Assessment of wound healing of tagged gray (*Eschrichtius robustus*) and blue (*Balaenoptera musculus*) whales in the eastern North Pacific using long-term series of photographs

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Abstract

Tags have been used to examine migration routes and habitat use of large whales for >40 yr, however, evaluation of tag wound healing has largely been short-term, anecdotal or generalized. This study developed methods for systematic photographic assessment of long-term external consequences of tag placement, to determine potential differences in wound healing between species and tag types and thus advise future tagging efforts to possibly minimize undesirable side effects. Tag site appearance and healing characteristics were evaluated by two reviewers and a time series evaluated by five veterinarians from photographs during 995 postdeployment encounters with 34 gray and 63 blue whales tagged in the North Pacific. Blue whale resightings were less frequent, but spanned a longer time period due to earlier tag deployments than the more frequent gray whale follow-up observations. Swelling occurred in 74% of reencountered gray whales, with the highest frequency 6 mo postdeployment. Swellings were common in blue whales with early tag designs but rare with current models.

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Depressions occurred in 82% of gray and 71% of blue whales. This study demonstrates the value of follow-up studies of tagged animals and systematic scoring of photographs to quantitatively compare tag response.

Key words: blue whale, *Balaenoptera musculus*, gray whale, *Eschrichtius robustus*, satellite tag, wound healing, North Pacific.

Tags have been used to examine migration routes and habitats of large whales for >40 yr, however, evaluation of tag wound healing has largely been short-term, anecdotal, or generalized with only limited studies conducted long-term (weeks to years). Evaluating health consequences of tag deployments in large whales has been difficult due to the logistical challenges with relocating tagged individuals that range over very large areas, identifying the tag site, and evaluating it. An early evaluation of the efficacy of longer-term satellite-monitored radio tags recommended their use for identifying critical aspects of the movements of large whale species (Montgomery 1987). Argos tagging studies were first conducted with two endangered large whales: North Atlantic right whales (*Eubalaena glacialis*) (Mate *et al*. 1997) to help identify their nearshore migration route from the Bay of Fundy summer feeding area to the southeastern United States calving areas, and the bowhead whales (*Balaena mysticetus*) to describe their westerly migration from arctic Canada to Siberia through areas of >90% ice cover (Mate *et al*. 2000). Since then tags have been used on other endangered whales: southern right whales (*Eubalaena australis*) (Best and Mate 2007, Mate *et al*. 2007, Zerbini *et al*. 2016), North Pacific right whales (*Eubalaena japonica*) (Wade *et al*. 2006, Zerbini *et al*. 2015), North Atlantic bowhead whales (Heide-Jørgensen *et al*. 2007), and western gray whales (*Eschrichtius robustus*) (Mate *et al*. 2015). The earliest studies of tagging effects on large whales were of conventional VHF radio tags anchored solely in gray whale blubber, which examined immediate or short-term physical effects of tag placement (Mate and Harvey 1983, Goodyear 1993). A few studies have attempted long-term follow-up to assess response of individual whales to tag attachments (Best and Mate 2007, Mizroch *et al*. 2011, Walker *et al*. 2012, Robbins *et al*. 2013, Irvine *et al*. 2014, Best *et al*. 2015). Most of the earlier follow-up studies were opportunistic and/or based on small sample sizes and tagging systems that have since undergone technological advances (Baker *et al*. 2012). Physiological responses to tag placement are still poorly understood, but are beginning to be examined by dedicated, longitudinal studies designed to relocate tagged animals on regular time intervals. For example, photographs of northwest Atlantic humpback whales (*Megaptera novaeangliae*), that include the tag site, were taken during postdeployment encounters to evaluate acute and chronic tag wound healing characteristics as well as overall body condition using scoring criteria specific to the target species (Robbins *et al*. 2013).

North Atlantic right whales were the first large whale population to be satellite-tagged, using a wide variety of tag types during early tag development to reveal tag site conditions from very minor depressions (also termed "divots") to more extensive swellings (Mate *et al*. 2007). Though interannual and individual variation in reproductive rates were observed in satellite-tagged southern right whales, a study by Best *et al*. (2015) demonstrated that reproduction and mortality rates were not affected by tagging. A case study of a blue whale (*Balaenoptera musculus*) tagged with an early generation externally mounted satellite tag suggested an association of long-term retention of a portion of a subdermal tag attachment with large swelling and possible altered reproductive success (Gendron *et al*. 2015). Despite the increasing number of

follow-up studies of tagged large whales, definitive evidence of long-term effects of tagging, such as resolution of tag wounds, characterization of factors influencing resolution, and possible risks to long-term health and reproduction, remain relatively unclear.

Two recent studies have provided insight into dart- or tag-related wounds in whales. The first study detailed the nature of a wound from a dart used to deliver sedatives to a mortally entangled North Atlantic right whale (Moore *et al.* 2013). The authors described the dart and a retained needle that spanned the blubber-muscle interface, resulting in necrosis and cavitation of the muscle observed upon postmortem examination. They suggested rigid devices such as darts, and satellite tags that may similarly span this interface, could affect the health of the animal. The second study, a long-term evaluation of tag sites on 21 southern right whales, found wounds healed within 5 yr of tagging or 2 yr after the tag was shed (Best *et al.* 2015).

This study focused on two species of baleen whales in the eastern North Pacific that represented excellent test cases for three primary reasons: (1) these animals included some with the longest histories of tag deployments observed repeatedly since 1986 (Calambokidis *et al.* 2009, 2012); (2) this data set included the largest number of implantable tag deployments on any whale population (*e.g.*, 185 eastern North Pacific blue whales tagged by Oregon State University [OSU]) with External and Implant tags (Mate *et al.* 2007, Irvine *et al.* 2014), with 63 of these animals resighted; and (3) individuals in both populations have been photo-identified and tracked during the last 25 yr (Calambokidis *et al.* 2009, 2012, 2017; Calambokidis and Barlow 2013). The Pacific Coast Feeding Group (PCFG) gray whales represent <2% of the total population and feed in coastal waters from central California to Kodiak, Alaska, and they have a high annual resighting rate in which over half the estimated population is seen annually (Calambokidis *et al.* 2002, 2012).

One of the goals of this study was to develop a method for systematic visual assessment of the long-term external consequences of tag placement on two whale species by examining photographs. The second was to determine potential differences in wound healing associated with species, tag type, reaction, placement, and time since tagging.

Methods

Tags Examined

We examined long-term, visible healing at sites of tag insertion following use of two general designs of tags on blue whales and PCFG gray whales. Tag types and attachments are defined as follows:

(1) Externally mounted trans-dermal intramuscular tag Telonics ST-6 (12.5 × 5.6 cm) and ST-10 (17.0 × 2.5 cm) attached with two attachments (14 × 0.6 cm), each consisting of a bladed entry tip that penetrates the skin, blubber, and potentially muscle (see Fig. 1, 2A), referred to as "External" in this manuscript.

(2) Transdermal intramuscular semi-implantable tag (most of tag embedded except for antenna endcap) Telonics ST-15 (25.5 × 1.9 cm) and Wildlife Computers Spot-5 (27.8 × 2.1 cm) and Wildlife Computers Spot-5 (22.8 × 2.1 cm) (see Fig. 1, 2B, C), referred to in this manuscript as "Long Implant" for those >25
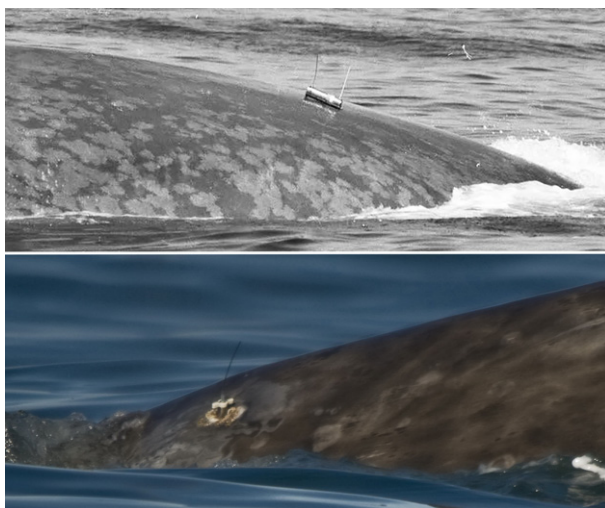
Figure 1. Photographic examples of External (top) and Short/Long Implant (bottom) tags. Photo credits: John Calambokidis (top) and Craig Hayslip (bottom).

cm long and "Short Implant" for those < 25 cm, consistent with Mate *et al.* (2007, 2010).

