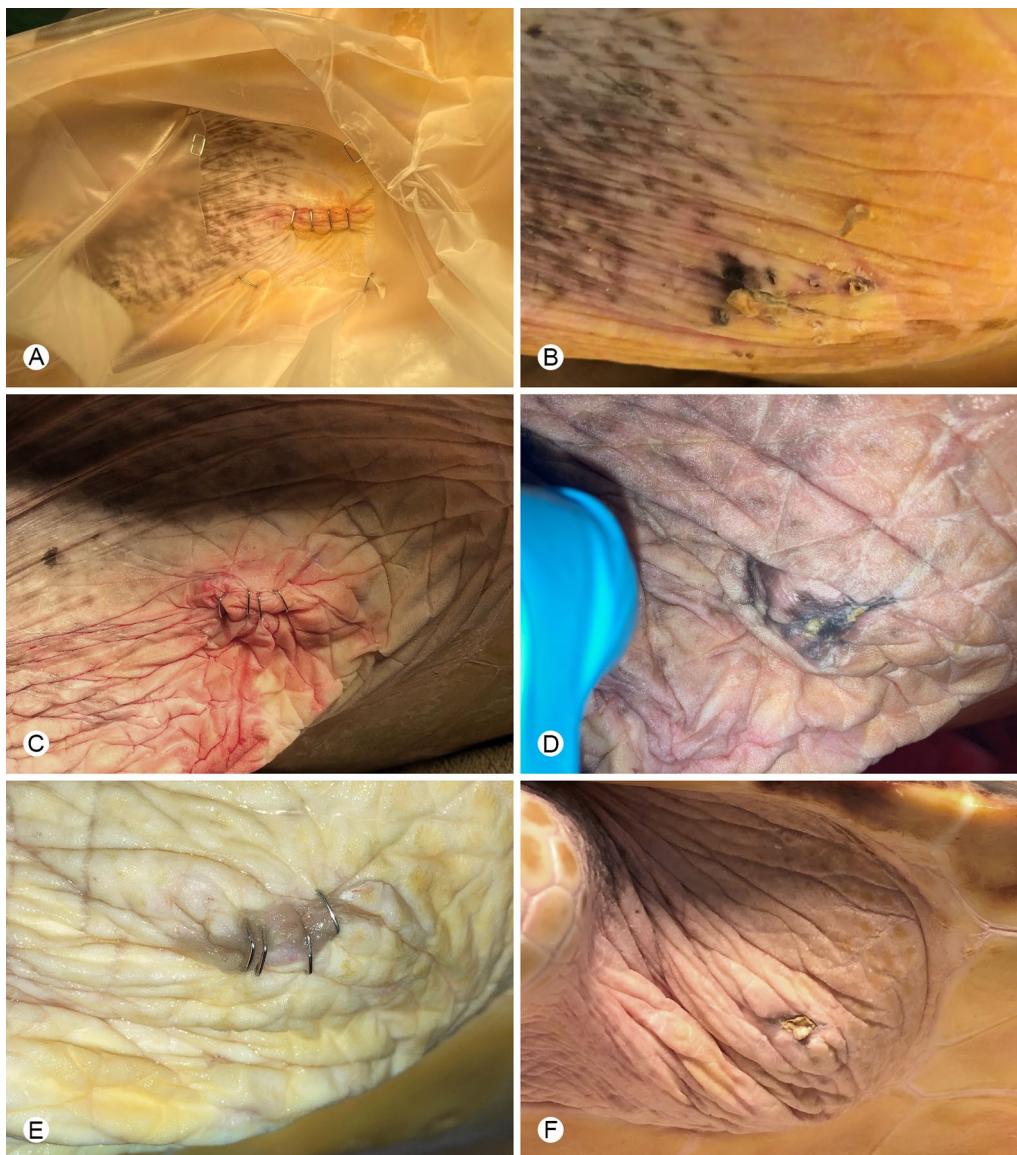


Fig. 6 Examples of good healing of uncomplicated surgical sites for three loggerhead turtles (*Caretta caretta*) in which subcutaneous acoustic transmitters were implanted in the right pre-femoral region. These cases have residual superficial eschar, irregular surface, and, or mild to moderate black pigmentation. In all images the turtle's head is to the right and its carapace is at the top. **A, B** Turtle 21-0784 day of surgery and 7 weeks post-operatively; **C, D** Turtle 21-0805 day of surgery; and 6 weeks post-operatively; **E, F** Turtle 21-1145 1 day post-operatively and 8 weeks post-operatively

was performed under intravenous and local anesthesia, and the turtle was intubated and provided with positive pressure ventilation. Upon surgically exploring the site, the tag and surrounding tissue were found to be covered with a thin membrane of tan exudate (Fig. 9). The transmitter was removed; the deep tissue was biopsied, cultured, debrided, and lavaged, then closed in two layers. Histopathologic evaluation of the biopsy specimen revealed fibroplasia, epithelialization, and mild heterophilic and histiocytic inflammation with intralesional

bacteria. Aerobic culture identified *Citrobacter freundii*, *Morganella morganii*, non-hemolytic *Streptococcus* sp., *Proteus mirabilis*, and *Providencia rettgeri*. Treatment with enrofloxacin (20 mg/kg orally [PO] q7 d×3 wk) was initiated based on susceptibility results. Despite transmitter removal and primary closure, the incision for this case opened again 21 days later, and the surgical site was then managed as an open wound until healed. Overall, for this case, the time from initial surgery to surgical site healing was 141 days.

