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Animal Welfare 2021, 30: 91-100
ISSN 0962-7286
doi: 10.7120/09627286.30.1.091

Can eye surface temperature be used to indicate a stress response in harbour seals (Phoca vitulina)?

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Abstract

*Infra-red thermography (IRT) is increasingly being used to estimate physiological stress responses in animals via changes in eye surface temperature. The aim of this study was to determine whether eye temperature of harbour seals (*Phoca vitulina*) changes in response to routine handling and the potentially painful procedure of flipper-tagging, and if responses to tagging can be mitigated by subcutaneous injection of lidocaine. Orphaned pups (n = 52) at a rehabilitation facility were assigned to one of four treatments: Lidocaine (handled twice, once for injection and once for tagging); Saline (also handled twice); Tag Only (handled once); Sham Tag (handled once). Eye temperature increased more when pups were first handled compared to pups that were not handled and increased further in pups that underwent a second handling. Eye temperature of pups that were tagged without any previous treatment (Tag Only) increased compared to pups that were sham-tagged. Eye temperature also tended to increase after pups were injected with lidocaine but not saline. These results suggest that: (i) handling causes a physiological stress response; (ii) increased eye temperature arising from the second handling suggests the first handling was likely aversive, resulting in sensitisation to further handling; (iii) the rise in eye temperature after tagging, but not sham-tagging, may reflect pain from tagging; and (iv) lidocaine, at the dosage tested, did not appear to reduce the physiological response to tagging. These results show promise for the use of eye temperature to monitor stress responses and for evaluating the potential aversiveness of routine procedures in seals.*

Keywords: *animal welfare, eye temperature, handling, harbour seal, pain, stress response*

Introduction

The assessment of potentially painful or aversive husbandry procedures typically relies on a combination of physiological and behavioural measures (Rutherford 2002). Unfortunately, many of these measures are time-consuming and may require handling or sampling which can themselves result in stress responses (Stewart *et al* 2005). As an alternative, infra-red thermography (IRT) is increasingly recognised as a reliable, non-invasive method to detect changes in heat emission, especially from around the eye, as an indication of physiological stress responses in animals (Stewart *et al* 2010).

For many years, IRT has been used successfully to identify inflammatory injury and disease in veterinary medicine (McCafferty 2007) and, increasingly, to measure stress responses in animals via changes in local vascular perfusion. There is evidence that both physiological and psychological stress are accompanied by changes in eye temperature (Cook *et al* 2001; Pavlidis & Levine 2002) and that eye temperature may reflect the activity of both the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis (Cook *et al* 2001; Stewart *et al* 2008a,b). Short-term eye temperature changes due to changes in peripheral blood flow are associated with the ANS and may be a physiological correlate of an animal's affective state, for example, in response to painful, stressful or arousing stimuli (Stewart *et al* 2008a). In such cases a change in the amount of blood flow through peripheral vasculature influences the temperature of the local skin and extremities leading to a change in the amount of heat radiated from affected surfaces that can be measured with IRT.

The eye and, in particular, the areas of the lacrimal caruncle and palpebral border of the ventral eyelid, have been recognised as being particularly sensitive to changes in blood flow from stress responses (Pavlidis & Levine 2002; Stewart *et al* 2008b). For example, changes in eye temperature have been recorded in horses (*Equus caballus*) (Dai *et al* 2015) and cattle (*Bos taurus*) (Stewart *et al* 2008a) in response to fear-producing stimuli, in horses after aversive procedures such as coat-clipping (Yarnell *et al* 2013) and the application of

https://doi.org/10.7120/09627286.30.1.091 Published online by Cambridge University Press

restrictive nosebands (McGreevy *et al* 2012; Fenner *et al* 2016), in elk (*Cervus elaphus canadensis*) and reindeer (*Rangifer tarandus*) from velvet antler removal (Cook *et al* 2005), in dogs (*Canis familiaris*) from veterinary examination (Travain *et al* 2015), and in cattle after castration and disbudding (Stewart *et al* 2008b, 2009, 2010). Hence, variations in eye temperature with IRT can provide a dynamic, real-time tool that can potentially be used for the evaluation of an animal's stress response to a range of handling and husbandry interventions.

Orphaned harbour seal pups (*Phoca vitulina*) are commonly admitted to rehabilitation facilities where they are hand-raised and, once deemed healthy and capable of independent survival, released (MacRae *et al* 2010). These animals are frequently admitted into rehabilitation with conditions or injuries that are potentially painful, and while in care they are subjected to many potential stressors (handling, restraint, veterinary interventions), some of which may cause pain. However, little research has assessed the stress responses of pinnipeds to such procedures. Moreover, there is limited information on identifying pain in any species of pinniped. Poor identification of pain in these species may thus result in inadequate or inconsistent pain therapies (Flecknell 2000). Additionally, exposure to prolonged or severe stress can have a range of negative health consequences, including impaired growth and immune competence (Moberg 2000). Since these orphaned pups are already vulnerable, it is important to identify and possibly moderate husbandry routines and procedures that may contribute to animals' distress.