OSU deployed 185 tags on blue whales between 1993 and 2008 (Mate *et al.* 1999, 2007; Irvine *et al.* 2014), starting with External tags from 1993 to 1995, followed by use of the Long Implant design. Beginning in 2009, OSU deployed Short and Long Implant tags on PCFG gray whales (Mate *et al.* 2010). Of the three tag types deployed for which tag sites were scored for this study, External tags were deployed on 12 occasions on blue whales (1993–1995), Long Implant on 51 and 25 of the blue and gray whales, respectively, and Short Implant tags on 9 gray whales. Starting in 2000, implant tags were partially coated with an antibiotic (gentamycin sulfate) embedded in methacrylate to provide a long-term (several months) dispersant release to reduce bacterial infections (Mate *et al.* 2007). Only four implant tags with follow-up observations were deployed without antibiotics, all on blue whales.

Photographic Sighting Database

Dedicated yearly photo-identification (photo-ID) surveys of varying duration for gray and blue whales have been conducted by Cascadia Research Collective (CRC) since 1986 off the Pacific Northwest and southern California. Detailed information about the study areas, data collection and analytical methods are described in Calambokidis *et al.* (2009, 2012). Oregon State University Marine Mammal Institute's Whale Telemetry Group provided photographs and video clips of blue whales being tagged and follow-up photos of previously tagged whales during additional OSU tagging efforts (Mate *et al.* 1999). Data from all available sources, including CRC and OSU were compiled for PCFG gray whales tagged in 2009, 2012, and 2013, and for blue whales tagged in 1993–2008.

Photographs, videos, and sometimes biopsies were taken at tag deployments. Photographs from tag deployments were used to reconcile identity of tagged animals
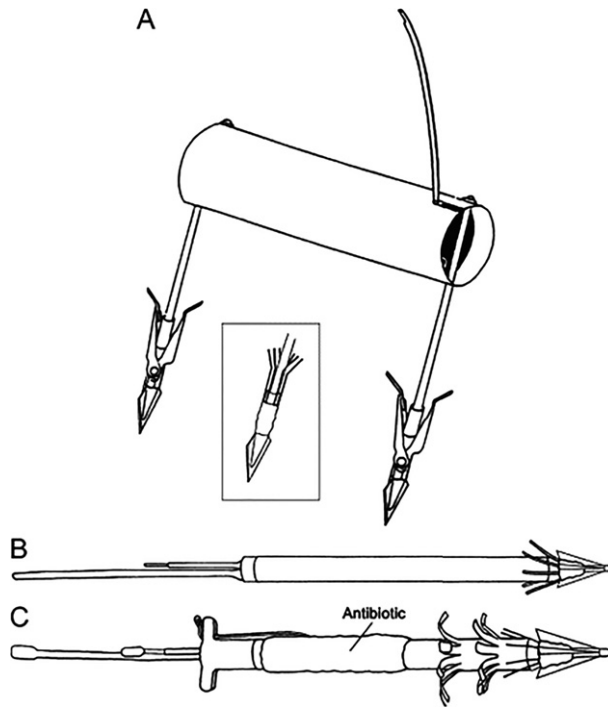
Figure 2. (A) A cylindrical surface-mounted projectile tag (12.5 cm long × 5.6 cm diameter) with two subdermal attachments (14 cm long × 0.6 cm) consisting of bladed entry tip and folding barbs (with wires to establish lateral spreading). Inset shows alternative wire rosette. (B) the first implant tag (19 cm long × 1.9 cm diameter housing), showing bladed entry tip at one end, and antenna and saltwater conductivity switch at the other. (C) implant tag, showing two rows of stainless steel petals (0.6 × 3.2 cm long) to prevent outward migration. A layer of antibiotic mixture (left of the petals) was added to tags beginning in 2000. Two lateral extensions (0.9 cm × 1.5 cm) of the Delrin endcap were incorporated in 2001 to act as a depth stop and prevent inward migration of the tag. Reprinted from Deep-Sea Research Part 2, Vol. 54, Numbers 3–4, Bruce Mate, Roderick Mesecar, Barbara Lagerquist, The evolution of satellite-monitored radio tags for large whales: One laboratory's experience, Pages 224–247, Copyright (2007), with permission from Elsevier.

with photo-ID data sets. Postdeployment follow-up photos came from a variety of camera types and sources (see Acknowledgments), though they were typically single lens reflex (SLR) photos, using film prior to 2004 and digital media thereafter, utilizing telephoto lenses. Where new encounters with previously tagged animals were determined by photo-ID, the best quality tag site photographs were selected and added to the tag site photograph time series. A sighting event was included in the study if it was represented by at least one image that was matched to a previously identified animal. Opportunistic visual documentation from naturalists and other researchers was also requested. Follow-up monitoring events were expressed in terms of years post tag deployment, with day of tagging designated as Day 0. Genetic analyses were performed from biopsies by Scott Baker's OSU Marine Mammal Institute Cetacean Conservation and Genomics Laboratory as an additional check for genetic

Table 1. Number of deployment events and encounters obtained for photographic tag wound healing scoring, with final number of tagging events (d) and encounters (e) used in the analysis.

Criteria	Blue whales	Gray whales
(a) Total tag deployment events	185	35
(b) Photo-ID determined for deployment events	85	35
(c) Number of unique individuals (1 gray and 2 blue tagged twice)	83	34
(d) Tagging events with follow-up >2 d posttagging	63	34
(e) Total encounters (including one pretagging encounter where available) used from (d)	449	546
(f) Max years between earliest tagging and latest follow up	20	4
(g) Total post tag years available to 2013 for all IDed tagged (b)	912	83
(h) Encounters per available year (e/g)	0.5	6.6
(i) Total unique individuals in Cascadia catalog through 2013	2,151	1,563
(j) Total encounters through 2013 of individuals in (f)	13,350	23,271
(k) Tag type (three whales were tagged twice)		
External	12	0
Short Implant	0	9
Long Implant	51	25

matches to previously biopsied whales. Table 1 summarizes the number of individuals tagged, those used in this study, and the number of encounters scored.

Tag Site Physical Scoring Criteria

A consistent terminology for describing the tag site appearance, and evaluating severity or extent of changes seen in the photographs was developed and used to create a set of 23 multiple choice questions with short-answer comments (Appendix S1). The six scoring criteria used in this study were considered most representative of physical characteristics of tag site wound healing likely to be visible in photographs. Except where indicated, attributes were scored on a three-point scale, with 0 indicating Could Not Be Determined, 1 indicating Yes, and 2 indicating No. In addition, three properties of tag placement on the body of the whale were evaluated and included as attributes in the analyses and are described below. For all scoring, Unknown was indicated if a determination could not be made due to angle or poor quality of the photo. Two of the authors (SAN and KRF) each scored all images.