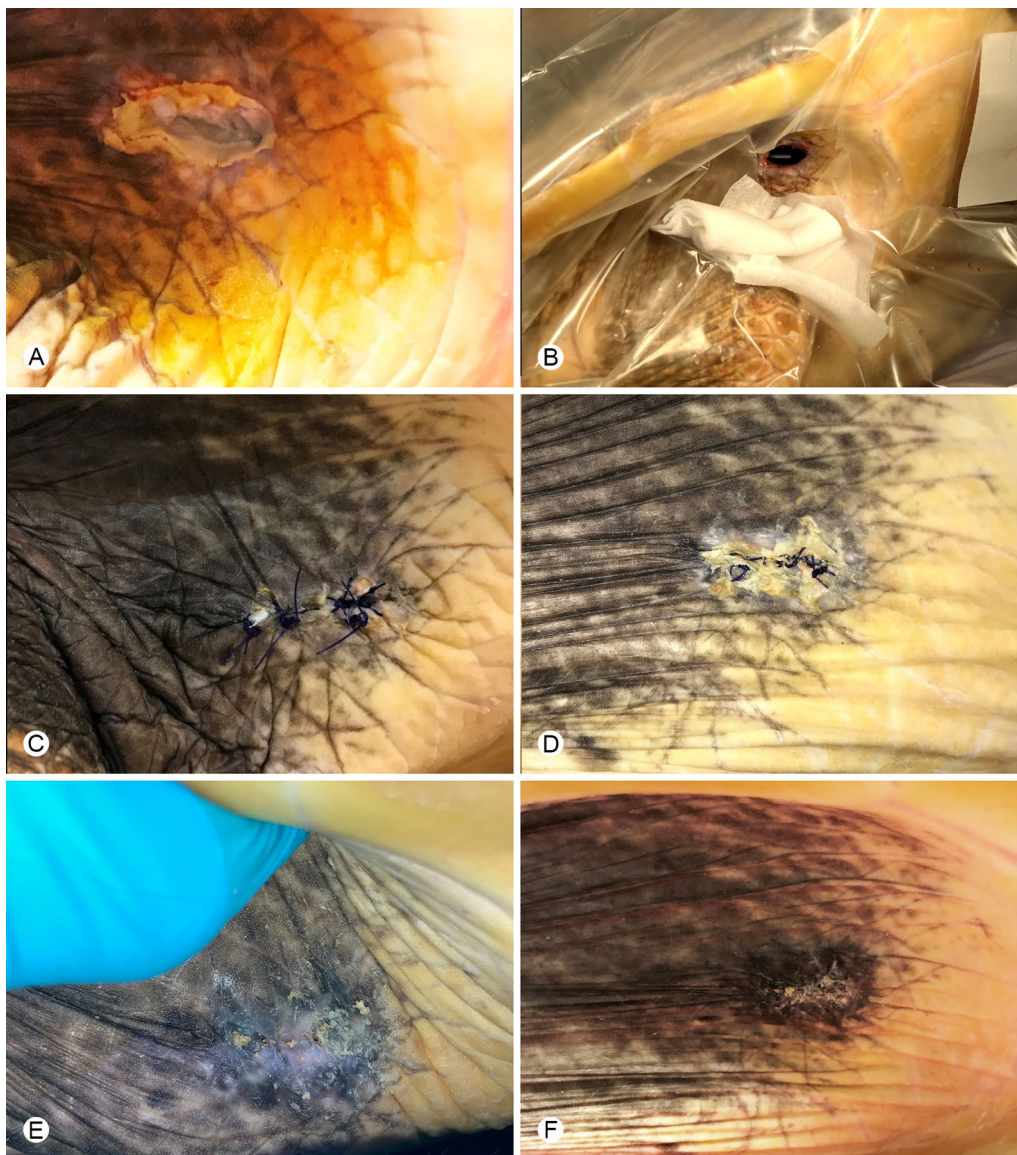


Fig. 7 Complication of surgical subcutaneous acoustic transmitter implantation in the right pre-femoral region of a loggerhead turtle (*Caretta caretta*). In all images the head is to the right and the carapace is at the top. Turtle 21–0719. **A** Four weeks after transmitter implantation, the skin sutures had loosened and been expelled, leaving only a thin eschar covering the gap in the skin incision; **B** under sedation on the same day, the acoustic transmitter is seen immediately deep to the skin after removing the thin eschar; **C** intact skin sutures 10 days after repair of the dehiscid surgical site; **D** surgical site 3 weeks after repair; **E** skin healing 5 weeks after repair, sutures have been removed; **F** skin healing 8 weeks after repair

Summary data for pre-operative and post-operative variables are provided in Table 2, including number of hospitalization days prior to surgery, number of days that systemic antibiotics were discontinued prior to surgery, number of days between most recent hematologic and plasma biochemical analysis at the commercial laboratory and surgery, number of days between the most recent point-of-care blood analysis and surgery, number of days between the most recent radiographs

and surgery, number of days to healing, and transmitter model. Turtles that had been under hospital care for a longer period of time prior to surgery typically showed faster wound healing of the surgical site ($r_s = -0.68$, $P = 0.005$). Shorter surgery duration (Firth logistic: $\chi^2 = 9.96$, $df = 1$, $P = 0.002$) was significantly associated with shorter healing time. Statistical analysis of pre-operative and total coliform data in comparison with