In Canada, the Department of Fisheries and Oceans (DFO) requires each rehabilitated pup to be marked prior to release; this is typically done by placing a tag in the web of a hind flipper and a microchip at the base of the tail. Similar identification procedures in other species have been associated with pain; examples include ear-tagging and microchipping in rodents (Dahlborn *et al* 2013) and ear-tagging (Leslie *et al* 2010) and ear-notching (Torrey *et al* 2009) in piglets (*Sus scrofa*). While being tagged and microchipped, seals have been observed to flinch, vocalise, exhibit escape behaviours, and curl their flippers after tagging — behaviours similar to those thought to reflect pain in many other species (Rutherford 2002; National Research Council 2009). Seals also demonstrate significant increases in orbital tightening after being tagged and chipped (a facial change indicative of pain in many species), as well as higher than normal breathing rates during these procedures, thought to be in response to the accompanying handling and restraint (MacRae *et al* 2018). Notably, it is not standard practice to provide analgesics for these procedures or sedatives for concomitant handling and restraint.

Measuring changes in eye temperature may be particularly useful in contexts that necessitate the temporary captivity of wild species. For example, in wildlife rehabilitation, handling and human proximity may be especially stressful to the animals, potentially dangerous to the handler, and may increase the risk of habituation to humans and thus potentially jeopardise the animals' biological fitness once released. IRT may therefore provide a practical method for identifying stress responses caused by fear and pain from routine procedures and husbandry practices in pinnipeds being hand-raised and rehabilitated. The aims of the current study were to determine: i) whether harbour seal pups showed a change in eye temperature after flipper-tagging; ii) whether such changes in eye temperature were the result of tagging, as distinct from possible effects of handling; and iii) whether any observed changes in eye temperature could be mitigated by the sub-cutaneous administration of a local anaesthetic (lidocaine).

Materials and methods

Ethical approval

This research was approved by the University of British Columbia Animal Care Committee (Protocol A16-0175) and by the Vancouver Aquarium Animal Care Committee.

Study animals and facilities

The 52 harbour seal pups (30 males, 22 females) had been recovered along the coastline of British Columbia, Canada, by Vancouver Aquarium Marine Mammal Rescue (MMR) staff, or been brought to the facility (49°14' 46.6512" N 123° 6' 58.4136" W) by members of the public, between June and August of 2016. Data were collected between August and December of 2016.

Animals were kept at MMR following the standard operating procedures of the facility. Handling of pups was confined to only essential husbandry and veterinary care. When initially admitted, pups were housed individually in plastic tubs (approximately 92 × 61 × 61 cm; length × width × height) for a quarantine period of at least 14 days. During this period, they were fed a commercial pinniped milk-replacer (Zoologic 30/55 Milk Matrix, PetAg, Hampshire, IL, USA, PMI Nutrition, St Louis, MO, USA) five times per day via gavage at approximately 10 to 15% of their bodyweight. Wounds were checked or cleaned twice per day and fluids given subcutaneously as needed. After approximately 14 days, animals were weaned onto whole herring (scatter-fed around 0900, 1500 and 2100h daily at approximately 12% of bodyweight) and moved out of quarantine into group-housing with groups of up to eight in a home pen with a fibreglass pool of approximately 4,500 l (2.4 × 0.8 m; diameter × depth) and a 3.7 × 4.0 m (length × depth) haul-out area. Once in group-housing, pups were captured once per week to be weighed but otherwise left unhandled and interactions with caregivers kept to a minimum.

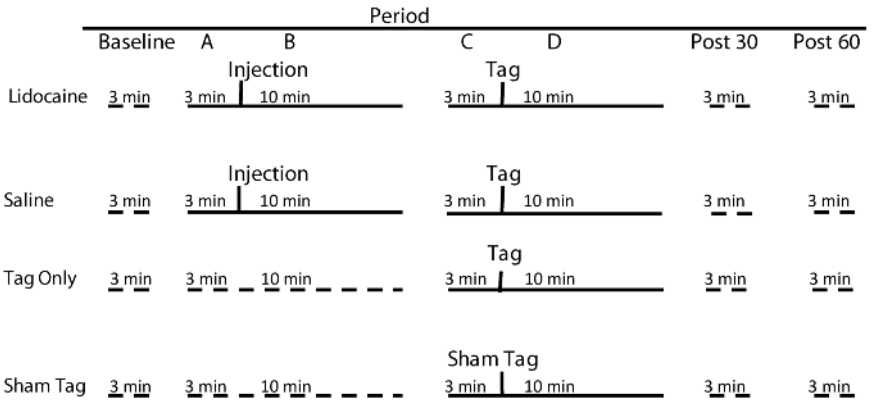
Once pups were deemed ready for release (weaned, estimated to be more than 90 days old, and free of disease or injury), they were assigned to one of four treatments using a random number generator. Mean (± SEM) bodyweight at the time of testing was 23.6 (± 0.3) kg (range: 18–30 kg). All pups had been housed in their home pen with the same pen-mates for at least one week prior to testing.