The first tag site attribute, presence of swelling, was defined as raised tissue adjacent to the tag (local) or more broadly distributed. The second attribute was tissue sinking (depression) at or near the tag site. The third and fourth attributes were extent of the swelling and/or depression, respectively, if present. The severity score was determined by the extent of convexity or concavity of the wound. When a swelling or depression was visible, the extent was determined relative to the size of the dorsal ridge or fin (Fig. 3, 4). The angle and quality of the image were scored as attributes to assess any role they would serve in evaluating the extent of the wound. Presence and evaluation of changes in skin coloration at or near the tag site comprised the fifth and six attributes, respectively (Fig. 5). The scale of the extent of discoloration ranged from dark gray to bright white and could be of any size or shape. In gray whales, it can be difficult to distinguish between skin color changes due to a tag and changes from
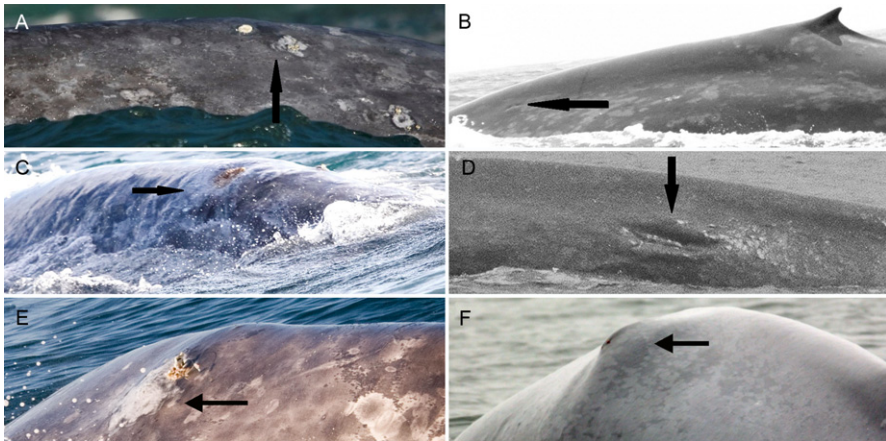
Figure 3. Examples of tag site swelling gradations (arrows) scored from gray (*Eschrichtius robustus*) and blue (*Balaenoptera musculus*) whales, left and right, respectively. A score of 1 was assigned to low-grade swelling (A, B). A score of 2 was assigned to medium-grade (localized area and/or less than height of dorsal ridge/fin) severity (C, D), and a score of 3 was assigned to high-grade swellings (broad area affected or width/length greater than height of dorsal ridge/fin) (E, F). Photo credits: (A, C, E) Craig Hayslip, (B, D) John Calambokidis, (F) Bernardo Alps.

those due to barnacles or other external causes such as fishery entanglement, predation, or propeller strikes. The scoring individual had to ensure the wound being evaluated was due to the tag and not another etiology. Extent of swelling, depressions, and discoloration were scored on a 4-point scale (0–3; no change, low-, medium-, and high-grade, respectively). Low represented a barely discernible reaction only at the immediate tag site; medium represented reaction clearly discernible, but still highly localized at the tag site; high-grade corresponded to the most extensive reactions that occurred beyond the immediate tag site.

Tag Site Veterinary Scoring Assessment

Five marine mammal veterinarians (coauthors: SAN, FMDG, MJM, SR, DSR) with large whale expertise provided independent evaluation of the long-term potential risk to the animal's health based on the inclusive time series of photos for each tagged whale. Images for 29 gray whales and 77 blue whales were presented to the veterinarians in two formats: as individual photos and as a series of images in a slide presentation format where the tag site was circled on each image for ease of location during evaluation. Of these, only 27 gray whale and 56 blue whale results were analyzed as longitudinal series, defined as at least 2 d of follow-up photographs. Each veterinarian was asked to review the images and subjectively answer the following question, based on the entire collective series of photographs for each whale: "To what degree does the evidence presented in this animal's set of tagging and posttagging photographs indicate a risk to the long-term health of this individual?" Responses were on a 5-point scale with 1 "Unlikely," 2 "Somewhat Likely," 3 "50-50 Likelihood," 4 "Likely," and 5 "Highly Likely."

Figure 4. Examples of tag site depression gradations (arrows) scored from gray (*Eschrichtius robustus*) and blue (*Balaenoptera musculus*) whales, left and right, respectively. A score of 1 was assigned to low-grade depression (A, B). A score of 2 was assigned to medium-grade (localized area and/or less than height of dorsal ridge/fin) severity (C, D), and 3 was assigned to high-grade depression (broad area affected or width/length greater than height of dorsal ridge/fin) (E, F). Photo credits: (A) Craig Hayslip, (B) John Calambokidis, (C) Craig Hayslip, (D) John Calambokidis, (E) Craig Hayslip, (F) John Calambokidis.

Statistical Analysis

Because the focus of the current study was to evaluate wound healing following tag deployment, scores from pretag deployment, those sites not discernible in photographs, or whales lacking follow-up photographs, were excluded from analyses. Sample prevalence and 95% exact binomial confidence intervals of wound type and severity scores were calculated for species and tag type. Agreement between the two physical scorers, was evaluated using a weighted Kappa statistic (κ) (Cohen 1960). This statistic was used to determine whether individuals may be trained to consistently characterize the presence, type, and severity of a tag-related wound from photographs of gray and blue whales (Cohen 1960, Viera and Garrett 2005).

Two types of statistical models were used to evaluate the association of tag, photograph, and whale demographic factors with wound severity scores. In the first
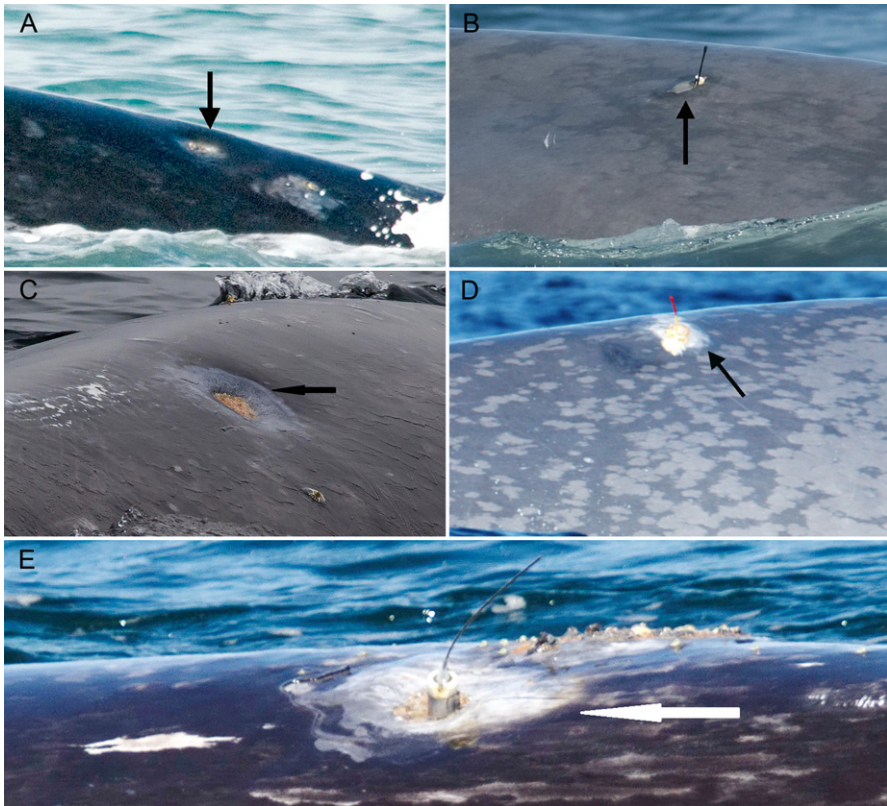
Figure 5. Examples of skin discoloration gradations at tag sites (arrows) scored from gray (*Eschrichtius robustus*) and blue (*Balaenoptera musculus*) whales, left and right, respectively. A score of 1 was assigned to low-grade discoloration composed of a small localized area and lighter gray (A, B). A score of 2 was assigned to medium-grade severity (brighter discoloration than low-grade and/or area of color change less than height of dorsal ridge/fin) (C, D), and 3 was assigned to high-grade coloration change where a broad area was affected (white oval) around the tag site (arrow) and/or very bright white or contrast to normal skin tone (E). High-grade discoloration was not observed in any blue whales evaluated in this study. Photo credits: (A–C, E) Craig Hayslip, (D) Kiirsten Flynn.

analysis, a generalized linear mixed model (GLMM), specifically multinomial mixed-effects logistic regression for ordinal responses (Agresti 2002), was used to assess the relationship between nine independent variables and the dependent ordinal outcome of swelling, depression, and skin coloration change severity at the tag site (none, low, medium-, high-grade for all three outcomes) (Table 2). The nine independent predictors that were evaluated for their association with the presence and severity of swelling, depression, and coloration change at the tag site included sex, species, tag type, vertical (high, medium, or low relative to the dorsal midline) and dorsal tag placement (relative to the dorsal fin/ridge caudal to blowholes) on the whale, tag insertion angle relative to skin surface, photograph quality, tag site photograph angle, and years postdeployment (Table 2). A random effect, used to account for variation across individuals, was included as a variable in the multinomial logistic regression models

Table 2. Summary of model covariates used in assessing wound healing responses in gray and blue whales. GLMM = generalized linear mixed-effects model, GLM = generalized linear model.

