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Assessment of flipper tag site healing in gray seal pups using thermography

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ABSTRACT

Infrared thermography was used to monitor the healing process at flipper tag sites in gray seal (*Halichoerus grypus*) pups. We tested the hypothesis that tagging would result in a rise in surface temperature associated with tag site healing processes compared with adjacent untagged areas of the flipper. Prior to tagging thermal images were recorded of the dorsal side of hind flippers of pups tagged in early lactation ($n = 20$) and at weaning ($n = 19$) on the Isle of May, Scotland ($56^{\circ}11'N$, $02^{\circ}33'W$) from October to December 2008. Pups tagged in early lactation were sampled again at late lactation, at weaning and then every 3 d for an average of 29 d post-tagging while pups tagged at weaning were sampled every 3 d for an average of 17 d post-tagging. Tag sites were also scored for signs of infection or swelling at each sampling. Results showed that (1) small temperature increases associated with wound healing processes around the tag site returned to pre-tagging levels before animals leave the island and (2) there was little evidence of tagging-related infections or tag loss irrespective of age at tagging.

Key words: tagging, marine mammal, wound, infrared imaging, gray seal, *Halichoerus grypus*.

Individual identification of animals is required for long-term population studies in pinnipeds and it is common to apply plastic flipper tags for this purpose (Lunn *et al.* 1994, Pomeroy *et al.* 2000, McMahon and White 2009). Although this method is effective there are problems with high tag loss rates (Bradshaw *et al.* 2000, Pistorius *et al.* 2000), particularly during the first year of life (Testa and Rothery 1992). Animals that have lost their tags may not be recognized in subsequent years and therefore not be counted as having survived and returned to a particular area. Increasing tag retention rates could therefore improve the accuracy of models of seal populations. Explanations of why pups lose tags have been based on limited observations of the enlargement of tag holes due to physical tearing or signs of infection around tags (Wilkinson and Bester 1997, Pistorius *et al.* 2000). Understanding the mechanisms and time course of tag loss is a precursor to refinement of current flipper tagging methods. Reduced tag loss would also be beneficial to the welfare of study animals by reducing the number of animals required to provide meaningful information on population dynamics. Normally, phocids are tagged in one or both of their hind flippers and otariids are tagged in their fore flippers using modified cattle ear tags. A common procedure is to remove any obvious dirt from the interdigital webbing then clean the skin with an antiseptic solution prior to skin puncture during tag application. Following this the tag site is sprayed with an aerosol antibiotic. This is similar to procedures for tagging cattle and is the recommended method of tag application in pinnipeds (Erickson *et al.* 1993).

Infrared thermography (IRT) for the assessment of injury and disease has proven useful in a number of applications. Human studies have included postoperative evaluations of skin grafts (Suominen and AskoSeljavaara 1996), digit reattachment (Saxena and Willital 2008), and the detection of febrile patients in mass screening during disease epidemics (Chiu *et al.* 2005, Chiang *et al.* 2008). In veterinary studies the technique has been similarly useful in assessing minor operative procedures in horses (Van Hoogmoed and Snyder 2002), pain responses in dairy cattle (Stewart *et al.* 2008), assessment of burns in pigs (Renkielska *et al.* 2006) and detection of disease in cattle (Schaefer *et al.* 2007, Rainwater-Lovett *et al.* 2009). Other physiological applications of IRT have included the detection of pregnancy in giant pandas (Durrant *et al.* 2006), stress response in rhesus monkeys (Nakayama *et al.* 2005) and heat production as a measure of feeding efficiency in cattle (Montanholi *et al.* 2008). The assessment of physiological processes in mammals using IRT has therefore proven to be a useful noninvasive research tool (McCafferty 2007). The precise measurement of infrared radiation emitted by an object using IRT allows the surface temperature to be determined according to relatively simple physical laws and known properties of the surface (Speakman and Ward 1998). This technique has been successfully used to study body surface temperature (Mauck *et al.* 2003) and increased heat production around application areas of telemetry tags and injection sites (McCafferty *et al.* 2007) in phocids. There is therefore the potential to detect signs of inflammation and infection associated with tagging using IRT.