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https://doi.org/10.7120/09627286.30.1.091 Published online by Cambridge University Press

Figure 1

Representation of the four treatments (n = 13 seal pups per treatment) over the different periods from Baseline (before any handling began) to Post-60 (60 min after all handling had ended). The dashed lines indicate periods when pups were not handled, and the solid lines indicate periods when pups were restrained.



Treatments

Twelve test days, approximately one per week for twelve weeks, were co-ordinated with the times when seals were ready for release. Four pups were tested per day (one pup per treatment) except on the first day, when eight animals were tested (two per treatment). Testing was carried out between 0800 and 1400h; daily feeding was delayed until testing was completed. The Baseline eye temperatures of all pups were first recorded for 3 min in their home pen before any handling began. The four treatments are shown in Figure 1 and described in the following text.

Lidocaine (n = 13, handled twice)

After the 3-min Baseline recording in the home pen, pups were captured and moved to the tagging area where they were immediately restrained (as described below). Eye temperature recording began as soon as pups were manoeuvered into the restraint position (which took approximately 20 s). After 3 min of restraint (Period A), they received 1 ml lidocaine (2%) injected subcutaneously into the tagging site. Restraint and eye temperature recording continued for 10 min after the injection (Period B). Pups were then placed in a plastic carrying tote (Rubbermaid®, Newell Brands, Atlanta, GA, USA) and left undisturbed in the tagging area for a 10-min rest when eye temperature was not recorded. Pups were then recaptured and restrained a second time with eye temperatures again being recorded. After 3 min of restraint (Period C), pups were tagged, and restraint continued for 10 min (Period D). Consecutive thermograms (one approximately every 10 s) were taken for the duration of each sampling period (Periods A, B, C and D). Then pups were returned to the home pen.

Saline (n = 13, handled twice)

This procedure was identical to the Lidocaine treatment except that 1 ml of saline solution was injected instead of lidocaine.

Tag Only (n = 13, handled once)

After the 3-min Baseline recording in the home pen, pups had eye temperatures recorded for a further 13 min while still unhandled in their home pen (Periods A and B). Ten min later, pups were captured and moved to the tagging area. After 3 min of restraint (Period C), pups were tagged and restraint continued for 10 min (Period D). Consecutive thermograms (one approximately every 10 s) were taken for the duration of each sampling period (Periods A, B, C and D). Then pups were returned to their home pen.

Sham Tag (n = 13, handled once)

This procedure was identical to the Tag Only treatment but instead of the animals being tagged, they were sham-tagged by touching the flipper with the tagging unit but without it piercing the skin. The sound from depressing and releasing the tagging unit was the same for both actual tagging and sham-tagging.

At the end of Period D, pups were returned to the home pen within 1 min. Eye temperature was then recorded for 3 min for each pup, starting 30 and 60 min after the pup had been released back into the home pen.

Testing locations

Testing was carried out in two locations: the home pen and the tagging area. Pups remained in their home pen (described above) during periods that required no handling. During the study the roof and sides of the home pen were covered with canvas in order to eliminate sunlight and minimise cross-draughts. The night before test days (at approximately 2200h), the pool in the home pen was gated off so pups would remain dry for eye temperature recording. Pups were then given access to the pool as soon as recording had been completed for the day. The tagging area was a 3 × 3 m (length × width) pavilion tent canopy with four sides where all testing that required handling was completed. The two locations were approximately 9 m apart. Pups were captured by hand and carried between locations in plastic totes (81 × 51.4 × 44.5 cm [length × width × height]). The ambient temperature and humidity of the relevant testing location were recorded at the start of each sampling period (Taylor Wireless Indoor/Outdoor Thermometer®, Oak Brook, IL, USA).

https://doi.org/10.7120/09627286.30.1.091 Published online by Cambridge University Press

Injection and tagging protocol

After being carried to the tagging area, individuals were placed on a foam mat where they were restrained in ventral recumbency, with the animal's body between the restrainer's knees, its head between the restrainer's feet and hind flippers secured in the restrainer's hands. The injection site was the webbing of the 2nd and 3rd digits of the hind flipper which was cleaned with chlorhexidine and alcohol. All injections were performed by the same person and consisted of either 1 ml of 2% lidocaine hydrochloride (20 mg ml^{-1}; Zoetis Canada Inc, Kirkland, QC, Canada) or 1 ml of sterile saline solution delivered with a 22-G × ¾ " (0.711 mm × 1.9 cm; width × length) needle (Kendall, Mansfield, Massachusetts, USA) on a 3-ml syringe (Terumo Medical Corporation, Somerset, New Jersey, USA) into the area targeted for insertion of the tag.

For tagging, animals were restrained, and tag sites cleaned as described. The time between injection and tagging was approximately 20 min. A 5-cm plastic tag bearing an animal identification number and DFO contact information was attached by piercing the webbing of the 2nd and 3rd digits of the hind flipper, as noted earlier. Males were tagged on the right hind flipper and females on the left. The same two operators (both members of the facility's animal care team) alternated between restraining and tagging the seals and their involvement was balanced across treatments.