Variable	Type	Notes
Species (gray or blue whale)	Categorical	
Sex (male, female, or unknown)	Categorical	Genetically determined where available

Tag type
    Externally mounted, Short
        implant, Long implant
	Categorical	See Mate *et al*. 2007 for complete tag descriptions

Vertical tag placement
    High
    Medium
	Categorical	Relative to dorsal midline
		        No tags were placed Low to the dorsal midline

Dorsal tag placement (Anterior, Middle, Posterior)	Categorical	Position between the blowhole and dorsal fin/hump

Tag insertion angle
    Shallow (0°–30°), Medium
        (31°–60°), Straight
        (61°–90°)
	Categorical	Relative to the skin surface

Photo quality (Excellent, Good, Fair, Poor)	Categorical	
Tag site photograph angle	Continuous	
Years postdeployment	Continuous	Used only in GLMM model

Years postdeployment
    (<0.299, 0.3–0.9, 1.0–2.9,
        3.0–4.9, 5.0–6.9, 7.0–8.9,
        9.0+)
	Categorical	Used only in GLM model since data collapsed by tagging event

Number of postdeployment encounters (≤3, 4–8, 9+)	Categorical	Used only in the GLM model

(whale ID number). Models with wound outcomes for both blue and gray whales combined were explored, but due to the lack of complete overlap across some variables, separate modeling of the two species was required. For example, tag type was confounded by species when modeling included both species combined because External tags were only deployed on blue whales; Short Implant tags were only deployed on gray whales; and shallow and medium tag angles were only observed on gray whales.

The second modeling analysis, a set of generalized linear models (GLM), specifically ordinal logistic regression, or proportional odds regression, was used to examine most of the same predictor variables as in the mixed-effects models (with the exception of years postdeployment as a categorical variable) but added the variable number of postdeployment encounters an individual was photographed to assess variation in the extent of maximum swelling, depression, and coloration change, collapsed for each tagged individual. This resulted in one point of data for each whale compared to multiple data points in the mixed-effects modeling (Anderson 1984).

For both the GLMM and GLM analyses, univariate models were developed to explore the relationships between single predictors and response (outcome) variables. Predictor variables associated with the response at a *P*-value of ≤0.25 on the Wald test on univariate analyses were included in the initial full model, as well as those variables of particular interest, even if not significant. Each of the covariates was

individually removed from the full and reduced models as needed to form the most parsimonious models based on likelihood ratio tests. If the removal of a variable caused another variable's coefficients to change more than 10%, then the latter variable was retained as the model was sensitive to its inclusion. For the GLM modeling, odds ratios (OR) were calculated, in addition to coefficient estimates (values for the regression equations for predicting the dependent outcome variable from the independent predictor variables) and standard errors (SE). Data analyses were performed using the Ordinal Package (Christensen 2015) in R (R Core Team 2015, Kuznetsova *et al.* 2015) and Stata version 12.0 (StataCorp LLC, College Station, TX).

<div align="center">RESULTS</div>

Photographs were scored for 34 gray and 63 blue whale tagging events (995 total images). The scoring of physical appearance by the two evaluators showed good (substantial) inter-rater agreement for assessing the presence and types of wounds (κ_w = 0.78), and moderate agreement for severity of swellings (κ_w = 0.68), depressions (κ_w = 0.65), and color change (κ_w = 0.60) at the tag sites (Landis and Koch 1977). Because of the different dates of tagging, tag site resightings were made from time of tagging to almost 17 yr for blue whales, and to slightly more than 4 yr for gray whales.

Physical Scoring

Of the 995 photos evaluated, 425 (229 blue, 196 gray) contained the tag in place, including photos of the day of tag deployment. Swellings and depressions were the most common changes observed during tag follow-up. Swellings were present in 25/34 (73.5%) of gray and 21/63 (33.3%) of blue whales and depressions in 28/34 (82.4%) of gray and 45/63 (71.4%) of blue whales. Both reactions were sometimes observed on an individual animal during the follow-up period, though they did not necessarily occur concurrently (gray: 19, 55.9%; blue: 16, 25.4%). The median duration ($\pm$SD, range) of follow-up years was: 1.1 ($\pm$1.6, 0.1–4.2) for gray and 5.4 ($\pm$4.8, 0.0–17.0) for blue whales, with median number of postdeployment encounters of 11.5 and 4.0, respectively. On average, blue whales were encountered 0.5, and gray whales 6.6 times per year, respectively, posttagging (*h* from Table 1), based on the number of encounters (*e* from Table 1) divided by the total number of years posttagging for each whale, including the tagging year (*g* from Table 1). Tissue swelling was first noted as early as 11 d posttagging in gray whales, with one blue whale recorded with tissue swelling as early as 30 d, but follow-up observations were less frequent for the latter. The earliest depression was documented 3 d postdeployment in a blue whale immediately surrounding the tag. In events where swelling and depression were both observed at some point during follow-up, depression occurred simultaneously adjacent to a swelling, or postswelling, but never preceded swelling. An example of photographic documentation of progression from swelling to depression is given in Figure 6.

A greater proportion of depressions only (*i.e.*, depression, but no swelling) occurred in blue whales with Long Implant tags compared to gray whales (Fig. 7). When analyzed on a species level, Long Implant tags were significantly associated with having a reaction (swelling or depression) (P = 0.03) compared to Short Implant tags on gray
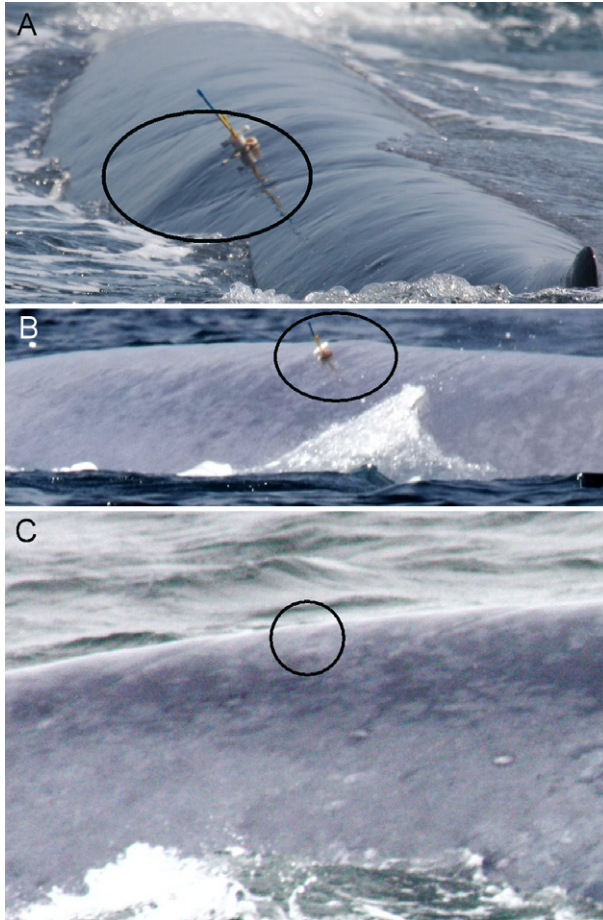
Figure 6. Progression of tag-associated wound from medium grade swelling (A; photographed 15 August 2008), (B; photographed 17 August 2008) to medium grade depression (C; photographed 18 June 2012) in a blue whale (CRC ID 536). The whale was tagged on 27 July 2008. Photo credits: (A) John Calambokidis, (B) Erin Falcone, (C) Channel Islands National Marine Sanctuary.

whales, and were also associated with swelling or depression in blue whales (Fisher's exact test = 0.05) (Fig. 7).

Maximum extent of swelling was scored in nine (26.4%) gray and five (7.9%) blue whales, and the deepest (*i.e.*, high-grade) depressions were present in five (14.7%) and three (4.8%), respectively. Determination of exact wound healing duration was not always feasible due to the challenge of consistently relocating individuals over time, especially blue whales, and the inability to examine the tag sites directly; however, approximations were possible for swellings in eight (23.5%) gray whales for which a relatively complete photographic visualization of wound healing duration was documented.