The aim of this study was to use IRT as a tool to assess heat production associated with local infections around tagging sites in gray seal (*Halichoerus grypus*) pups. The hypothesis examined was that tagging would result in a rise in surface temperature associated with wound healing processes compared with adjacent untagged areas of the flipper. We investigated whether skin surface temperature around the tag site was related to visual indications of infection and the age of pup when tagged, once account had been taken of local environmental conditions.

MATERIAL AND METHODS

Study Site

This study was undertaken at the gray seal breeding colony on the Isle of May (56°11'N, 02°33'W), Scotland from 31 October to 6 December 2008.

Meteorological Measurements

General meteorological conditions were recorded by an automatic weather station (Minimet, Skye Instruments Ltd., Powys, UK) situated at the center of the island (50 m a.m.s.l). Air temperature ($\pm 0.1^\circ\text{C}$), relative humidity ($\pm 1\%$), and global solar radiation ($\pm 0.1 \text{ W m}^{-2}$) were measured at 1.2 m and wind speed (ms^{-1}) at 2 m above the ground. Wind speed was also measured locally at the site of each sampling at a height of 0.1–0.5 m using a hand held anemometer (Kestrel 3000, Lymington, UK).

Study Animals

Measurements were made of pups during field studies on the long-term breeding success of gray seals (see Pomeroy *et al.* 2000). Two groups of pups were followed. Pups were tagged either early in lactation (aged $< 5 \text{ d}$, $n = 20$), or around the time of weaning (aged approximately 20 d, $n = 19$). Estimations of pup age at tagging were based either on direct observations of birth date or on developmental stages described by Kovacs and Lavigne (1986). Study pups were individually identified at late lactation with a paint mark on the animal's mid-dorsal region and followed through to weaning by daily observations. For the purposes of this study, pups were defined as being weaned on the third consecutive day of being seen without their mother. Pups were weighed $\pm 0.5 \text{ kg}$ on a spring balance (Salter Industrial Measurements Ltd., West Bromwich, UK) at each sampling.

Imaging Protocol

Early lactation pups—Thermal images of the early lactation study group were taken before tagging, at late lactation ($\bar{x} = 11 \text{ d}$ post-tagging), at weaning ($\bar{x} = 18 \text{ d}$ post-tagging) and thereafter at 3-d intervals where possible.

Weaned pups—Thermal images of the weaned study group were also taken before tagging then every 3 d post-tagging.

Pups were captured and restrained using standard catching procedures for gray seals (see Pomeroy *et al.* 2000). Thermal images were taken of the dorsal side of the hind flippers from a distance of approximately 0.6 m by holding flippers against the ground surface and perpendicular to the camera. The influence of solar radiation on surface radiative temperature of the hind flippers of pups (see McCafferty *et al.* 2005) was minimized by shading flippers during measurement and avoiding imaging during the middle of the day. Images were recorded using a hand-held infrared camera (ThermaCAM E300, FLIR Systems Ltd., UK) with a 24° lens. This model has a spectral range of 7.5–13 μm with a measurement accuracy of 2% and thermal sensitivity of $< 0.1^\circ\text{C}$. In addition, a digital photograph (6 megapixels) was also taken as a visual aid to assessing thermal images.

Measurements and Treatment of Flippers

Pups were tagged in each hind flipper with numbered plastic tags (Dalton, Henley-on-Thames, UK). Before tags were applied the interdigital web was cleaned either using surgical gauze soaked in Videne Alcoholic Tincture (povidone-iodine 10%w/w) (Ecolab, Leeds, UK) or Savlon Antiseptic Solution (chlorhexidine-gluconate 0.3%w/w) (Novartis Consumer Health UK Ltd., Horsham, UK) diluted to approximately 1:17 Savlon:water. Where Savlon was used tag wounds were then sprayed with Terramycin Aerosol Spray (oxytetracycline 3.92%w/w) Pfizer Ltd., Kent, UK). Tag sites were examined at each recapture and scored for wound type (1 = closed, 2 = open, no bleeding, and 3 = open and bleeding), signs of swelling (1 = none, 2 = localized around tag site only, and 3 = regional: extending over interdigital web and/or flipper) and exudates (1 = none, 2 = minor: thin thread like discharge around tag wound, and 3 = major: discharge extended >3 mm from tag site). Scores were summed as an indicator of tag site condition for analysis. Wounds showing any serious inflammation or swelling were cleaned using dilute chlorhexidine solution and sprayed with oxytetracycline. Flippers were also scored as either being wet or dry.

