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Implantation of subcutaneous heart rate data loggers in southern elephant seals (*Mirounga leonina*).

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Abstract

Unlike most phocid species (Phocidae), *Mirounga leonina* (southern elephant seals) experience a catastrophic moult where they not only replace their hair but also their epidermis when ashore for approximately one month. Few studies have investigated behavioural and physiological adaptations of southern elephant seals during the moult fast, a particularly energetically costly life cycle's phase. Recording heart rate is a reliable technique for estimating energy expenditure in the field. For the first time, subcutaneous heart rate data loggers were successfully implanted during the moult in two free-ranging southern elephant seals over 3 to 6 days. No substantial postoperative complications were encountered and consistent heart rate data were obtained. This promising surgical technique opens new opportunities for monitoring heart rate in phocid seals.

Key words: marine mammals, Pinnipeds, surgery.

Introduction

Measurement of heart rate is commonly used to estimate the metabolic rate of laboratory and free-ranging mammals (Butler *et al.* 2004; Green 2011). In laboratory studies, fully implantable heart rate devices are usually used (Morhardt and Morhardt 1971; Mills *et al.* 2000). However, for studies on free-ranging pinnipeds ECG electrodes can be attached to shaved areas of skin and heart rate monitors glued to the fur (Thompson and Fedak 1993; Boyd *et al*. 1995; Ponganis *et al.* 1997). Implantation of subcutaneous electrodes to measure heart rate has previously been carried out in both otariid and phocid species (Otariidae and Phocidae; McPhee *et al*. 2003; Greaves *et al.* 2005) with the limitation that electrodes had to be wired externally to loggers and glued to the hair. Moreover, externally glued devices can be unreliable because they are prone to falling off as animals move or moult. To maximise data collection, devices are deployed when animals are not moulting to ensure longer-term attachment to fully grown hair. Alternatively, harnesses have been used to attach telemetry devices to otariid species in captive facilities (Dalton *et al.* 2014). This technique avoids the necessity to glue devices to hair, but has never been used with phocids. Recently, fully implantable subcutaneous data loggers were used successfully to record heart rate in large mammal species (*Ursus americanus*, black bear; Laske *et al*. 2011). However, in *Mirounga angustirostris* (northern elephant seal), subcutaneous loggers had to be removed after causing marked inflammation and infection (Green *et al*. 2009). Implantable devices have been used without complication in *Zalophus californianus* (Californa sea lion; Green *et al*. 2009) and *Phoca vitulina* (harbour seal*;* Blundell *et al*. 2014). Those studies indicated some variability in subcutaneous implantation responses within and between species, depending on their lifestyle. The aim of this study was to determine if implantable devices can be used in free-ranging *Mirounga leonina* (southern elephant seal) and to develop this method for future deployments.

Materials and methods

Biological models, study site and devices

Heart rate recorders were implanted in two wild adult female *M. leonina* on Kerguelen Archipegalo (49°26'S; 70°23'E) during the moult. A third female was implanted with a heart rate recorder in 2016 and followed postoperatively for ten days, but the logger migrated from the incision site before the individual was recaptured and was therefore excluded from the study. We used two different loggers: in 2015 a Reveal® XT Insertable Cardiac Monitor (ICM, Model 9529; Medtronic; 8 mm × 19 mm × 62 mm; 15 g; developed for humans) was used with a custom-made software patch to increase the frequency of data recording for heart rate and activity (Ditmer *et al*. 2015), and in 2016 a heart rate sensor (DST milli-HRT heart rate and temperature logger; Star Oddi; 13 mm x 39.5 mm; 11.8 g; for animals) was deployed.

Surgical procedure with Reveal® XT ICM

Female 37 (tag #37) was captured in early January 2015 (Table 1) using methods of McMahon *et al*. (2000) and intravenously sedated with 2 ml of tiletamine and zolazepam (0.65 $mg.kg^{-1}$; Zoletil® 100;