Within 6 mo of deployment, just over 70% of tagged gray whales had evidence of swelling, and 50% were scored as medium to high-grade extent (Fig. 8A). In blue
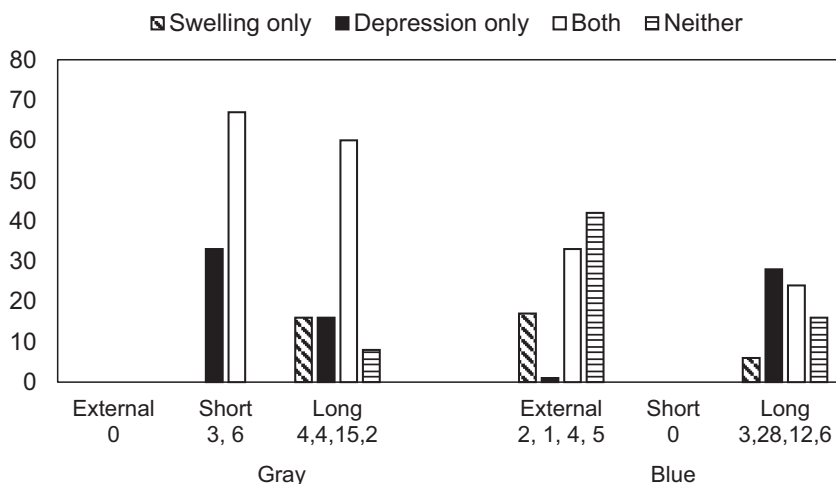
Figure 7. Percentage of wounds associated with satellite-tagged gray and blue whales at any point in time (1993–2013). Number of events for each tag and wound type are listed under the *x*-axis labels. External = externally mounted, Short = Short Implant, Long = Long Implant.

whales, for which it was not often possible to examine progression of swelling within time frames <1 yr, an initial "peak" of swellings was observed 1 yr postdeployment in 25% of all tagging events, with a subsequent peak at 9 yr (Fig. 8B). Of the five (5/ 63; 7.9%) blue whales that reached a high-grade swelling, three either "peaked" or were first noted to have high swelling between 6 and 9 yr after tagging and a fourth that was noted with medium-high swelling 11 yr posttagging. Four of these five blue whales were tagged with the shallower External type tags used early in the development of tags and only one with the deeper Long Implant tag, despite much larger numbers of the latter being applied.

Depressions appeared in gray whales after swellings peaked and persisted for the duration of the study period in almost all of the tagged individuals (Fig. 8C). For one gray whale, the healing of a depression was noted in a little over 4.5 mo; however, most depressions were of longer duration and likely became a permanent skin defect such as a scar. Cyamids (*e.g.*, *Cyamus scammoni*) were observed at or adjacent to the tag site on 17 (50.0%) gray whale posttagging follow-up observations, often disappearing when the wound had contracted down to a small depression or scar. In blue whales, the proportion of individuals with a depression peaked 3–4 yr postdeployment, with the proportion variably declining over the following years (Fig. 8D). Because many gray whales were more recently (2013) tagged and blue whale resightings were sporadic, wound healing continues and is reflected by ongoing observations of tag-associated wounds in more recent encounters. Mean duration from the date a tag-associated gray whale wound was first observed, regardless of whether the tag was still present or had been lost, to its resolution, as defined by absence of swelling or the presence of a low depression (scar), was a little over a year (1.014 ± 0.741).

Changes in coloration of the epidermis at or immediately adjacent to the tag site were observed for some of the whales. Gray whales appeared to have more reactive skin based on the degree of epidermal color change. At least 39 blue (70%) and 33
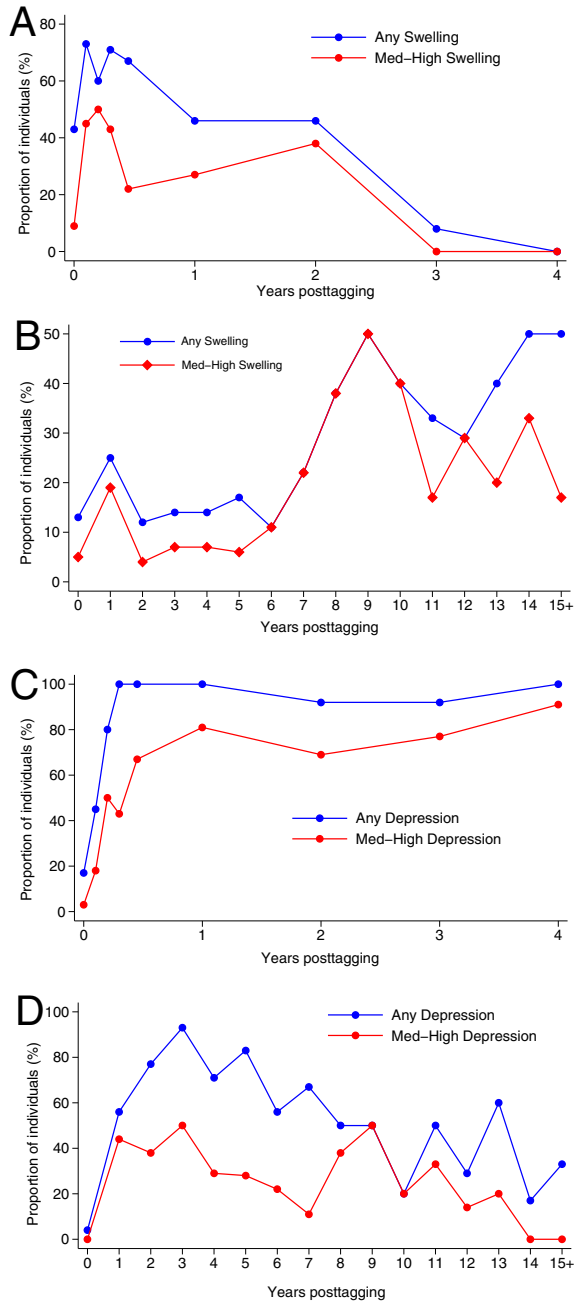
Figure 8. Duration of tag site swelling and depressions in Pacific Coast Feeding Group (PCFG) gray whales (A, C), blue (B, D). Proportion of individuals with any wound (blue lines) and medium- to high-grade severity wound (red lines) are plotted over increments of 6 mo (gray whales) and 2 yr (blue whales) posttagging.

gray (97%) whales experienced at least a mild degree of skin discoloration at or near the tag site. The mean length of time to resolution of discoloration was 4.4 yr in blue whales. Mean time could not be calculated for gray whales as the progression of color change was still ongoing at the last encounter.

Veterinary Assessments

In general, subjective scoring among the veterinarians agreed on whether some whales were at elevated potential health risk. However, there was often disagreement on the magnitude of that elevated potential risk using our subjective scoring levels (five levels from Unlikely to Highly Likely). Only 10% of the scores for either species was thought to have 50% Likelihood or greater potential health risk (Fig. 9). Differences between blue and gray whales were apparent, however, in the distribution of the scores for the Unlikely or Somewhat Unlikely categories (Fig. 9). In 56% of gray whales, the scores indicated that there was a Somewhat Likely potential risk to the animal's long-term health, whereas that was only 9% for blue whales. Though 85% of blue whales scores were judged as Unlikely potential risk, three whales (5%) were assessed as having the highest subjective risk (Highly Likely), primarily as a result of these being three blue whales with large swellings of longer duration (discussed below).