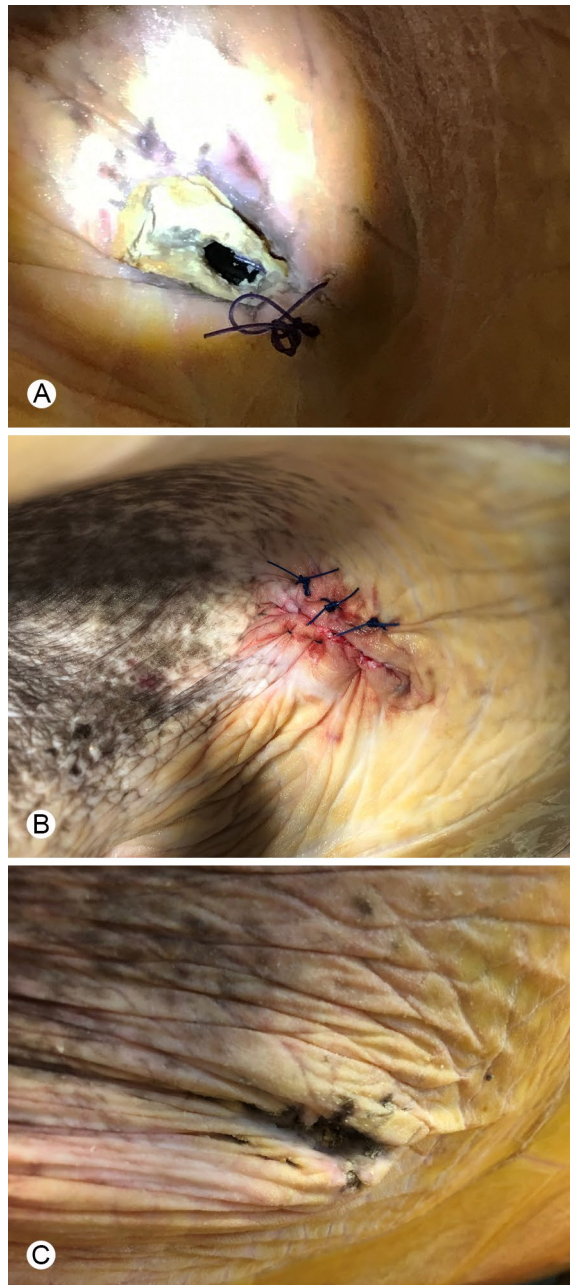


Fig. 8 Complication of surgical subcutaneous acoustic transmitter implantation in the right pre-femoral region of a loggerhead turtle (*Caretta caretta*). In all images the head is to the right and the carapace is at the top. Turtle 21–0838. **A** Seven weeks after transmitter implantation; the majority of skin sutures have been expelled and the single remaining skin suture is loose, leaving only a thin eschar partially covering the skin incision; the transmitter is visible immediately deep to the eschar and skin; **B** closure of the repaired surgical site after debridement and lavage; **C** healed incision 12 weeks after repair

the occurrence of complications revealed no significant differences for the majority of variables.

Prior to release, of the 14 turtles that had transmitters present, 13 turtles' transmitters were detected by hydrophone in the rehabilitation hospital. The transmitter for one turtle was dysfunctional despite being functional prior to implantation. Discussion with the transmitter manufacturer provided minimal insight regarding potential reasons for loss of function, and attempts to activate the transmitter through transcutaneous magnet exposure were not successful. Potential removal of the dysfunctional transmitter was discussed with federal officials but in light of the already healed status of the surgical site, an agreement was reached to leave the transmitter in situ to avoid further surgical trauma and delayed release.

Post-release monitoring

As of March, 2023, we have obtained detections from 100% of turtles with functional acoustic transmitters ($n=13$). A total of 915 detections have been obtained from 40 different acoustic receiver locations off the coasts of Massachusetts, Rhode Island, New York, Virginia, North Carolina and in southern New England offshore waters, with the majority of detections (90%) from Nantucket Sound (Fig. 10, 11). The 13 tagged turtles generated 5–235 detections (mean \pm SD; 70 ± 73) on 1–13 individual acoustic receivers (5 ± 3) for periods of 3–400 days post-release (118 ± 123). Eleven out of 13 turtles were detected within the Nantucket Sound array during the days to months following release, while two turtles have only been detected by acoustic receivers deployed in other regions via data shared by MATOS researchers. In total, seven turtles have been detected outside of Nantucket Sound via MATOS, including all four turtles released in 2021 and three turtles released in 2022. The loggerhead with the maximum detection duration of 400 days post-release was detected in New England waters in successive seasons.

Of the six satellite-tagged turtles, four had functional acoustic transmitters, one had a dysfunctional acoustic transmitter, and one's acoustic transmitter had been removed to expedite incision healing and release. Three turtles were tracked for >100 days, with two turtles still transmitting at the time of this report. Three turtles had shorter than expected satellite tracking durations of 5, 35, and 70 days, respectively. Satellite tag records from the 5 day track showed temperature and location data consistent with removal from the water, indicating probable mortality of this turtle. The 35 day track had no evidence

of tag biofouling or battery failure based on our assessment of the tag's status files, and was due to either tag loss or antenna breakage, as this turtle continued to be successfully tracked by its acoustic transmitter for at least 321 days after the satellite tag stopped reporting. The turtle with the 70 day track also had no evidence of tag biofouling or battery failure in the status files, and the last transmissions occurred in the fast ferry lane between Hyannis and Nantucket. We have not had additional acoustic transmitter detections from this turtle, which suggests the turtle sustained satellite tag damage just before departing Nantucket Sound or may have died. Future acoustic detection of this turtle would be informative of its fate. Turtles with short tracks remained in Nantucket Sound for the duration of their tracking period, while the other three turtles made seasonal migrations to coastal waters of Virginia, North Carolina, and Florida, respectively. Summary data for acoustic transmitters and satellite tags can be found in Table 3.

Discussion

Acoustic telemetry is an important and widely used tool for the study of sea turtle biology [11]. However, external acoustic transmitters are prone to premature detachment in some cases [17]. Based on successful long-term retention of surgically implanted acoustic transmitters in other vertebrate taxa, we conducted this pilot study to surgically implant acoustic transmitters subcutaneously in the pre-femoral region of 15 loggerhead sea turtles. The pre-femoral region is routinely used for surgical procedures in turtles, allowing access to the coelomic viscera for procedures, such as enterotomy, oophorectomy, visceral biopsy, and laparoscopic sexing. Pre-femoral surgical sites, even those that fully enter the coelom, generally heal within 10 weeks, often much sooner [42–44]. We expected a similar outcome after subcutaneous transmitter implantation, especially considering the minimally invasive nature of the procedure (i.e., a small skin incision, and no entry into the coelom). Among the 12 turtles in this study that did not experience complications, incision healing occurred at a median of 7 weeks post-operatively, consistent with expectations. Three turtles experienced surgical site complications that required

repair, two of which healed with the transmitter still implanted, while the third required transmitter removal. All turtles' surgical sites healed, and all turtles were released to the wild.