Eye temperature measurements

Infra-red images of each pup's eyes were collected with an IRT camera (FLIR T300, FLIR Systems AB, Danderyd, Sweden) which has a thermal sensitivity of < 0.05°C and is able to detect temperatures between –20°C and 650°C. The camera self-calibrated at regular intervals. Consecutive thermograms (one approximately every 10 s) were taken from approximately 1 m from seals' eyes for the duration of each sampling period (Baseline, Periods A, B, C, D, Post-30 and Post-60). When in the home pen, seals stayed relatively stationary, thus allowing easy capture of eye images despite the animals being unrestrained. All thermal images were taken by the same person and the animals were dry at the time of recording. Once collected, the thermal images were uploaded to FLIR Tools+ analysis software. They were analysed to determine maximum eye temperature (°C) within the area of the medial posterior palpebral border of the ventral eyelid and the lacrimal caruncle (Figure 2). The person analysing the images was blind to treatment.

Statistical analysis

Thermograms were binned by minute to obtain a mean of the maximum eye temperature in each minute of each period for each seal. No temperature difference was found between the left and right eyes, so these images were pooled. For Periods A through D each minute was treated as a separate datapoint. Three minutes of data were collected for each seal in the Baseline period and in the two periods after all handing had been completed (Post-30 and Post-60 min). In the absence of significant differences in eye temperature between the respective minutes of these periods, data were pooled to make a single average maximum eye temperature for each seal for each of these three periods. Subsets of the data were analysed separately for each of the main comparisons (ie to determine the effects of treatment, tagging, handling and of receiving an injection). All results were expressed as means (± SEM). For each model, normality and homoscedascity of residuals were assessed graphically.

Data were analysed using R version 3.2.1 (R Core Team 2015). Linear mixed-effects models (LMM) were calculated with the R package lme4 (Bates *et al* 2015) and lmerTest (Kuznetsova *et al* 2017). The models included the seals' maximum eye temperature (as the response variable), and tested the effects of sex, bodyweight, treatment, treatment period (Baseline, Periods A, B, C, D, Post-30 and Post-60) within the treatment, minute within treatment period, and handling (capture, restraint, and/or injection, tagging), as fixed-effect variables, as well as interactions between treatment and treatment period and between treatment period and minute. Seal was included as a random effect. Results were considered significant at $P \leq 0.05$.

Results

The pups showed large and consistent individual differences in eye temperature throughout the experiment. For example, one of the Sham Tag pups ('Bubbles') had eye temperature values from 30 to 34.4°C throughout the study whereas another ('Peter') remained in the range of 27–29°C.

Eye temperature values correlated weakly with ambient temperature in some periods and more strongly in others. For example, the correlation of eye temperature and ambient temperature was 0.38 in the Baseline period and 0.80 in the final readings taken 60 min after pups had been returned to their home pens. As a result of the correlation with ambient temperature, all comparisons of eye temperature values were made after including ambient temperature in the model, except when comparing Periods A to B and C to D because the same ambient temperature applied during those periods.

Handling (capturing, restraining, injecting) varied across treatments. This allowed us to test the effect of different handling events separately. Treatments were grouped when possible to increase power as described below. To assess the effect of handling events on eye temperature change, the interactions between period and treatments were included. When not statistically significant, the interaction term was removed, and only main effects reported.

Eye temperature changes after handling

To examine the effect of handling (capture and restraint) versus no handling, we compared the change in eye temperature from Baseline to Period A for the 26 pups that were moved to the tagging area (Lidocaine and Saline treatments) versus the 26 pups that remained in the home pen (Tag Only and Sham Tag treatments). To explore the effect on seals of being handled a second time, eye temperatures for the Lidocaine and Saline treatments were compared for Period A (first handling) and Period C (second handling).

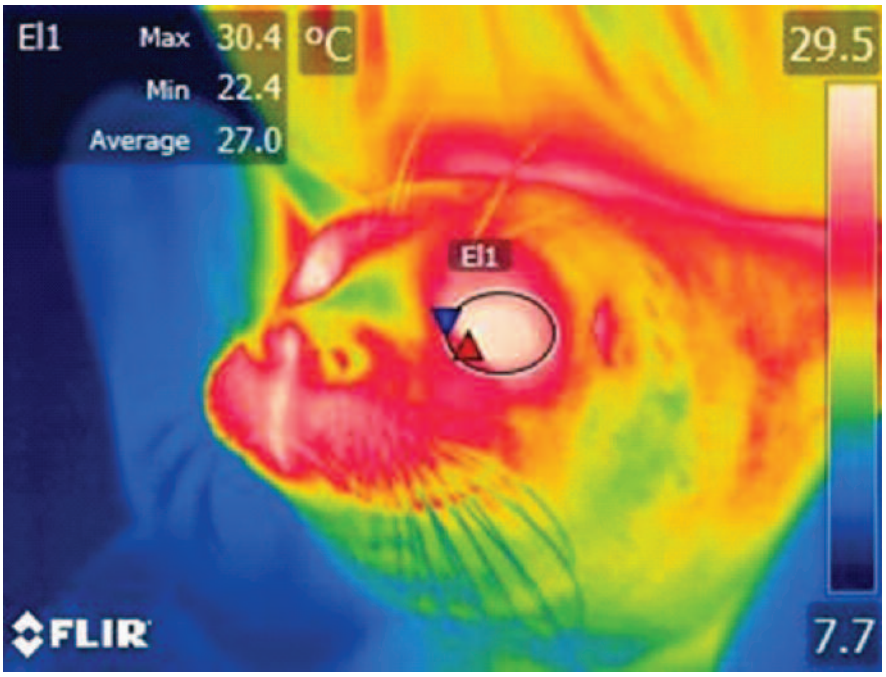
Eye temperature increased from Baseline to Period A in all pups. However, there was an interaction between treatment