In two blue whale tagging events (CRC IDs 1573 and 2208), with External tags, the high mean veterinary scores suggested a Likely risk (scores of 4 and 4.4 out of 5, respectively) to the animals' long-term health based on the photographic evidence (see next section for details on these two animals). Scoring of tag wound healing in
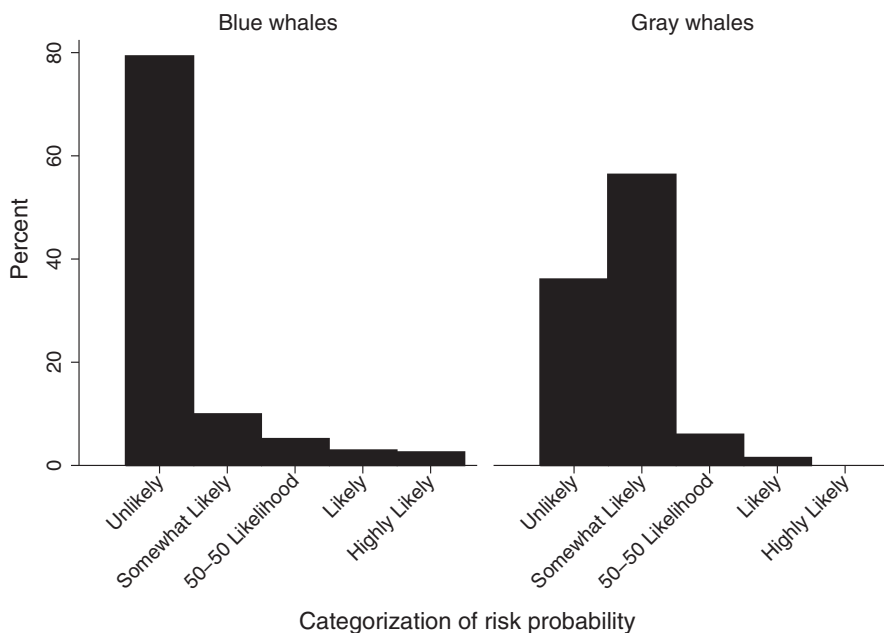

Figure 9. Proportion of scores by a veterinary panel assessing the probability of adverse risk to long-term health of an individual whale posttagging in blue and gray whales.

Table 3. Comparison of physical scoring of tag wound severity and veterinary assessment scores for gray and blue whales by reaction type. For each group of severity scores (*n*), the proportions of veterinary scores ≥3 and ≥4 are presented.

	n for severity scoring	Proportion of veterinary scores ≥3	Proportion of veterinary scores ≥4
Swelling: blue whales			
Severity score ≥2	10	30	18
Severity score <2	46	6	3
Swelling: gray whales			
Severity score ≥2	13	11	3
Severity score <2	14	4	0
Depression: blue whales			
Severity score ≥2	19	14	5
Severity score <2	35	9	6
Depression: gray whales			
Severity score ≥2	23	8	0.9
Severity score <2	4	5	5

these animals revealed an External tag that resulted in high-grade swelling and medium-grade depression formation. Comparisons between wound scoring and veterinary assessment scoring revealed that animals with greater severity scores were those the veterinarians tended to be more concerned about as revealed by their higher scores (Table 3). This was particularly noticeable in swellings compared to depressions. Swelling and depression severity scores ≥2 on physical scoring corresponded with higher (*i.e.*, >50–50 Likelihood, or ≥3) scoring by the veterinary panel, particularly in gray whale swellings and for both wound types in blue whales. On 12 occasions (*n* = 9; 32.1% of gray and *n* = 3; 5.4% of blue whale tagging events) physical scoring of tag wound healing was assigned a high-grade score for swelling and/or depression; however, the corresponding veterinary scoring of risk was ≤50–50 Likelihood risk to long-term health of the individual, particularly if the wound progressed toward resolution as evidenced by smooth edges, absence of any swelling or medium to large-sized depressions, and lack of discharge or soft tissue protrusion.

Modeling Results

A summary of significant species-specific multinomial mixed effects (GLMM) modeling results revealed that vertical tag placement, tag type, years postdeployment, and photo quality were significant predictors of swelling assessment in blue whales (Table 4, 5). The presence of an interaction term between tag type and years postdeployment accounted for differences in timing of swelling associated with External tags (deployed only in early years of tag development) and the more recent Implant tags. Swellings associated with External tags were more pronounced later and for longer periods than those associated with Implant tags. The most supported model to assess factors influencing severity of swelling in gray whales (Table 5) indicated more severe swelling was less likely to occur for those tags with a higher insertion angle (more vertical) relative to the body surface. In gray whales, the probability of swelling decreased as time increased.

Models assessing severity of depression in blue whales indicated that tag type, vertical placement and year postdeployment were important explanatory covariates. The

Table 4. Summary of significant factors associated with tag site wound healing in large whales using two different modeling approaches: generalized linear mixed-effects models (GLMM) and generalized linear models (GLM), more specifically proportional odds logistic regression. The GLMM were fitted to evaluate variation in the severity of swelling and depression wound responses associated with implant satellite tags in gray and blue whales with more than one line of data for each tagging event. Results of these models were compared and contrasted to those from the GLMs fitted to evaluate wound healing, collapsed by individual; ypd = years postdeployment.

Wound	Model type	
	GLMM	GLM
Swelling		
Blue	Vertical tag placement, tag type, ypd, photo quality; interaction: tag type × ypd	Species, vertical tag placement, number of postdeployment encounters
Gray	Tag angle insertion, ypd	
Depression		
Blue	Vertical tag placement, tag type, ypd	Species, vertical tag placement, number of postdeployment encounters
Gray	Ypd, tag angle insertion, photo angle	
Coloration		
Blue	Dorsal tag placement, ypd, photo angle	Species, vertical tag placement
Gray	Vertical tag placement, ypd, photo angle	

probability of more severe depressions increased for tags placed lower in the body, for Implant tags and with time after deployment. In gray whales, the likelihood of more severe depressions increased with time. Photo angle appears to influence identification of depressions in gray whales, with more severe reactions being more difficult to identify with an increase in the angle of the photograph taken, relative to a perpendicular photo.

Models of epidermal color change at, and immediately adjacent to, the tag site showed that discoloration scores in blue whales were influenced by the extent of the tag's dorsal placement on the body (*i.e.*, between the blowholes and dorsal fin/ridge), year postdeployment and photo angle. The probability of higher discoloration scores was greater with more posterior placement and with increased time after tag deployment. Greater photo angle appeared to reduce the likelihood of seeing discoloration. For gray whales, vertical tag placement and time postdeployment and vertical photo angle all were associated with higher color change score. The probability of higher discoloration scores in gray whales was greater for tags placed vertically lower on the body and with increased time postdeployment. Similar to blue whales, photo angle negatively influenced the coloration scores.

GLM models used to assess maximum severity scoring of swellings, depressions, and coloration changes at the tag site, collapsed over individual tagging events, revealed that species, maximum years postdeployment, vertical placement of tag, and number of postdeployment encounters were significant predictors of reaction severity when included with dorsal tag placement and tag type. For examining maximum grade of coloration change, the same covariates were included in the model as those used for assessing maximum grade of swelling and depression; however, the additional variable tag type was included in the final model for discoloration severity. The model for maximum swelling severity revealed that, compared to gray whales,

Table 5. Maximum-likelihood estimates from fitting generalized linear mixed-effects models (GLMM) to evaluate variation in severity of reactions (swelling or depression) associated with satellite tags in gray (*Eschrichtius robustus*) and blue (*Balaenoptera musculus*) whales. Multinomial logistic regression models were used to model wound healing characteristics with an interaction between tag type and years postdeployment in the blue whale swelling model. Significant predictor coefficients ($P < 0.05$) are shown in boldface type. Tag type = External (blue whales), Tag type = Short Implant (gray whales), Vertical tag placement = high, and Dorsal tag placement = posterior, serve as the reference categories. SE = standard error.

Wound	Species	Variable	Estimate	SE	Z	P
Swelling	Blue	Vertical tag placement	15.112	3.553	4.253	**<0.001**
		Tag type: Long Implant	1.595	1.216	1.312	0.19
		Years postdeployment	0.234	0.084	2.805	**0.005**
		Photo quality	−0.957	0.299	−3.303	**0.001**
		Tag type: Long Implant × years postdeployment	−0.499	0.149	−3.351	**0.001**
	Gray	Angle of tag insertion	−0.651	0.259	−2.514	**0.012**
		Years postdeployment	−1.377	0.182	−7.580	**<0.001**
Depression	Blue	Vertical tag placement	6.358	2.956	2.151	**0.031**
		Tag type: Long Implant	3.332	1.061	3.141	**0.002**
		Years postdeployment	0.316	0.052	6.131	**<0.001**
	Gray	Angle of tag insertion	−0.586	0.291	−2.011	**0.044**
		Years postdeployment	0.696	0.099	7.038	**<0.001**
		Photo angle	−0.458	0.165	−2.783	**0.005**
Coloration	Blue	Dorsal tag placement: anterior	−0.837	0.62	−1.350	0.177
		Dorsal tag placement middle	−1.447	0.579	−2.499	**0.012**
		Years postdeployment	0.095	0.041	2.342	**0.019**
		Photo angle	−0.995	0.222	−4.481	**<0.001**
	Gray	Vertical tag placement	6.535	2.467	2.649	**<0.001**
		Years postdeployment	0.562	0.083	6.782	**<0.001**
		Photo angle	−0.620	0.14	−4.414	**<0.001**

blue whales had significantly less severe swelling, while higher vertical tag placement on the whale resulted in significantly less severe swelling (Table 6). Regardless of species, the increasing number of encounters was significantly associated with increased maximum severity of swelling, but probably reflects more follow-up sighting opportunities to document swellings and their progression. For maximum depression severity, increasing number of encounters were associated with higher-grade depressions, again likely reflecting a greater number of opportunities to observe a wound over time (Table 6). Maximum depression severity significantly decreased for individuals with increased years of follow-up observations. Maximum severity of discoloration was greater in gray whales compared to blue whales and for whales with lower vertical tag placement (Table 7).