Image Analysis

Images were analyzed using the software package FLIR Thermacam Reporter (Version 7.0). The average surface temperature immediately surrounding the tag was recorded by selecting a circular area ($\sim 1,130 \text{ mm}^2$) with diameter equal to two times the tag width (19 mm) centered on the tag post and excluding all parts of the tag within that area. This standardized the measurement area to account for small differences in measurement distance between the camera and flipper. An identically shaped area was copied and transferred to the adjacent nontagged interdigital web symmetrical in location to the tagged interdigital web. In this way an equivalent average temperature from the adjacent nontagged interdigital web could be recorded (Fig. 1). Average temperature is calculated by taking the absolute temperature recorded in each pixel within a given area and averaging over the number of pixels within that area. The difference in average temperature between the tagged interdigital web and the adjacent untagged web (dTave) was used to examine changes in surface temperature patterns. To examine any possible effects of small differences in viewing angle when taking images, mean dTave of untagged pups without injuries were categorized into two groups; images that were perpendicular to the flipper and those that had deviated from this. Digital photographs were used to score for the presence and absence of wounds or other injuries on flippers outside the immediate tag region.

Statistical Analysis

Generalized additive mixed models (GAAMs) (Wood 2006) were used to account for variation in dTave over time. Models were fitted using the multiple generalized cross validation (mgcv; Wood 2006) and nonlinear mixed effects (nlme; Pinheiro *et al.* 2009) libraries in the statistical package R (R Development Core Team 2009). Pseudo-replication due to repeat measures on individual animals was accounted for by the use of an autoregressive correlation structure (Pinheiro and Bates 2000). This controlled for measurements within the same individual being more similar than measurements between individuals. Visual inspection of residuals showed violation

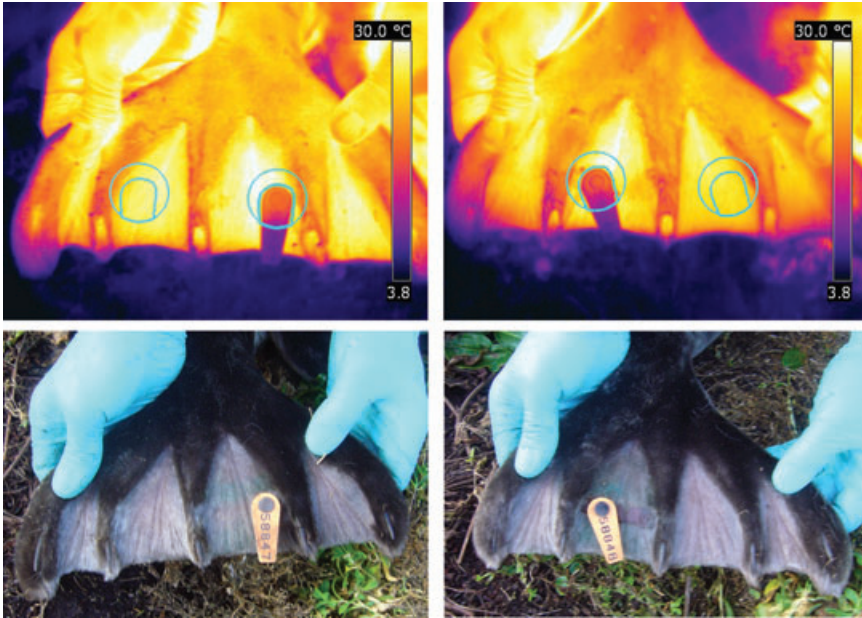


Figure 1. Infrared images of left (top left) and right (top right) flippers with associated digital photographs beneath. Areas sampled to get average temperature for differentials of tagged minus untagged interdigital webs (dTave) are delineated by blue lines. Images are of a weaned pup tagged 9 d previously.