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Figure 2

Example of a thermal image of a seal's eye. Maximum and minimum eye temperature indicated by red and blue triangles, respectively.



and period ($P = 0.014$); the magnitude of the eye temperature change was greater ($P < 0.05$) for pups that were handled (adjusted mean during Baseline of 29.0 [± 0.2] vs 29.8 [± 0.2]°C in Period A) than those that remained in the home pen (adjusted mean during Baseline of 29.2 [± 0.2] vs 29.6 [± 0.2]°C during Period A). There was no effect of bodyweight or sex on eye temperature.

An apparent effect of capture and restraint was also seen when pups in the Lidocaine and Saline treatments were restrained a second time in Periods C and D. These animals, which had already been restrained and injected in the tagging area during Period B, showed a further increase of 0.7 (± 0.08)°C ($P < 0.001$) in eye temperature from Period B (adjusted mean of 29.7 [± 0.2]°C) to Period C (30.4 [± 0.2]°C) with no difference between the Lidocaine and Saline groups ($P = 0.79$).

Eye temperature also increased after the pups were returned to the home pen. The first reading, taken 30 min after the pups were returned to the home pen and reunited with pen-mates (adjusted mean of 30.3 [± 0.2]°C), was 1.1 (± 0.2)°C ($P < 0.001$) higher than the during Baseline (29.2 [± 0.2]°C), regardless of treatment and controlling for changes in ambient temperature. Eye temperature declined 0.5 (± 0.15)°C ($P < 0.001$) at the final reading taken 60 min after the pups were returned (adjusted mean of 29.8 [± 0.2]°C) but still remained higher than Baseline (by 0.6 [± 0.26]°C; $P = 0.002$), regardless of changes in ambient temperature. Treatment did not affect the change in eye temperature from Baseline at either 30 or 60 min after treatment.

Due to the apparent effect of capture and restraint, treatments were compared only for Lidocaine versus Saline and for Tag Only versus Sham Tag because pups in these pairs of treatments had been handled in the same way (Figure 1).

Eye temperature changes after injection

After lidocaine injection, some pups showed an upward trend in eye temperature over the 10 min of Period B (Figure 3). Of the 13 seals, ten had a higher mean eye temperature after lidocaine injection (Period B) than before (Period A), the mean increase of all 13 seals being 0.3 (± 0.16)°C ($P = 0.07$). No similar change was seen in the pups injected with saline (0.0 [± 0.16°]C; $P = 0.9$).

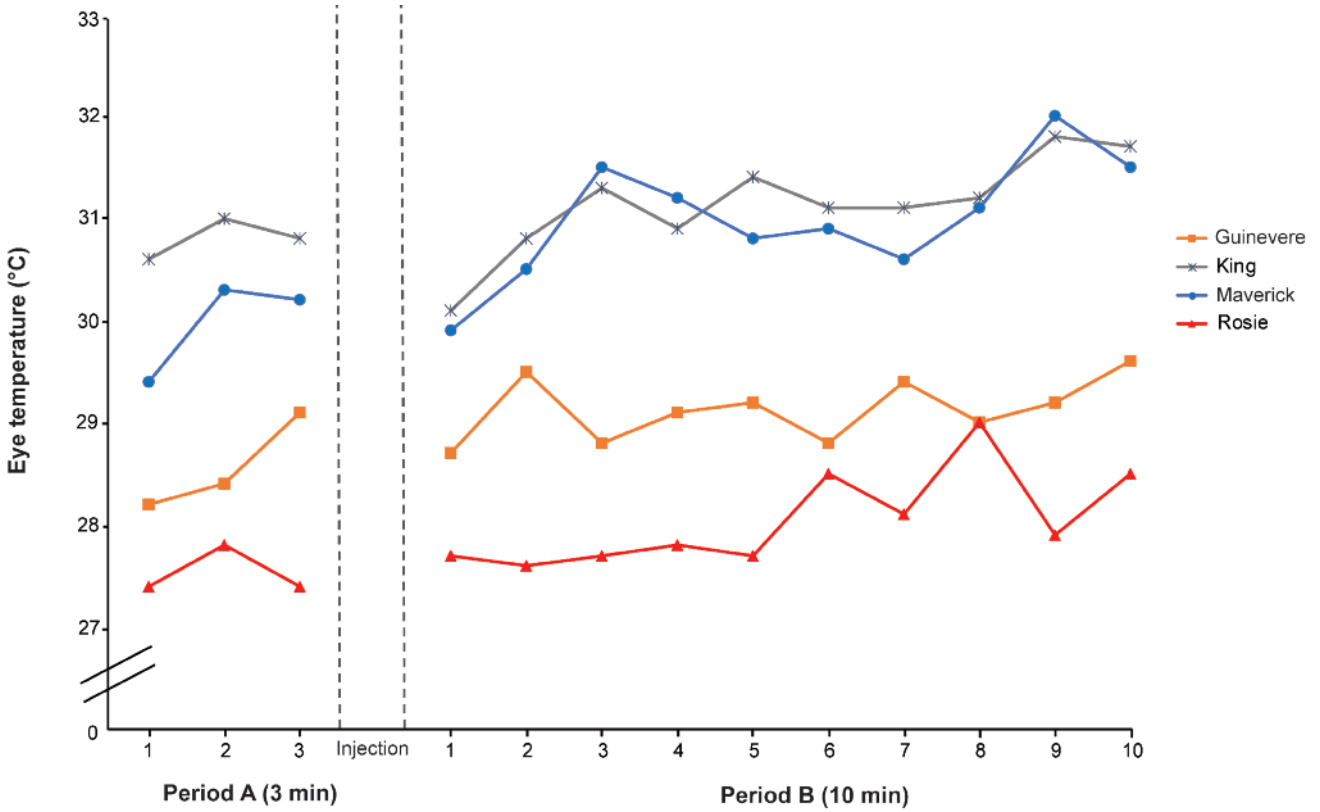
Eye temperature changes after tagging or sham-tagging