Interpretation of associations between the predictor and response (outcome) variables was also achieved with ORs, which are derived from exponentiation of the model regression coefficients. For example, in a given tagging event (Table 6), the odds of more severe swelling in a gray whale was approximately 4.4 (1/0.228) times that of such a swelling in a blue whale (with all other predictor variables set to zero); the odds of greater swelling severity during the latter 2/3 of the first year postdeployment compared to that of the initial 1/3 of the year was approximately 8.3 (1/0.121); the odds of greater swelling severity with a tag placed lower compared to higher

Table 6. Results of fitting generalized linear models (GLM) proportional odds models to evaluate variation in the severity of swelling and depression wound responses associated with satellite tags in gray and blue whales, given species, years postdeployment follow-up, vertical placement of tag relative to the whale's dorsal midline, and number of postdeployment encounters. Species = gray, years = <0.299, vertical tag placement = medium, and encounters = ≤3, were reference categories. Significant predictors ($P ≤ 0.05$) are bolded. SE = standard error, OR = odds ratio.

Variable	Estimate	SE	OR	Wald z	P
Swelling					
Species (blue *vs.* gray)	−1.477	0.626	0.228	−2.360	**0.018**
Maximum years postdeployment					
0.300–0.999	−2.111	1.118	0.121	−1.890	0.059
1.000–2.999	−1.079	0.913	0.34	−1.180	0.238
3.000–4.999	−0.953	0.931	0.385	−1.020	0.306
5.000–6.999	−1.174	1.128	0.309	−1.040	0.298
7.000–8.999	−2.443	1.578	0.087	−1.550	0.122
9.000+	−1.708	1.146	0.181	−1.490	0.136
Vertical tag placement (high *vs.* medium)	−2.714	0.929	0.066	−2.920	**0.003**
Number of postdeployment encounters					
4–8	2.363	0.73	10.625	3.24	**0.001**
9+	2.766	0.83	15.9	3.33	**0.001**
Depression					
Species (blue *vs.* gray)	−0.211	0.636	0.809	−0.330	0.74
Maximum years postdeployment					
0.300–0.999	3.409	1.116	30.23	3.05	**0.002**
1.000–2.999	2.804	1.016	16.507	2.76	**0.006**
3.000–4.999	2.625	1.037	13.802	2.53	**0.011**
5.000–6.999	2.774	1.146	16.027	2.42	**0.015**
7.000–8.999	2.591	1.487	13.348	1.74	0.081
9.000+	0.816	1.223	2.262	0.67	0.504
Vertical tag placement (high *vs.* medium)	−0.232	0.771	0.793	−0.300	0.764
Number of postdeployment encounters					
4–8	1.331	0.525	3.785	2.54	**0.011**
9+	2.486	0.686	12.014	3.63	**<0.001**

vertically was roughly 15 (1/0.066); and the odds of high-grade swelling with 4–8 and 9+ postdeployment encounters, compared to <4 encounters, was approximately 11 and 16, respectively, reflecting the greater ability to detect responses with more follow-up.

Discussion

This study uses follow-up photographs to evaluate long-term tag wound healing associated with body penetrating satellite tags. Physical parameter scoring agreement between two independent reviewers indicates the scoring methodology described here can be consistently applied to multiple species, and can thus be used by other cetacean researchers interested in tag site wound healing. Swelling and depression were more frequently and proportionately severe for gray whales than blue whales, while mean duration of change was longer, but sparser in blue whales. The largest and most

Table 7. Results of fitting generalized linear model (GLM) proportional odds models to evaluate variation severity of coloration changes at postdeployment tag sites in gray and blue whales, given species, years postdeployment follow-up, type of tag, vertical placement of tag relative to the whale's dorsal midline, and number of postdeployment encounters. Species = gray, years = <0.299, tag = External, vertical tag placement = medium, and encounters = ≤3 were reference categories. Significant predictors ($P \leq 0.05$) are bolded. SE = standard error, OR = odds ratio.

Variable	Estimate	SE	OR	Wald z	P
Species (blue *vs.* gray)	–3.027	0.809	0.048	–3.740	**<0.001**
Maximum years postdeployment					
0.300–0.999	0.626	1.055	1.87	0.59	0.553
1.000–2.999	0.471	0.919	1.601	0.51	0.609
3.000–4.999	0.673	0.991	1.96	0.68	0.497
5.000–6.999	0.096	1.025	1.101	0.09	0.925
7.000–8.999	0.685	1.376	1.984	0.5	0.619
9.000+	–0.682	1.33	0.506	–0.510	0.608
Vertical tag placement (high *vs.* medium)	–3.189	1.402	0.041	–2.270	**0.023**
Tag type					
Short Implant	2.781	1.968	16.143	1.41	0.158
Long Implant	2.81	1.764	16.605	1.59	0.111
Number of postdeployment encounters					
4–8	0.329	0.536	1.39	0.61	0.539
9+	1.081	0.662	2.947	1.63	0.103

persistent high-grade swellings were observed in two blue whales, associated with early External tag types, and were likely due to retention of a broken subdermal tag attachment rod (see Fig. 2A). The difference in prevalence and severity of tag site wounds between the two species is further apparent in the modeling of factors related to wound healing characteristics when considering other covariates such as tag type and placement characteristics.

Species Differences

Differences in reaction characteristics were noted between species, including wound severity and duration as confirmed by significant modeling results. There are marked body size differences between the two subject species (12 m, 35 tons and minimum of 21 m, 100 tons for gray and blue whales, respectively; Mizroch *et al.* 1984; Rice *et al.* 1984). Gray whales have a greater prevalence of swellings and depressions compared to blue whales suggesting greater reactivity to skin and blubber layer insults. There are a number of differences in the skin and parasite loads among whale species (Reeb *et al.* 2007). In gray whales, extensive scarification and skin reactions have been reported with parasite infestation from cyamids (typically *C. scammoni* and *C. ceti*) and barnacles (Samaras and Durham 1985), as well as entanglements in fishing gear (Bradford *et al.* 2009). Swelling and depression severity relative to vertical tag placement was only a factor for blue whales and may reflect interspecies differences in blubber thickness, which can also vary across the body (Slipjer 1948, Naess *et al.* 1998, Konishi 2006, Lockyer and Waters 2006). The higher rate of swellings noted in gray whales are similar to humpback whales in which swellings were noted posttagging in 47% of tagged individuals (Robbins *et al.* 2013). While blue

whales in our study generally did not have extensive scarring and pock marks this has been reported on blue whales in other areas (Brownell *et al.* 2007). These distinctions between species may reflect differences in foreign body response or in the metabolic composition of blubber. For example, wound healing is faster and more successful in bottlenose dolphins (*Tursiops truncatus*) compared to other marine mammal species due to higher concentrations of the short-chain fatty acid, isovaleric acid within the blubber (Zasloff 2011). The role of this metabolite in wound healing in gray and blue whales is unknown, but might shed light on the observed differences.

Tag Placement

The GLMM and GLM models predict tag placement relative to the dorsal midline (*i.e.*, vertical placement) which is an important predictor of swelling, depression, and discoloration severity, where lower vertical tag placement is associated with higher grades of these reactions. Tag placement location and/or angle may influence tag attachment duration because of the tissue properties (*e.g.*, stiffer, more fibrous collagen tissue is found closer to the dorsal midline or fin than lower on the body) and potential physical damage to underlying tissues including underlying muscle movement during flexion and extension of the caudal portion of the body (Pabst 1993, Moore *et al.* 2013). Higher vertical tag placement is also desirable for a number of reasons unrelated to tag impact, including saltwater switch detection at surfacing to control transmissions, and antenna clearance of saltwater for stronger transmissions. Tag locations caudal of the dorsal fin are also not preferred for tag performance because these areas do not surface as reliably for transmissions to reach the satellites compared to areas farther forward for most species. Proximity of the wound to muscle tendons may also influence the severity of damage caused by the tag.