Among the fourteen turtles that were released with implanted transmitters, thirteen had functional transmitters. One turtle had a satellite tag with location data (inshore) and temperature data (rapid change of $7+^{\circ}\text{C}$) that was consistent with a human interaction/mortality event soon after release. This turtle was one of the three cases with surgical complications, but its cause of death (inferred from satellite tag data) appears to be unrelated to its acoustic transmitter. Results to date have been extremely promising, with all thirteen turtles detected multiple times over periods of up to 400 days. Due to the quarterly schedule of MATOS data uploads and sharing, the detection data for the 2022 cohort is limited so far. We anticipate more detections for these turtles from outside research projects as new data are made available through MATOS in the coming months and years, as well as from the redeployment of our Nantucket Sound receiver array in 2023 and beyond. Our high detection success supports the findings of Barco & Lockhart [25] that the carapace does not completely inhibit transmission of subcutaneously implanted acoustic transmitters in cheloniid sea turtles. Nonetheless, the extent to which the carapace may affect transmitter detection range for live turtles remains unknown. In previous studies, individual tracking duration for sea turtles with externally attached acoustic transmitters averaged 186 days [11]. Since our internal tags have only been deployed for <1 (2022) to <2 (2021) years, it is premature to conduct a comprehensive comparison of tracking durations between internal and external methodologies, but our preliminary results are encouraging with minimum tracking durations of 356 and 400 days for two individuals released in 2021. High detection success, long-term transmitter retention/transmission, annual deployment of New England Aquarium acoustic receivers, and the continued expansion of acoustic receiver arrays along the U.S. east coast [45], will provide new opportunities to monitor the movements and survival of sea turtles across broad regions of the northwest Atlantic.

(See figure on next page.)

Fig. 9 Complication of surgical subcutaneous acoustic transmitter implantation in the right pre-femoral region of a loggerhead turtle (*Caretta caretta*). In all images the head is to the right and the carapace is at the top. Turtle 21–0781. **A** Surgical site closure on the day of initial transmitter implantation; **B** incompletely healed surgical site 8 weeks after transmitter implantation; the site was debrided, lavaged, and closed; **C** 4 weeks after repair, the site is again poorly healed and the transmitter is visible immediately deep to the skin; **D** transmitter and tan membranous capsule that were removed from the surgical site 4 weeks after repair (12 weeks after implantation); **E** surgical site closure on the day of transmitter removal; **F** surgical site 3 weeks after tag removal, again healing poorly; **G** several days later the site has opened, at which time sutures were removed and the site was allowed to heal as an open wound by granulation; **H** 8 weeks after tag removal (20 weeks after initial implantation) the site has granulated