To examine whether there was a change from before to after tagging or sham-tagging, the difference between average eye temperature before tagging (Period C) and after tagging (Period D) was compared for each of the Tag Only and Sham Tag treatments separately, using a paired *t*-test. This approach was used because the assumption of homogeneity of variance was not met and because it was not necessary to adjust for ambient temperature when comparing Periods C and D because the same ambient temperature applied in both periods.

After tagging, pups showed large individual differences in eye temperature response but no uniform pattern (Figure 4). For example, of the pups that were tagged with no previous intervention (Tag Only), two ('Oscar' and 'Satchi') showed an acute 1° increase in eye temperature in min 2–4 after tagging, whereas most others did not. However, mean eye temperature increased gradually during the 10 min, with mean (± SEM) temperature being 0.3 (± 0.11)°C higher after tagging (Period D) compared to before (Period C) ($t = 2.58$, df = 12; $P = 0.02$), and increased 0.4 (± 0.17)°C from min 1 to min 10 after tagging (Period D) ($t = 2.43$, df = 12; $P = 0.03$). Pups in the Sham Tag treatment showed no similar increase in average eye temperature (mean change of 0.0 [± 0.19]°C; $t = –0.11$, df = 12; $P = 0.92$) between Periods C and D.

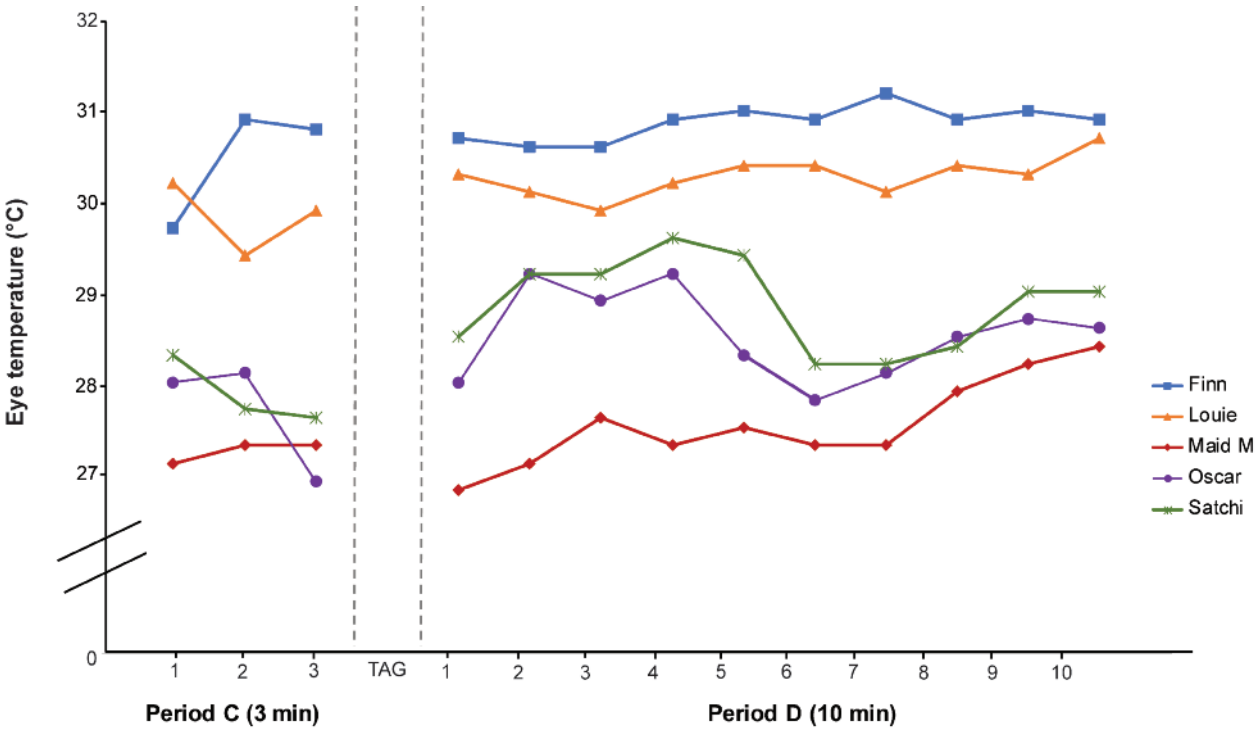
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Figure 3