Field *et al*. 2002). The female was weighed (using a net and hoist, suspended from a tripod, with a portable scale; ± 0.5 kg), measured (nose-tail length) and placed in a right lateral decubitus position (Lander *et al*. 2005). We visually detected the apical heart beat projection area (i.e. the point of maximum cardiac impulse) on the left ventral thorax, vertically to the caudal extremity of the scapula, and we selected a 6 x 8 cm incision site, 10 cm caudally to this projection area, behind the lateral flipper insertion. Old hairs had already been shed over this patch, thus the incision area did not need to be shaved. The implantation site was surgically cleaned using iodinated povidone 7.5% surgical scrub and iodinated povidone 10% solution (Vétédine®). A vertical skin incision (5 cm) was made in the centre of the patch using a sterile scalpel blade. We then used non-traumatic Mayo scissors to break adipose tissue until reaching the muscle layer and then carved the blubber to create a tunnel in the cranial direction, along the blubber muscle interface, to the apex beat projection area. We inserted the ICM in the tunnel with a non-traumatic forceps to place it under the blubber layer and against the thoracic muscle, above the apex beat projection area. A simple interrupted suture with PDS® II D2T monofilament absorbable (3-0, 3/8, 24 mm; Ethicon) was used in the deep adipose tissue to close the tunnel. We then applied a simple running subcutaneous suture with PDS® II D3T monofilament absorbable (2-0, 3/8, 24 mm; Ethicon) to appose skin incision edges. Butyl cyanoacrylate tissue adhesive (Surgibond®) was applied on the incision surface and simple interrupted cutaneous sutures with Prolène® D3T monofilament non-absorbable (2-0, 3/8, 24 mm; Ethicon) sealed the skin. The wound was cleaned with iodinated povidone 10% solution (Vétédine®) and protected with an aluminium healing spray (Alumisol®). Postoperatively, the female received an intramuscular (IM) injection of amoxicilline (Clamoxyl®) at 15 mg.kg^{-1}. The whole capture and surgical procedure lasted 1.5 hours.

Surgical procedure with DST milli-HRT

Female 56 (tag #56) was captured in mid-February 2016 (Table 1). The implantation procedure was similar to that in 2015. However, an initial anaesthetic dose was administered via an IM injection of 3 ml of tiletamine and zolazepam (0.97 mg.kg^{-1}; Zoletil® 100) using a blowpipe and dart and left for 10 minutes for the drug to take effect. Heavy sedation was then completed using an IM dose of 1 ml of the same drug (0.32 mg.kg^{-1}) and maintained throughout the procedure with additional doses (0.5 ml; 0.16 mg.kg^{-1}) as required. The female was left on ventral decubitus and a local anaesthesia, which included a mixture of lidocaine and adrenaline (Lignol® 2% w/v), was administered along the intended area of implantation (three separate boluses of 1 ml at the blubber muscle interface along the future tunnel position and a 1 ml bolus at the subcutaneous layer of the future incision position). The skin incision was shorter (2-3 cm) than with ICM due to the smaller size of the logger. A non-steroidal anti-inflammatory drug (carprofen; Carprox Vet®; 4.0 mg.kg^{-1}) was administered IM, along with antibiotics. The whole capture and surgical procedure lasted 1.5 hours.

Monitoring and retrieval

Wound healing was monitored in the field for signs of inflammation, swelling and exudate with digital and thermal images (ThermaCAM^{TM} P25 FLIR Systems) for prediction of infection, as increased local temperature is a significant sign of wound infection and can be early detected with infrared thermography (Schaefer *et al*. 2004; Fierheller & Sibbald 2011). In 2015, female 37 was observed once, two days after the operation, whereas in 2016 female 56 was observed daily, during 6 days, until instrument retrieval.

For retrieval of devices, the same capture and surgical procedures were applied with the exception that the incision site was located directly above the position of the devices and that new hair was shaved.

Data collection and analysis

From 9-12 January 2015, the ICM recorded mean heart rate (bpm) every two minutes. This was calculated from continuous monitoring of the subblubber electrocardiogram recorded from a differential voltage between two titanium electrodes. It also recorded the animal’s physical activity (%) from a built-in accelerometer as the number of minutes spent in motion during 15 min intervals, and stored the timing of each heartbeat and daily activity (Laske *et al*. 2011).

From 16-22 February 2016, the DST milli-HRT logger recorded heart rate (bpm) and temperature (± 0.2°C) every 5 min and stored a 5 min continuous electrocardiogram every 24 hours. The heart rate was derived from a single channel electrocardiogram with leadless electrodes as part of the housing material. The logger took a burst (graded with a quality index for validation purposes) of 600 measurements on any set time interval and calculates the mean heart rate for each recording. These data were analysed using Mercury 4.22 software (Star Oddi).

Thermal images were analysed with ThermaCAM® Researcher Pro 2.10 (FLIR Systems) and all statistical analysis were performed with R statistical software (R Core Team, GNU GPL, version 3.3.0).