of homogeneity that was accounted for by use of a power variance function (Pinheiro and Bates 2000) allowing residual spread to vary with time. Air temperature, solar radiation, relative humidity, wind speed and mass were included as linear terms in the full model. Tag site score, age group, whether or not flippers were wet or dry and whether or not flippers had injuries outside the site of tagging were included as factors. A nonlinear smooth term was used to capture any patterns in changes with the number of days post-tagging. Wind measurements recorded by the weather station were chosen over those recorded by the handheld anemometer to avoid collinearity and to provide consistency with the method for obtaining the other meteorological measurements. Stepwise backwards model selection was carried out using Akaike information criterion (AIC) to identify the best model of the data.

RESULTS

Study Animals

Early lactation pups ($n = 20$) were followed throughout lactation and the post-weaning period for an average of 29 d post-tagging (range = 21–35 d). Weaned pups ($n = 19$) were followed throughout the post-weaning period for an average of 17 d post-tagging (range = 6–24 d). The mean body mass \pm SE at first capture was 18.7 ± 0.7 kg and 44.6 ± 1.3 kg for the early lactation and weaned groups, respectively.

Meteorological Data

Throughout the study period air temperature, relative humidity, daytime solar radiation, wind speed (1.2 m), and wind speed (ground level) averaged $6.7 \pm 0.04^\circ\text{C}$, $99.6 \pm 0.04\%$, $75.7 \pm 1.65 \text{ W m}^{-2}$, $7.7 \pm 0.06 \text{ ms}^{-1}$, and $2.0 \pm 0.08 \text{ ms}^{-1}$, respectively. There were 20 d of rain ($>0.2 \text{ mm}$) with rainfall totaling 55.9 mm over 37 d.

Summary of Tag Site Scores

Early lactation study pups showed signs of exudates at the tag site in 28% of flippers and partially open wounds in 6% of flippers ($n = 36$) between 8 and 14 d post-tagging (Table 1). Between 18 and 24 d post-tagging the same group of animals showed signs of exudates in 23% of flippers and partially open wounds in 5% of flippers ($n = 100$). Only one animal in this group showed signs of major swelling in one flipper at 11 d post-tagging which was not evident at the next sampling of this animal at 19 d post-tagging. No tag sites showed any signs of exudates, swelling, or being open after 24 d post-tagging.

Weaned study pups showed signs of minor exudates in 4% of flippers ($n = 74$) between 3 and 7 d post-tagging (Table 1). Minor exudates were again observed in 3% of flippers ($n = 76$) between 8 and 14 d post-tagging but not in the same animals as at 3–7 d post-tagging. No tag sites showed any signs of exudates, swelling or being open between 18 and 24 d post-tagging.

For the two groups combined a total of 10 animals were scored as having both tag sites simultaneously showing signs of exudates, swelling or being open at any given time.

Variation in dTave

For early lactation and weaned pup study groups combined a fitted smooth curve of dTave over time (significance of smooth term $P = <0.001$) showed there was

Table 1. Percentage of flippers with different wound types, swelling, and exudate scores at tag sites for pups tagged at early lactation and as weaned pups ($n =$ number sampled). Results were grouped into different periods post-tagging.