Examples of the change in eye temperature (°C) showing four seals before (Period A) and after (Period B) receiving an injection of lidocaine (Lidocaine treatment). The four seals were chosen to illustrate the inter-individual differences in eye temperatures and responses.

Figure 4



Examples of change in eye temperature (°C) showing five seals in the Tag Only treatment before (Period C) and after (Period D) the animals were flipper-tagged. The five seals were chosen to illustrate the inter-individual differences in eye temperature and responses.

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Eye temperature changes after lidocaine injection

As noted above, pups that had been restrained and injected in Period B (Lidocaine and Saline treatments) showed a further increase in eye temperature when restrained a second time for Period C. These animals then showed a decrease in eye temperature in the minutes immediately after tagging (decrease of 0.5 [± 0.11]°C from Period C to the first 3 min of Period D; $P < 0.001$), possibly as their initial response to handling diminished. Thereafter, mean eye temperature showed no clear changes or differences between treatments. Specifically, eye temperature tended to increase during the remainder of Period D for the Lidocaine treatment (increase of 0.3 [± 0.17]°C from min 1–3 to min 7–10 of Period D; $P = 0.1$) but not for the Saline treatment (decline of 0.1 [± 0.2]°C; $P = 0.7$).

Discussion

The changes in eye temperature after the putatively stressful events of restraint and tagging suggest that eye temperature is a promising non-invasive means of monitoring stress responses in this species. That said, eye temperature is influenced by a range of physiological responses involving both the ANS and the HPA axis (Cook *et al* 2001; Stewart *et al* 2008a,b). Hence, more research would be needed to identify the specific physiological events that lead to changes in eye temperature in harbour seals. Such understanding might also help to explain the large individual differences seen both in baseline levels and responses.

When captured, moved and restrained for the first time, pups had higher eye temperatures than non-handled pups, suggesting that these handling events caused a physiological stress response detectable via IRT. Restraint, in particular full body immobilisation, is known to be aversive and result in a stress response in several species (Buynitsky & Mostofsky 2009). Full body restraint is a common technique for seals in captivity. Increased breathing rate can be associated with a stress response (Gulland *et al* 2001) and has been recorded in grey seals (*Halichoerus grypus*) (Lapierre *et al* 2007) and harbour seals (MacRae *et al* 2018) in response to handling.

Interestingly, pups that were not handled also had an increase in eye temperature from Baseline to Period A, although the change was not as great as in the handled group. Social transmission of fear has been demonstrated when animals observe pen-mates in distress (Kim *et al* 2010) and can cause behavioural and physiological changes indicative of a stress response in the observing animal (Olsson & Phelps 2007). Also, because the pups were group-housed and handlers were required to enter the enclosure, both to capture pups and to record IRT images, it is possible that the proximity of the handlers and/or observing the capture and removal of pen-mates amounted to a stressor that resulted in a modest eye temperature increase in pups that were not themselves handled in this period.

The further increase in eye temperature when pups were handled a second time (Lidocaine and Saline treatments) suggests that the first handling, which was presumably aversive, sensitised pups to subsequent handling. A similar response has been reported in calves that underwent two separate jugular catheterisations, known to be aversive for cattle (Alam & Dobson 1986). In that case, calves showed no change in eye temperature or plasma cortisol concentration after initial catheterisation, but both measures increased after the second catheterisation one week later, possibly because the calves were anticipating an event that they had learned would be aversive (Stewart *et al* 2007). Together, the increase in eye temperature after the first handling, and the further increase after the second, suggest the seals' experience of the first handling was aversive. Also, seals that were handled twice received injections (of either lidocaine or saline) during their first handling which may have further contributed to a negative association with handling. Seals' reactions to repeat handling suggest it may be advisable to combine necessary procedures and interventions into single handling events whenever possible, but more work is needed to determine if this is indeed a better practice.