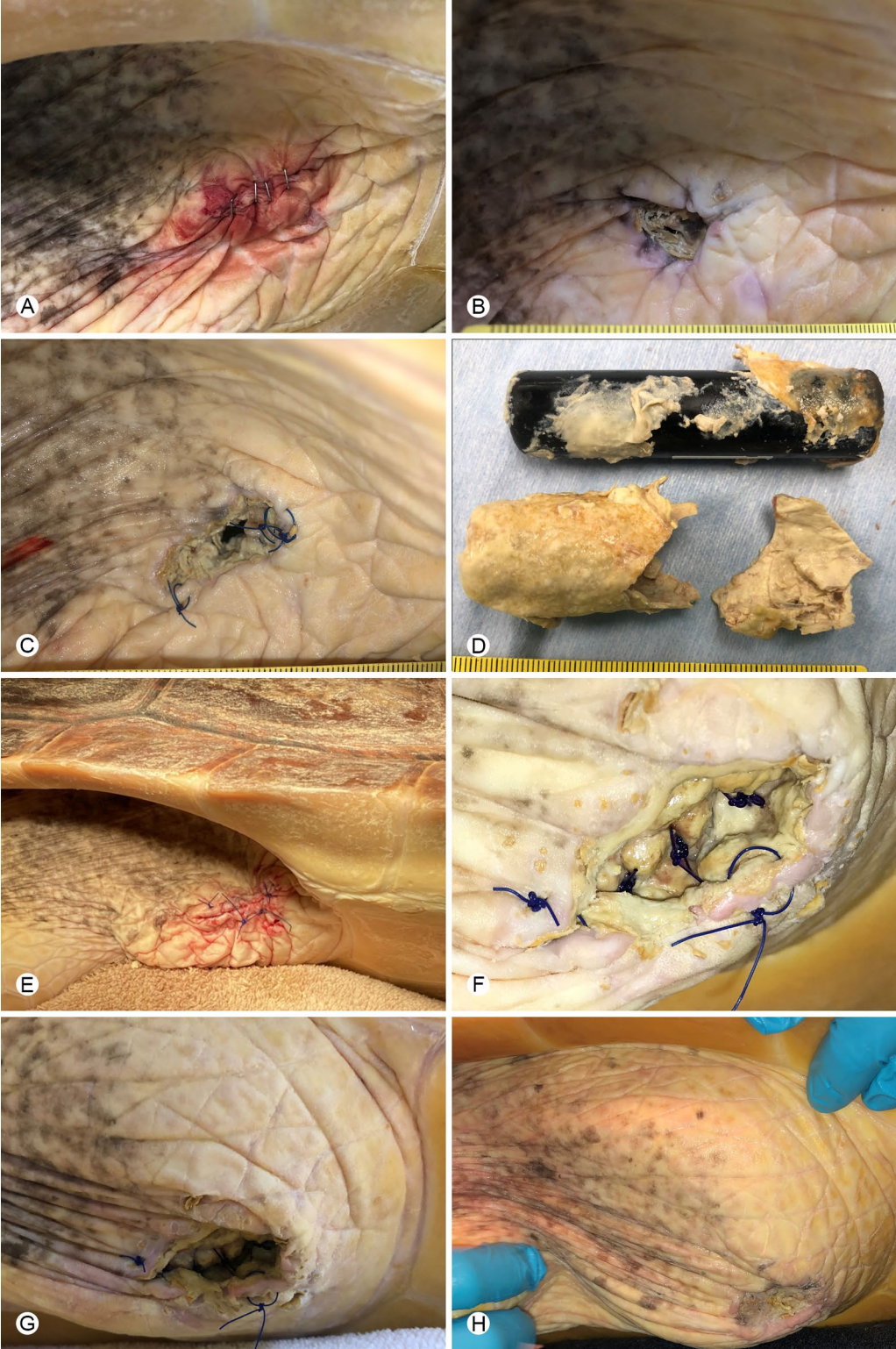


Fig. 9 (See legend on previous page.)

Table 2 Summary data for events associated with acoustic transmitter implantation for 15 loggerhead turtles (*Caretta caretta*)

	Uncomplicated (n = 12) Mean (median; range)	Complicated (n = 3) Mean (median; range)
Days since admission to hospital at time of surgery ^a	122 (116; 58–172)	84 (84; 57–111)
Days until surgical site considered healed (all skin staples or sutures removed)	55 (47; 41–100)	112 (136; 60–141)
Surgery duration (min) ^{a, b}	14 (14; 10–23)	37 (33; 30–47)
Days since last therapeutic use of antibiotic	79 (74; 35–133) (n = 8); four cases had no prior antibiotic	56 (n = 1); two cases had no prior antibiotic
Days since most recent hematologic and plasma biochemical panel at referral laboratory prior to implant	89 (89; 85–93) (n = 2); ten cases had no prior referral labwork	86 (n = 1); two cases had no prior referral labwork
Days since most recent point-of-care blood chemistry panel prior to implant	113 (113; 48–164) (n = 11); four cases had no prior point-of-care analysis	76 (83; 52–92) (n = 3)
Days since most recent radiograph prior to implant	16 (13; 6–29) (n = 12)	7 (9; 3–10) (n = 3)
Transmitter model	V13 (n = 7), V16 (n = 5)	V13 (n = 2), V16 (n = 1)

^a Significant difference between uncomplicated and complicated cases by non-parametric analysis.

^b Significant difference between uncomplicated and complicated cases by Firth regression

The anesthetic and surgical procedures used in this study allowed for efficient transmitter implantation, rapid anesthetic recovery, and rapid return of normal biological functions. With a median surgical time of 15 min, and median intubation to return-to-water time of approximately 90 min, this procedure was safe and practical. Surgery for eight turtles was conducted without need for inhalant anesthesia (but with intermittent positive-pressure ventilation). Turtles returned to their pre-operative swimming and feeding behavior within 2 days, at most, often accepting food on the same day of surgery when offered. We saw no evidence that transmitter implantation inhibited limb function. Given the speed of recovery, it is possible that more rapid return to the wild could be considered for future projects that use this methodology. For certain field studies, for example, methods could likely be developed and refined, such that turtles could be released later the same day or the following day. Major trade-offs would include more rapid return to natural environmental conditions, diet, and behavioral repertoire, lower cost and lesser resource allocation for long-term holding, vs. thorough post-operative monitoring and assessment of incision healing.

Of substantial concern are the complications seen in three turtles in this study. Here, we discuss many factors that may have influenced surgical outcome, yet given the small sample size and relatively large number of variables (some of which co-vary), it is not possible to draw definitive conclusions. One of the more intriguing results of our post-hoc evaluation is that turtles that had been hospitalized for at least 100 days prior to surgery typically experienced faster wound healing (i.e., considered

healed within 50 days). Whereas, those turtles that had been hospitalized for a shorter period prior to surgery (84 days or less) showed protracted wound healing (mean 92 days). This occurrence was due to the timing of project approval in year 1, which somewhat delayed the procedures relative to the procedures in year 2. While both cohorts of turtles met inclusion criteria, it is possible that turtles that experienced complications had not yet recovered as thoroughly from their stranding events. If so, it is possible that a not-yet-robust immune response and diminished healing capacity could have influenced outcome. This observation, however, is complicated by confounding factors of surgical duration and surgeon.

Surgery durations longer than 20 min had a higher incidence of complication, with the three complicated cases experiencing the longest surgical durations. Subjectively, prolongation of the procedure generally occurred when the surgeon was not satisfied with the initial positioning of the transmitter, taking additional time for dissection and re-positioning. It is possible that some inherent aspect of these turtles' anatomy affected surgical duration, or it is possible that the additional surgical trauma may have resulted in greater post-operative inflammation, longer healing time, and greater risk of infection. Surgery duration was inherently increased for one of these cases by the use of skin sutures (requiring time to place and tie individual suture knots) vs. staples (rapid deployment).

Differences in complication outcomes between surgeons were considered in data analyses to help understand the transferability of the procedure. However, decisive factors of surgical technique (aside from the