Days post-tagging		Percentage of flippers					
		Early lactation pups			Weaned pups		
		8–14	18–24	25–35	3–7	8–14	18–24
<i>n</i>		36	100	70	74	76	32
	Total <i>n</i>		206			182	
Wound type	Closed	94	95	100	100	100	100
	Open	6	5	0	0	0	0
	Bleeding	0	0	0	0	0	0
	None	97	100	100	100	100	100
Swelling	Localized	0	0	0	0	0	0
	Regional	3	0	0	0	0	0
Exudates	None	72	77	100	96	97	100
	Minor	28	21	0	4	3	0
	Major	0	2	0	0	0	0

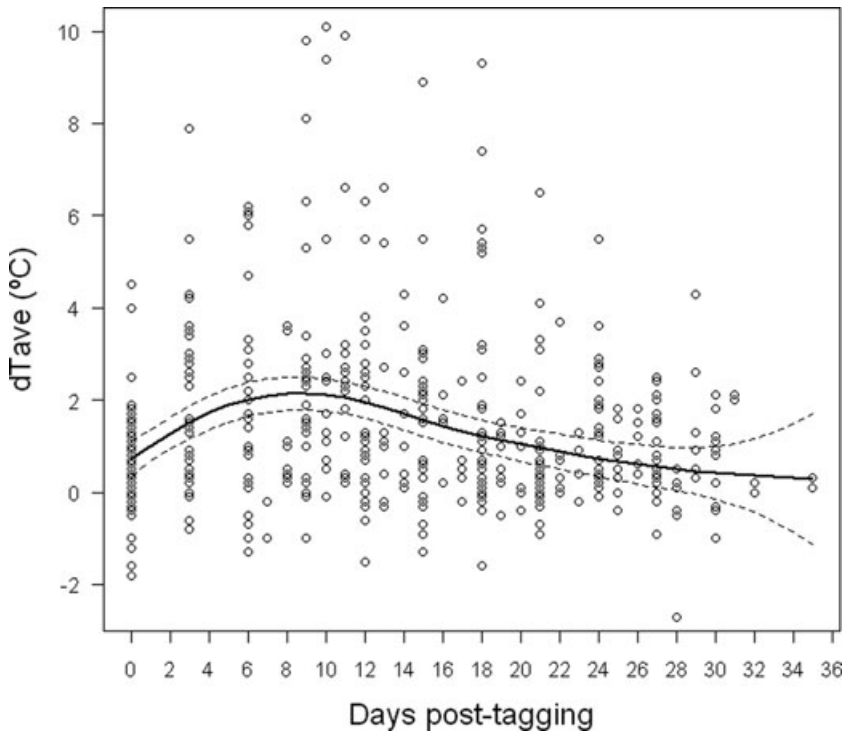


Figure 2. Plot of GAAM model showing predicted temperature differentials of tagged minus untagged interdigital webs (dTave) over time for all study pups. The solid black line represents the smoother for dTave over days post-tagging with dashed lines as the 95% CIs. Data points are raw values for dTave.

a significant increase in dTave that returned to pre-tagging levels by the end of the sampling period (Fig. 2). Significant explanatory variables included in the final model were air temperature, solar radiation, whether or not flippers were wet or dry and presence/absence of injuries (Table 2). dTave decreased with increasing air temperature, increasing solar radiation and when flippers were wet and increased when animals had other injuries. Age group was not found to be a significantly influencing factor on dTave over time. Tagging pups in early lactation or at weaning therefore had no significant effect. Small differences in viewing angle were not significantly different when comparing mean dTave of images taken perpendicular to the flipper and those that had deviated from this (t -test on pooled early lactation and weaned study groups $t = 0.41$, $df = 62$, $P = 0.69$). This was therefore not a significant source of error in the variation in dTave.

DISCUSSION

In this study our approach was to use IRT to examine the physiological response of gray seal pups to the application of flipper tags that are used during long-term population studies. Results showed that (1) there was little evidence of

Table 2. Summary of GAMM results for GAMM model. (Significance levels, n.s., * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.) Tag site scores were wound type (1 = closed, 2 = open, no bleeding, and 3 = open and bleeding), signs of swelling (1 = none, 2 = localized, and 3 = regional), and exudates (1 = none, 2 = minor, and 3 = major).