We had anticipated that seal eye temperatures would quickly return to Baseline levels upon their return to their home pens for recovery. For example, elevated eye temperatures recorded in dogs in response to a 4–5 min veterinary examination decreased to pre-examination values within 5 min post-examination (Travain *et al* 2015) and decreased in horses within 10 min after wearing a tight noseband for 10 min (Fenner *et al* 2016). However, regardless of treatment, the eye temperature of seals when returned to their home pens remained higher than the Baseline levels at both 30 and 60 min after handling. This change in eye temperature was also seen in the sham-tagged animals, ruling out a prolonged response to tagging. It is possible that the arousal of pups as they were reunited with their pen-mates amplified the effect of the physiological disturbance. Alternatively, the sampling times (30 and 60 min after treatment) may have been too brief to capture complete recovery in this species. After surgical castration of calves, eye temperature had not started to decrease by the end of a 20-min observation period (Stewart *et al* 2010). The slower recovery of the seals suggested by the current data may reflect the more prolonged exposure to stressful handling and proximity to humans, or alternatively a species difference. In particular, the seals are of a wild species whereas most comparable research has been done on domesticated animals which typically show reduced flight behaviour and a general decline in environmental responsiveness (Hemmer 1990; Price 1998; Künzl & Sachser 1999).

Eye temperature has been shown to increase in response to potentially painful procedures in multiple species, for example, in elk and reindeer after velvet antler removal (Cook *et al* 2005) and in cattle after castration and disbudding (Stewart *et al* 2008b, 2009, 2010). In our study, eye temperature was higher in the 10 min after tagging but not after sham-tagging. Tagging for identification causes tissue damage and is associated with pain in other species (Leslie *et al* 2010). Harbour seal pups show several behaviours

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indicative of pain after being tagged, including orbital tightening (MacRae *et al* 2018). The increase in eye temperature after tagging in the current study is consistent with the view that flipper-tagging is painful for seal pups.

Cattle show a rapid, transient drop in eye temperature within the first few seconds of presentation of a stressor, likely because of an initial sympathetic response (peripheral vasoconstriction) (Stewart *et al* 2007, 2008a,b). It is possible that seals had similar initial decreases in eye temperature. However, because sampling did not start until several minutes after seals had been captured, any immediate thermal responses would have been missed. As noted above, pups that had been restrained and injected in Period B (Lidocaine and Saline treatments) showed an increase in eye temperature when restrained and recorded for Period C. These animals then showed a decrease in eye temperature in the minutes immediately after tagging. However, as the decrease in eye temperature was seen only in the pups that already had elevated eye temperatures in their second handling period, it is possible this decrease indicates their initial response to handling diminished as distinct from reflecting an acute response to the pain from tagging.

Lidocaine is known to provide effective pain control in many species for a range of procedures (Valverde & Gunkel 2005), but in the present study its administration had little moderating effect on the increase in eye temperature after tagging. While lidocaine has been shown to effectively control pain in pinnipeds (Gutiérrez *et al* 2016), an effective dosage and route of administration have not been established for flipper-tagging. Moreover, lidocaine injection is known to produce pain due to its acidity (Cepeda *et al* 2012). For example, although lidocaine reportedly provided analgesia for velvet antler removal in elk and reindeer, its application appeared to initiate an additional attendant stress response in control animals that were not subjected to antler removal (Cook *et al* 2005). Similarly in calves, lidocaine reduced changes in eye temperature after disbudding and surgical castration (Stewart *et al* 2008b, 2010), but its initial injection caused an increase in eye temperature (Stewart *et al* 2008b) and heart rate (Stewart *et al* 2010), suggesting lidocaine administration itself is stressful. In the current study, there was an upward trend in eye temperature in the 10 min after injection of lidocaine but not of saline (Period B) and there was no clear difference between the eye temperatures of lidocaine- and saline-treated pups after tagging (Period D). Our injection of lidocaine appears not to have effectively blocked the nerves at the tag site, may have caused pain, and required a second handling. For these reasons, lidocaine, as used in this study, cannot be recommended for the flipper-tagging of seals. Further investigation to establish an effective pain therapy for this procedure is warranted.

Animal welfare implications and conclusion

This study indicates that eye temperature measured by IRT can be used as an immediate and non-invasive way to reveal responses to routine husbandry procedures in harbour seals, thus enabling refinement of such practices in the future. The increased eye temperature after tagging but not sham-tagging, and after lidocaine injection but not saline injection, suggests that eye temperature is increased by pain. However, handling also had a significant effect on eye temperature; hence, future use of IRT to monitor states such as pain will need to control for handling. The results provide no evidence that lidocaine as used in this protocol diminished pain from tagging.

Acknowledgements

We thank the staff and volunteers at the Vancouver Aquarium Marine Mammal Rescue Centre and, in particular, Lisa Baskett, Lauren Cee, Janet Bauer and Duncan MacRae for help with animal handling and data collection, Tiffany Lin and Kathryn Gibb for data processing, David Rosen for use of the camera and Robin Steen and Meghann Cant for their helpful comments on our manuscript. We also thank Drs Martin Haulena and Cathy Schuppli for their help with the experimental protocols. This work was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) postgraduate scholarship awarded to Amelia Mari MacRae.

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