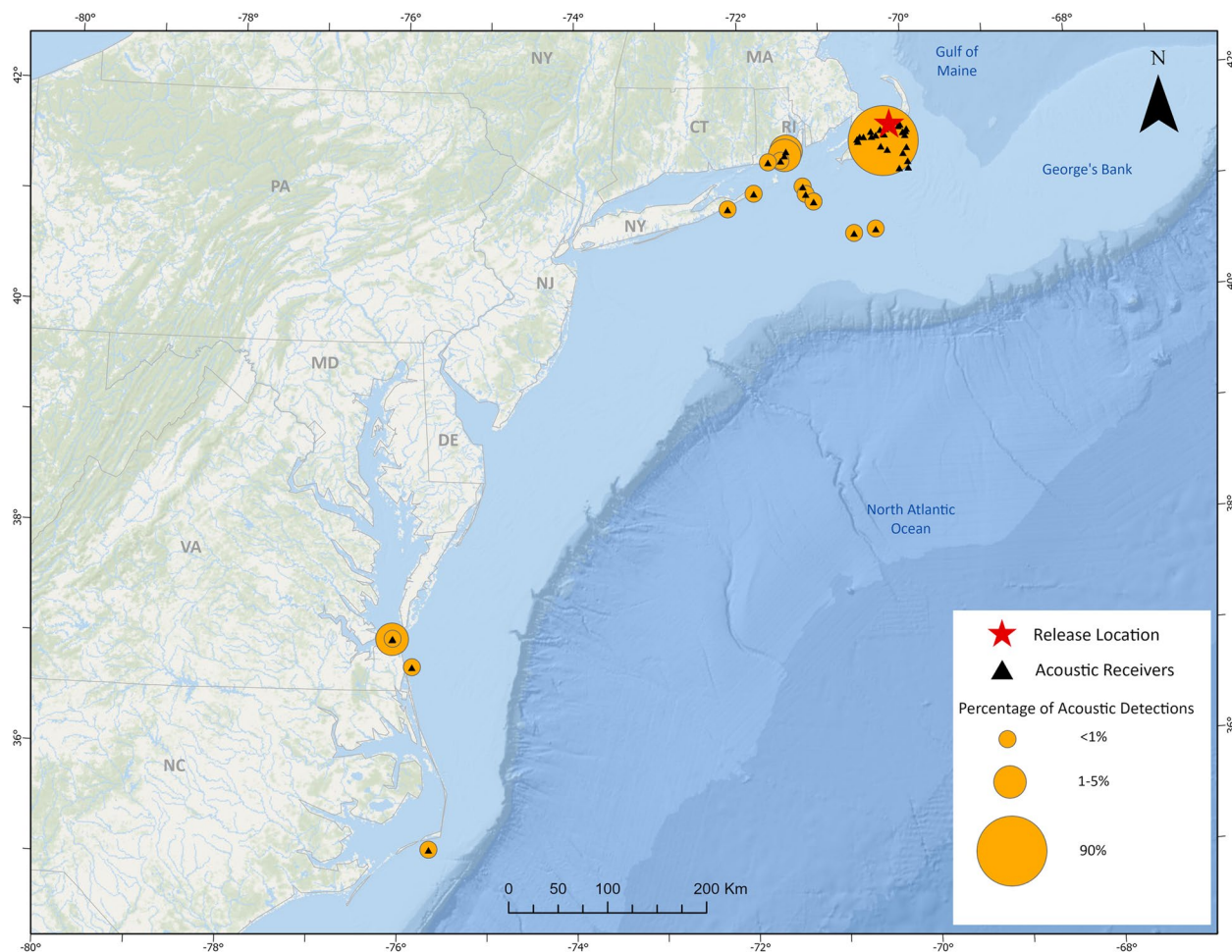


Fig. 10 East coast acoustic detection data for 13 loggerhead sea turtles from August, 2021 to October, 2022. Red star represents the release location of all turtles. Black triangles represent acoustic receiver locations that had detections, and the percentage of acoustic detections is represented by the size of the circle. Detections from receivers that were in close proximity were clustered to calculate the detection percentage

use of skin sutures) were not identified. Although we attempted to use consistent methods among surgeons, it is possible that variations in the depth of tag implantation, exact positioning of the tag, etc. could have affected outcome. The limited number of cases (small sample size), and presence of confounding factors (i.e., duration of prior hospitalization, duration of surgery) prevent clear understanding of the influence of the surgeon on outcome, but comparative studies of transmitter implantation in fish have demonstrated that surgical experience influences procedure duration and complication rate [46, 47]. Transferability of this procedure to future investigators will require refinement of methods and careful training.

The surgical methods and materials used in this study were based on routinely used methods for sea turtles, including the choice of poliglecaprone suture based on its comparatively good performance in this species [48].

While we are unsure which exact poliglecaprone product we had in stock across the timeframe of these procedures (i.e., which manufacturer’s product), it seems unlikely that the products would vary so greatly as to affect outcome. Other minor surgical variations in several cases, as detailed in the Results, included the number of subcutaneous closure layers and the use of cyanoacrylate skin adhesive, all of which showed no clear trend among the complicated cases.

Surgical complications could be caused by primary infection, or infection could have occurred secondary to poor tissue healing. Rates of infection in some types of human surgery increase incrementally with surgical duration [49], and this phenomenon may explain the infections seen in this study. To limit the risk of infection, pre-operative antibiotics were provided, skin was prepared following surgical convention, sterile drapes and instruments were used, and transmitters were disinfected