	Significance level
f (Days post-tagging)	***
Air temperature ($^{\circ}\text{C}$)	**
Solar radiation (W m^{-2})	*
Relative humidity (%)	n.s.
Wind (ms^{-1})	n.s.
Mass (kg)	n.s.
Age group (early lactation or weaned pups)	n.s.
Tag site score	n.s.
Wet or dry	*
Other injuries	**
Deviance explained (%)	13.3

tagging-related infections or tag loss before animals went to sea irrespective of age at tagging and (2) small temperature increases associated with wound healing processes around the tag site returned to pre-tagging levels before animals leave the island.

Gray seal pups have been shown to have age-related differences in blood chemistry and hematology from birth to the end of the post-weaning fast (Hall 1998). Young pups have also been shown to have lower levels of immunoglobins than weaned pups (Baker and Baker 1988) and a reduced response to phytohemagglutinin (PHA) skin tests (Hall *et al.* 1999) indicating, not surprisingly, that the first few weeks of life for gray seals are important in developing a robust immune system. Previous studies on gray seals have indicated that conjunctivitis (Waters 1971) and peritonitis (Baker and Baker 1988) are more prevalent in younger pups than in weaned pups. This would imply that tags applied to animals at an early age have a higher risk of developing infection at the tag site or have a reduced healing capacity than for animals tagged at or after weaning. In this study, pups tagged at early lactation did seem to have a higher prevalence of minor exudates persisting around the tag site. This may have been due to molted lanugo accumulating around the tag and thereby trapping dirt and pathogens. Pups tagged during the post-weaning fast will have lost most if not all of their lanugo and so do not accumulate lanugo and dirt in the same way. We found no evidence based on thermal images that animals tagged at early lactation suffered any greater adverse affects compared to pups tagged at weaning.

While efforts are made to clean interdigital webs prior to the application of flipper tags, carrying out this task in field conditions is inevitably a nonsterile procedure. Living in a contaminated environment and the risk of aggression from other lactating females exposes pups to infection immediately from birth. This is evident in mortality and morbidity rates associated with bacterial infection in pinniped pups (Baker 1984, Castinel *et al.* 2007). Baker (1984) found that groups of potential pathogens (*Streptococcus* spp. and *Corynebacterium* spp.) found on the body surface and in the orifices of gray seal adults and pups were also found in the substratum of breeding colonies on North Rona, the Monach Isles, and Shillay. On the Isle of May these same groups of pathogens were found to be associated with bite and/or trauma wounds in

postmortem examination of deceased gray seal pups (Baker and Baker 1988). In this study pups that had injuries other than the tag wound itself showed an increase in dTave indicating that injuries inflicted by bites or in other ways may have a negative effect on the healing response even when injuries seem quite remote from the actual tag site. It may be that pups in denser parts of the colony, where aggression from other animals is more prevalent, are more prone to such injuries and subsequent infection.

Previous studies using IRT to assess injury in animals have been carried out under controlled environmental conditions (Van Hoogmoed and Snyder 2002, Renkielska *et al.* 2005). The fact that the model presented in this study explained only 13.3% of the variance suggests that the use of IRT to study small, localized changes in temperature in animals in wild conditions has limitations. This is especially true in seals that have such variable rates of perfusion of blood to flippers during thermoregulation (Reynolds and Rommel 1999). However, the significance of the model allowed for the investigation of a number of covariates and to explore the time course of healing of tag sites. The model also highlights the importance of other unrelated injuries that may have an effect on tag site healing. Further field applications of IRT on pinnipeds could be improved by reducing effects of environmental conditions and differences in animal behavior and activity on surface temperature patterns (McCafferty 2007). Parallel studies on captive pinnipeds where these variables can be more easily standardized/controlled for, may therefore provide further opportunities of using IRT to more accurately detect inflammation, infection, and other physiological processes.

Conclusions

Overall, IRT was successfully used to pick up changes in skin surface temperature associated with flipper tag application. Tagging of animals had no overall adverse effect as tag sites were at an advanced stage of healing before animals went to sea regardless of age when tagged. It would appear that tagging of gray seal pups is therefore not a significant challenge to welfare. There was no evidence to suggest that further refinement to current tagging protocol is required and that the retention rate of tags in the period up to and immediately after leaving for sea should be quite high for gray seals.

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