Results

Postoperative monitoring

Females 37 and 56 recovered quickly after anaesthesia was discontinued and resumed normal behaviour. No unusual behaviours or signs of discomfort were observed during the healing period.

In 2015, female 37 was observed to have a clean healing site, two days after implantation. Upon retrieving the device, the female had lost 5 kg since deployment (i.e. 1.7 $kg.d^{-1}$). The healing site was tainted with a small amount of blood, but the sutures were still in place with the wound well apposed and showing no signs of swelling or exudate. There was no sign of increased skin temperature visible with infrared imaging and a normothermic rectal temperature was measured (36.8°C).

In 2016, the healing site of female 56 was visible and clean, with no sign of inflammation (wound skin temperature was 2.0°C below maximal body skin temperature; Online Resource 1), 2 days and 3 days after implantation. The female was recaptured 6 days post-surgery. Upon retrieving the device, the female had lost 18.5 kg since deployment (i.e. 3.1 $kg.d^{-1}$). The skin sutures had broken due to skin swelling during the healing process. The cutaneous layer was still slightly open at the surface, but the deep tissues were healed and closed with fibrous tissue. Cicatricial tissue was covered with a clear exudate in low amounts and not purulent. The healing site was cleaned with iodinated povidone 10%

solution (Vétédine®) and new simple interrupted cutaneous stitches were applied with Prolène® D3T monofilament non-absorbable (2-0, 3/8, 24 mm; Ethicon). No marked skin inflammation was measured upon the wound (less than +1.0°C above maximal body skin temperature) and rectal temperature was within normal limits (37.7°C). The longer deployment of the device had resulted in the device being encapsulated in thin connective tissue over time, but without retrieval complication.

Heart rate

Temporal and heart rate data per individual are detailed in Table 1. Distribution of heart rates showed a non-normal distribution (Shapiro-Wilk normality test, in 2015: $W = 0.98935$, $p < 0.0001$; in 2016: $W = 0.98641$, $p < 0.0001$; Fig.1). Female 37 spent 74% of its time inactive (physical activity = 0; n = 277 activity records) in 2015, with a mean heart rate of 67.8 ± 12.3 bpm, lower than when it was active (80.2 ± 12.4 bpm; Wilcoxon test: $W = 11092$, $p < 0.0001$). In 2016, only 2% of the heart rate data measured with the DST milli-HRT on female 56 were defined as “poor quality” (91% of “high quality”) by the device and were discarded (presumed muscle artefacts). Electrocardiograms recorded helped to confirm extreme heart rate values. The subblubber temperature was 36.5 ± 0.5°C (mean ± SD), range 35.6-38.1°C (n = 1722 temperature records).

Discussion

Mass losses of both females were below maximal mass loss observed in moulting female *M. leonina* (Boyd *et al.* 1993; Carlini *et al.* 1999; Postma *et al*. 2013). No sign of acute infection was observed in the few days after surgeries, suggesting that surgical procedures of asepsis in the field were effective. A longer postoperative monitoring would be necessary to assess long-term tolerance of devices by *M. leonina*. During the moult, while *M. leonina* is hauled out, blood is shunted through the blubber layer via anastomoses (Ashwell-Erickson *et al*. 1986). Increased blood perfusion possibly could facilitate the healing process, which may explain why we did not observe the same inflammatory responses reported by Green *et al*. (2009) with a similar sample size (*M. angustirostris*, n = 3) and for the same duration of observation (3-5 days after implantation). Additional hypothesis to explain the success of implantation could be that animals during moulting show reduced physical activity and that the logger shape and implant localisation facilitated healing process and decreased infection risk. Cutaneous sutures breaking, probably due to postoperative skin swelling, may have accounted for the non-successful surgical implantation in 2016. Skin swelling around the surgical wound might be reduced with the addition of non-steroidal anti-inflammatory drugs during the healing period. We recommend the use of dart and blowpipe for remote injection renewals to avoid recapturing the individual and minimise stress' causes during the moult process and heart rate monitoring. Recapture of the seal in the middle of the moult, or of the study, should only be resorted to in presence of signs of rejection to urgently remove the device. Multifilament (braided) sutures may be considered for the cutaneous layer to better adapt to skin surface movements in free-ranging animals (although monofilament sutures are usually used to limit risk of skin infection). We also suggest that fixing the logger with a non-absorbable thread at a deep subcutaneous level could help retrieval, particularly in free-ranging animals and for a longer-term implantation.