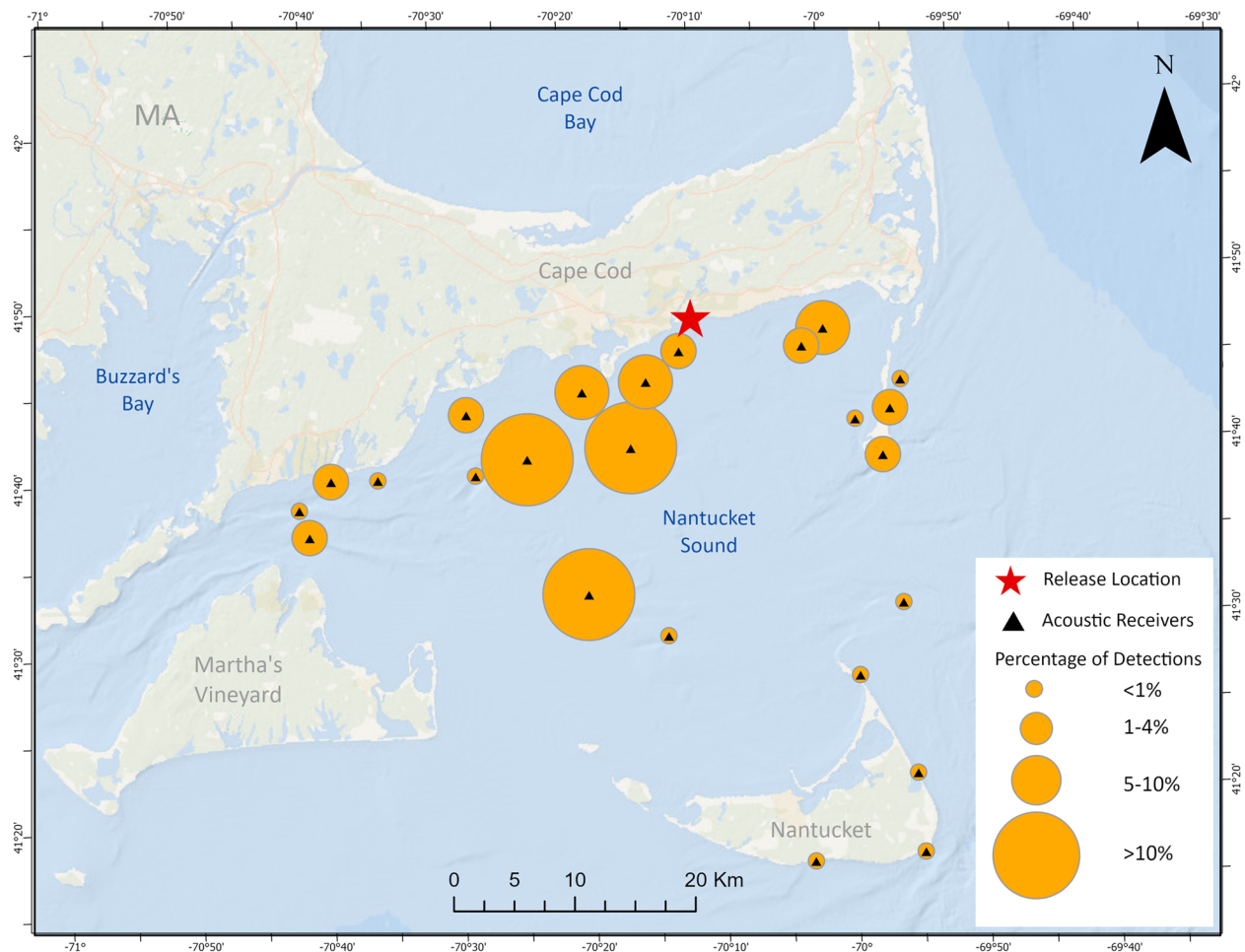


Fig. 11 Detection data in Nantucket Sound and south of Nantucket for 11 loggerhead sea turtles from August, 2021—October, 2022. Red star represents the release location of all turtles. Black triangles represent acoustic receiver locations that had detections, and the percentage of acoustic detections is represented by the size of the circle

prior to insertion. Oxytetracycline was chosen as the pre-operative antibiotic due to its known pharmacokinetic profile and clinical safety in this species, and its efficacy for skeletochronological evaluation in the event of future carcass recovery [29, 50]. Based on pharmacokinetic data for oxytetracycline in this species, therapeutic plasma concentrations were likely present at the time of surgery [29]. Other antibiotics with greater gram-negative bacterial efficacy could also be considered for future work ([40, 41, 44]). Skin disinfection, while completed conventionally, could be enhanced for future procedures, including thorough pre-cleaning with warm water and soap, longer duration of disinfectant exposure, and the use of multiple disinfectant types. Although the transmitter was disinfected prior to insertion, it would be preferable to use a transmitter that had truly been sterilized. Currently, transmitters are not supplied sterilely from the manufacturer, and the manufacturer recommends povidone

iodine or chlorhexidine solution for disinfection prior to implantation. Seeking higher level disinfection, we chose to disinfect the transmitters with ortho-phthalaldehyde solution, as commonly done for certain instruments for other chelonian surgical applications (e.g., laparoscopy). The transmitters are not tolerant of autoclave conditions, but we did explore the possibility of ethylene oxide gas sterilization. Neither the transmitter manufacturer nor the gas sterilizer manufacturer would vouch for the safety or efficacy of this method (citing safety concern due to the presence of a battery). Manufacturers should validate methods to sterilize transmitters that are intended for surgical implantation, such that sterilely packaged transmitters can be provided directly to the user.

The size of the transmitters and the size of the turtles were considered as related to complications. Notably, the five smallest turtles in this study all healed without complications. In assessing the smaller V13 vs. the larger

Table 3 Summary data for acoustic transmitters and satellite tags for 15 loggerhead turtles (*Caretta caretta*)

Turtle identification	Acoustic transmitter model	Case type	Release date	Acoustic detection number	Receiver number	Acoustic tracking duration (d)	Satellite tracking duration (d)	Last date in Nantucket Sound
20-1083	V13-1H	U	8/5/21	92	13	400	NA	9/8/22
20-1128	V13-1H	U	8/5/21	43	5	85	120	10/27/21
20-1141	V13-1H	U	8/5/21	18	3	155	NA	8/5/21
20-1145	V13-1H	U	8/5/21	28	3	356	35	10/7/21
21-0719	V13-1H	C	7/16/22	235	8	96	NA	10/19/22
21-0784	V13-1H	U	7/6/22	55	2	87	NA	9/30/22
21-0805	V16-4H	U	6/29/22	20	3	39	NA	6/29/22
21-0810	V16-4H	U	6/29/22	172	5	29	70	7/27/22
21-0829	V13-1H	U	7/6/22	53	8	98	NA	9/3/22
21-0833	V13-1H	U	7/6/22	157	6	109	NA	10/22/22
21-0837	V16-4H	U	6/29/22	27	6	75	NA	9/11/22
21-0839	V16-4H	U	7/6/22	10	1	4	NA	7/6/22
21-0838	V13-1H	C	7/27/22	5 ^a	2	3	5	8/1/22
21-0834	V16-4H	U	6/29/22	0 ^b	0		355 ^d	10/5/22
21-0781	V16-4H ^c	C	9/6/22	NA	NA	NA	286 ^d	9/17/22

Acoustic tracking durations should be considered a minimum, since transmitters can remain active for 1,113 days (V13-1H) and 2435 days (V16-4H)

U uncomplicated transmitter implant; C Complicated transmitter implant; NA not applicable; ^a = probable mortality; ^b = dysfunctional acoustic transmitter; ^c = transmitter removed; ^d = actively transmitting as of June 20, 2023.