The desired length of tag retention time should be linked to study objectives. They often involve longer-term tracks to identify home ranges, core areas, and migrations to link widely separated seasonal feeding and breeding habitats. The tags described here are not specifically designed to eject, although archival tags storing amounts of data too vast to send *via* satellite have been, so that those data could be recovered to examine underwater behaviors and dive details, such as duration, depth, and foraging lunges (Mate *et al.* 2016).

Timing and Progression of Tag Wounds

Documented initial tissue swelling posttagging was noted earlier in gray whales (11 d) compared to blue whales (30 d), but is imprecise due to the sparse posttagging encounters with blue whales to better estimate when swellings start. The significance of temporal differences in initiation of swellings and depressions between the two species are not readily quantifiable, but are likely due to variability in resighting likelihood. Once the tag is shed, wound healing and some restoration of the depression occurs from mature collagenous fibers filling the defect left by the tag's penetrating tract (Albert *et al.* 1980). Depressions occur due to the damage to fat cells that do not replace themselves in the blubber layer. Depressions may persist for many years or be permanent for some whales (Best *et al.* 2015). One persisted for 14 yr in a blue whale (CRC 306), though others ($n = 3$, 5%) disappeared with complete certainty based on photographic evidence (over 2, 6, and 8 yr postdeployment).

Wound healing can be ideal in which normal anatomic structure, function, and appearance are achieved, or as acceptable, in which sustained anatomic and functional

continuity, but not necessarily appearance, are restored (*i.e.*, permanent depression or scar) (Lazarus *et al*. 1994). Wounds that are not ideally or acceptably healed are a general concern because they may remain an open port for microbes, and could predispose the animal to systemic infection, chronic pain, or suboptimal health. Introduction of a fungal infection from the tag site or remnant petals from the tag attachment has been raised as a recent issue with the death of a killer whale (*Orcinus orca)* due to disseminated mucormycosis in the Pacific Northwest after deployment of a LIMPET satellite tag into the dorsal fin (National Marine Fisheries Service 2016*a*, *b*; Department of Fisheries and Oceans Canada 2016). Whales are also exposed to other injuries that take time to heal, including parasites, cookie-cutter sharks (*Isistius brasiliensis*) (Jones 1971), larger sharks, killer whales, entanglements, and vessel strikes (Lazarus *et al*. 1994, Harding *et al*. 2002, Nunan *et al*. 2014).

Differences in Wound Healing by Tag Type and Placement

Observations of tag wound healing only relate to visible external tissues surrounding the tag site, with little understanding of possible effects on unobservable underlying tissues. Results in one tag wound healing study suggested no significant long-term damage, as demonstrated by absence of swelling in almost all resightings, decreased cyamid presence at the tag site, and skin repigmentation (Best *et al*. 2015).

In the present study, the most extreme swellings tended to be associated with earlier tag designs. Four of the five blue whales exhibiting high-grade swellings were associated with External tags. One of these (CRC 1573), was tagged in 1995 and is described in Gendron *et al*. 2015. Observed and photographed annually in Mexico and California since 1994, swelling was documented at this whale's tag site from 1999 to 2005, including an embedded prong that was apparently expelled by 2006 (Gendron *et al*. 2015). A scar was finally observed in 2009, and the female was again seen with a calf in 2011. The authors suggested reproductive success may have been disrupted by the swelling, based on this animal having been seen with a calf in 3 of the 5 yr it was seen prior to and after the swelling, and in none of the 7 yr it was encountered during the period of swelling or open wound. High-grade swellings may result from infection or foreign body response (Weller 2008) such as appeared to be the case due to a remnant portion of the tag in this animal.

Early External tags (Fig. 2A) were surface-mounted cylinders subject to large hydrodynamic forces, creating both drag and lift. The 14-cm-long attachment rods may or may not have extended into the muscle layer depending on the angle of insertion, (8–11 cm blubber layer reported for blue whales by Mackintosh and Wheeler 1929), and certainly not as deep as the >25 cm Long Implant tags did. After three years of using External tags (1993–1995), electronic miniaturization made implantable tags possible (Mate *et al*. 2007). We suspect these larger swellings from these earlier External tags, even though they did not penetrate as deeply, were the result of the attachment rod remaining embedded in the animal. The problem of breakage was minimized with the redesign of attachments to eliminate moving individual parts in favor of a captured "petal" system once electronic miniaturization took place (Mate *et al*. 2007).

This study found several patterns of association between tag type, reaction severity, and length of time postdeployment. There was no statistical significance between tag types, but the interaction terms indicated the probability of swelling decreased for Implant tags on blue whales with time, when compared with External tags.

Comparing Scoring Approaches

The veterinary scoring showed a higher proportion of gray whales having at least some potential risk agreed with the findings of higher grade response in gray whales. This was most apparent for the animals that had high-grade swellings or depressions during physical scoring which were also rated by the veterinarians as more likely to affect long-term health. This makes sense given that the veterinarians were more likely to notice and score the more severe reactions as higher risk than less severe reactions which were not as pronounced, and tended to provide fewer comments on the latter reactions.

Study Limitations

There are several limitations to this visual wound healing assessment study. Tagged animals must be photo-identified to allow resighting over time, something that was only possible with less than half the tagged blue whales. Additionally, sufficient tag site follow-up requires an adequate number of quality photographs or video stills. Individuals for which there were only poor-quality photographs of the tag site or for which insufficient follow-up encounters existed (*i.e.*, blue whales) could not be included in the analyses. The omission of a high proportion of tagged animals from evaluation raises the question of how representative the remaining animals are that were evaluated. This study only examined photos of whales available during field opportunities, and, therefore, does not include animals that may have died prior to resighting effort. In addition, several wound criteria (*e.g.*, skin texture over the swelling or depression; determining presence of any blood or pus draining from the tag site) could not be routinely scored from photographs due to difficulty in interpretation or visualization, therefore, they were not found to be useful for assessing wound healing (Appendix S1). Photo quality and angle appear to play a role in evaluating physical scoring of tag wounds, emphasizing the need for photographs that are as high quality as feasibly possible. The lack of significance for some predictor variables could be due to sample size, as well as exclusion, or inadequate inclusion, of essential covariates that were not recognized or could not be measured, such as presence of comorbidities, including malnutrition or subclinical disease.

Conclusion

This study demonstrated that the fate of tagged whales and their wounds can be tracked over the long-term, facilitated by collaboration among research groups conducting photo-identification. It provided some of the most powerful analyses conducted to date examining tag effects, qualitatively and quantitatively demonstrating a range of physiological reactions at the tag site. Based on our results, we recommend placement of tags higher on the whale's back (closer to the mid-line). In general, wounds tended to go through a progression, in which some developed swelling that usually resolved, but in a few cases persisted for several years. Based on the two whales with the largest, most persistent swelling, broken tag parts left in animals (from the earlier tag designs) appear to have the greatest visible long-term consequences until they are expelled. Multiple ongoing efforts are underway to improve tag designs, minimize breakage and eliminate parts being left behind in animals. Characteristics of populations being considered for tagging must be evaluated when developing a tagging protocol and study. Gray whales appear to be more reactive in their wound

response to tagging compared to blue whales. Additionally, this study demonstrated the typical challenges of these types of studies with the need for: larger sample sizes, populations with high resighting rates, high-quality photos for identification of tagged animals, as well as more frequent and longer-term follow-up observations. Long-term posttagging follow-up, combined with robust quantitative assessments, will afford a greater understanding of the potential effects of tagging. While this study focused on quantitative evaluation of wound development and healing, a separate analysis of survival rates is being undertaken to compare resighting rates of tagged and representative untagged animals (as controls).

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Supporting Information

The following supporting information is available for this article online at http://onlinelibrary.wiley.com/doi/10.1111/mms.12443/suppinfo.

Appendix S1. Physical evaluation of tag attachment sites in large whales.