Heart rates measured with both data loggers in 2015 and 2016 were similar to the range previously reported by Andrews *et al*. (1997) and Green *et al*. (2009). We measured a difference in heart rates between the two females while they are of similar initial body mass at capture and similar body length (Table 1). However, this variation could be due to other differences in biological factors: females were captured at similar moult stage (at the beginning of their moult) and recaptured in the middle (2015) or the end of their moult (2016), but not at the same time. Indeed, female 37 hauled-out in early moult period in 2015, as female 56 hauled-out in late moult period in 2016 (Table 1). We did not estimate age of adult females captured (supposed ≥ 4 years old) but based on their body mass, both could have been breeders (Arnbom *et al*. 1994). However, physiological stages between the two females could have been different: gestating or not, different success and duration of the previous foraging period at sea, impacting on their heart rate. This difference in heart rate could also be linked to differences in physical activity during the study (movements on land) or to the use of two different loggers between 2015 and 2016. Even though both loggers (or similar models) were previously used to record animals' heart rate (Reveal® XT ICM on *Ursus americanus*, Laske *et al*. 2011; DST *micro*-HRT on rats, Dudek *et al*. 2015), further species-dependent studies are required to fully validate such data collection.

To our knowledge this study represents the first promising use of fully implanted subcutaneous heart rate monitors in free-ranging *M. leonina*. The implant method is certainly more invasive than external tag attachments and requires surgery skills and specialised equipment, but allows data collection on freely moving *M. leonina*. This technique allowed data collection in 2 out of 3 deployments in *M. leonina* with few complications and hold promise for heart rate assessment of free-ranging seals in the wild.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval

Elephant seal work (Program IPEV 1037, HENERGES) was approved by The Antarctic Committee for Environmental Protection and the Ethic Committee (Cometh ANSES/ENVA/UPEC n°16: n°14-055 and n°15-061). The program was also authorised by the French Southern and Antarctic Lands (Decree 2014-131, 15th October 2014; Decree 2015-110, 4th September 2015).

All applicable international, national and institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Table and figure captions

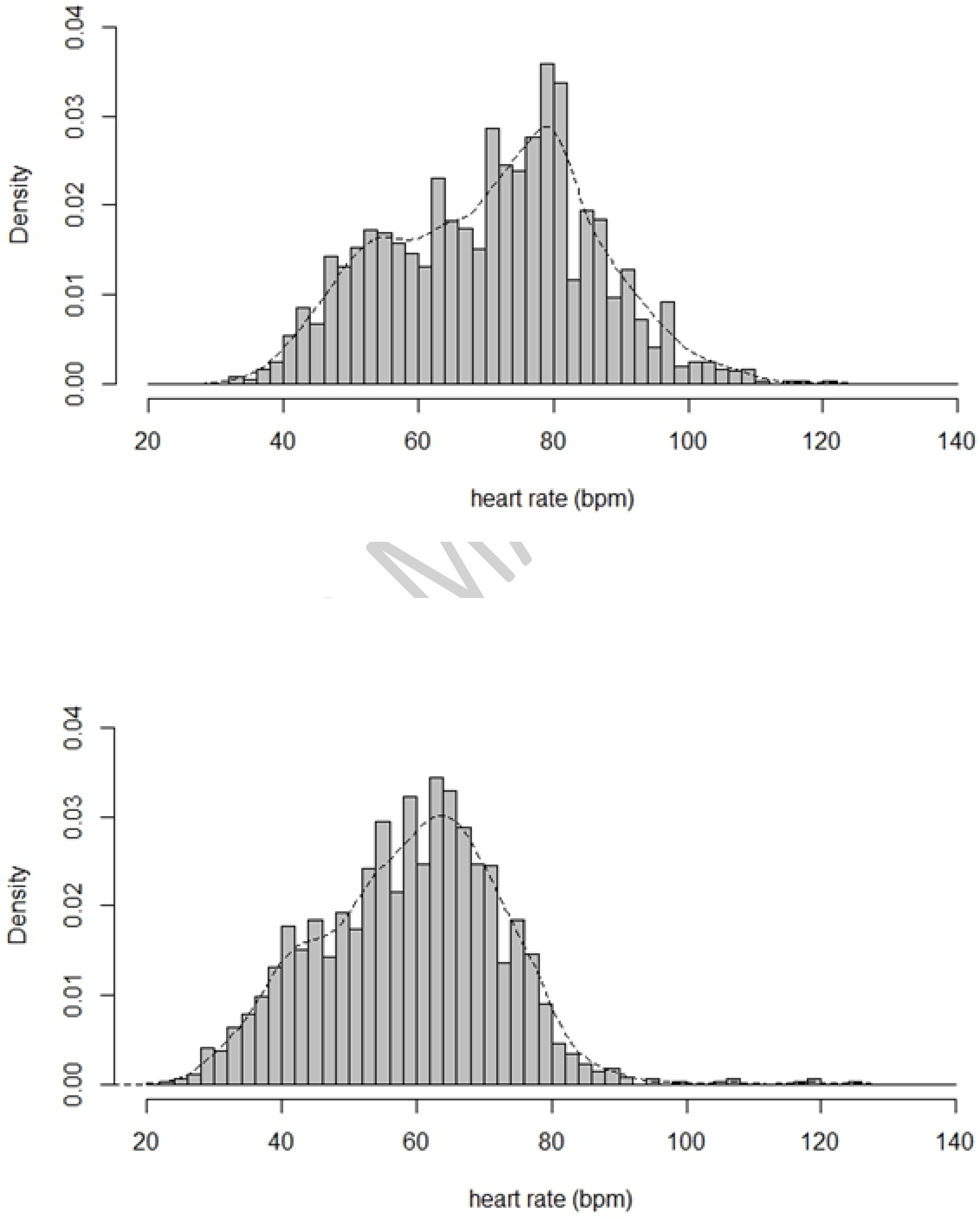
Table 1 Biometric, temporal and heart rate data per year and individual