V16 transmitter, two of the three complicated cases were implanted with the smaller transmitter. In addition, among the turtles ≥ 50 cm SCL that were implanted with the larger V16 transmitters, the two smallest turtles healed without complication. Overall, while we cannot completely define the effect of transmitter size on healing, there is no clear trend.

In comparison with most pre-femoral surgical procedures, the current study resulted in a relatively large foreign body intentionally placed within the surgical site. Despite our expectations, it is possible that there is enough subcutaneous movement of the transmitter in this region to interfere with healing, favoring expulsion of the transmitter (e.g., in response to movement of the coelomic muscle wall during ventilation or locomotion). Expulsion of subcutaneous transmitters has been reported in some studies of pinnipeds and fish [51, 52]. Based on results of this pilot study, several ideas should be considered for future investigations. First, if using very similar methods as those described here, surgeons should attempt to create a subcutaneous tunnel that results in the tag being placed as far as possible from the incision site, taking care to close the tunnel well to prevent migration of the tag toward the incision. Second, while a small skin incision may intuitively seem best for promoting transmitter retention, a small incision limits the amount of surgical exposure, and may increase the difficulty of orienting the transmitter as desired. It is possible that an alternative method using a larger incision

could result in more precise, deeper positioning of the transmitter, and more thorough closure of deep subcutaneous tissue over the transmitter. It is also possible that other anatomic locations could better protect the transmitter from movement. For example, we have recently explored several loggerhead turtle cadavers, and determined that a cranial pre-femoral insertion site would allow the transmitter to be implanted deep to the bridge of the carapace, possibly limiting its movement. While more invasive, one could alternatively consider intracoelomic transmitter implantation. As related to the risk of transmitter expulsion, Horning et al. [19, 24] suggest that intraperitoneal implantation (for pinnipeds) may allow the transmitter to more freely settle into a position of least resistance, rather than being held in the more confined subcutaneous space. Finally, while the use of subcutaneous sutures in sea turtles is routine, it is possible that these sutures stimulate tissue reaction that could result in complication; and delayed breakdown of such sutures at sub-mammalian body temperatures could result in prolonged suture presence [53, 54]. It would be interesting to assess healing in the absence of subcutaneous sutures, relying only on a very robust skin closure with suture material, perhaps incorporating subcutaneous tissue within skin sutures to reduce dead space overlying the transmitter (Craig Harms, DVM, North Carolina State University College of Veterinary Medicine, personal communication).

While not prospectively documented as part of this study, future similar studies may consider documentation of the phases of healing as described for green sea turtle (*Chelonia mydas*) skin biopsies [55]. In comparison with those criteria, healing that was characterized as “excellent” in the present study had achieved stage 4 healing (healed, visible) at the time of release. Those that had “good” healing progressed more slowly, and had achieved stage 2 to 3 healing at the time of release (closed, indented; closed, discolored, respectively). Stage 5 healing (healed, indistinguishable) was not seen in this study. It is possible that stage 5 healing may have been seen with a greater duration of post-surgical monitoring (minimally achieved at 176 days in green turtles after skin biopsy) [55]. It is also possible that truly “indistinguishable” surgical sites may not be achieved with the methods used in this study given the larger and deeper incisions in comparison with skin biopsies.

Conclusions

We found that 80% of turtles healed well after initial implantation of subcutaneous acoustic transmitters, all turtles healed well with additional management, and all turtles were released to the wild. This study demonstrates the importance of thorough monitoring of healing when evaluating novel surgical techniques in wildlife. To date, 100% of loggerheads with functional acoustic tags have been detected both within Nantucket Sound by the New England Aquarium receiver array (77%), as well as along the US northeast coast (MA to NC) by other acoustic telemetry projects (85%). Given the promise of acoustic telemetry for chelonian biological investigations, additional studies are warranted to establish the safest and most effective methods for transmitter implantation. These methods can likely be improved by the efforts of a variety of investigators, trialing a variety of methods. Future detections of the turtles described in this study are likely, and updates regarding the long-term outcome of this cohort will be produced over time.

Abbreviations

SCL	Straight carapace length
SC	Subcutaneously
SD	Standard deviation
IM	Intramuscularly
PO	Orally
TC	Total coliform
MPN	Most probable number
ATON	Aid to navigation
MATOS	Mid-Atlantic Acoustic Telemetry Observation System

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Author contributions

CI, KD, JK conceived and designed the study; CI, KT performed surgery; CI, EB analyzed medical and surgical data; KD, JK, EJ, SP acquired and/or analyzed transmitter data; NF acquired and VZ analyzed total coliform data. AK, SP, LL, SD, AB, KL, DE, CS, MBT, NN, MJ performed anesthesia and medical management. All authors read and approved the final manuscript.

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Availability of data and materials

Medical, surgical, and hospital environmental data are retained in the Tracks medical record system at New England Aquarium and are available from the corresponding author on reasonable request. Acoustic transmitter detection data are owned by the New England Aquarium and may be available upon reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the United States Fish and Wildlife Service as part of Permit Number ES69328D, Part VIII, effective April 12, 2021 through January 13, 2024; and the New England Aquarium’s Institutional Animal Care and Use Committee, Proposal 2021–04.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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