Seal ID (tag number)	**Mass at capture (kg)**	**Body length (m)**	**Capture/recapture data**			**Heart rate data (bpm)**			
			Implant date	**Explant date**	**Period**	**Total records (n)**	**Mean ± SD**	**Minimal-maximal range**	**Median [interquartile range]**
Female 37	306	2.3	Jan 9, 2015	Jan 12, 2015	Early moult	2076	71.1 ± 15.1	33-122	73.0 [59.0 ; 81.0]
Female 56	308.5	2.2	Feb 16, 2016	Feb 22, 2016	Late moult	1717	59.2 ± 13.4	23-125	60.0 [50.0 ; 68.0]

Fig.1 Distribution and density curves (dotted lines) of heart rates (bpm) recorded in female 37 in 2015 for 3 days (a) and female 56 in 2016 for 6 days (b)

a)

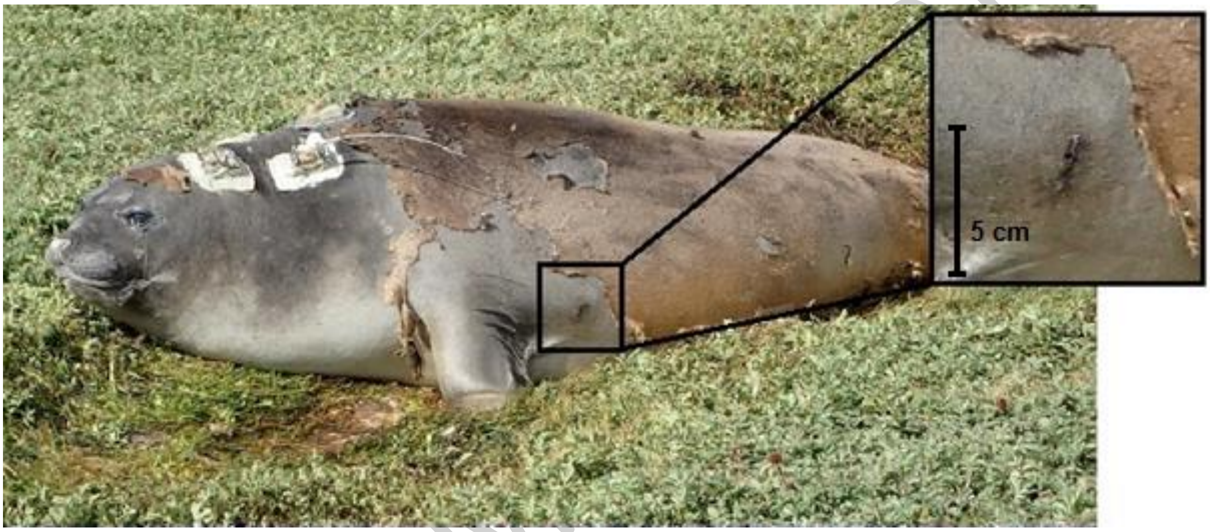
b)



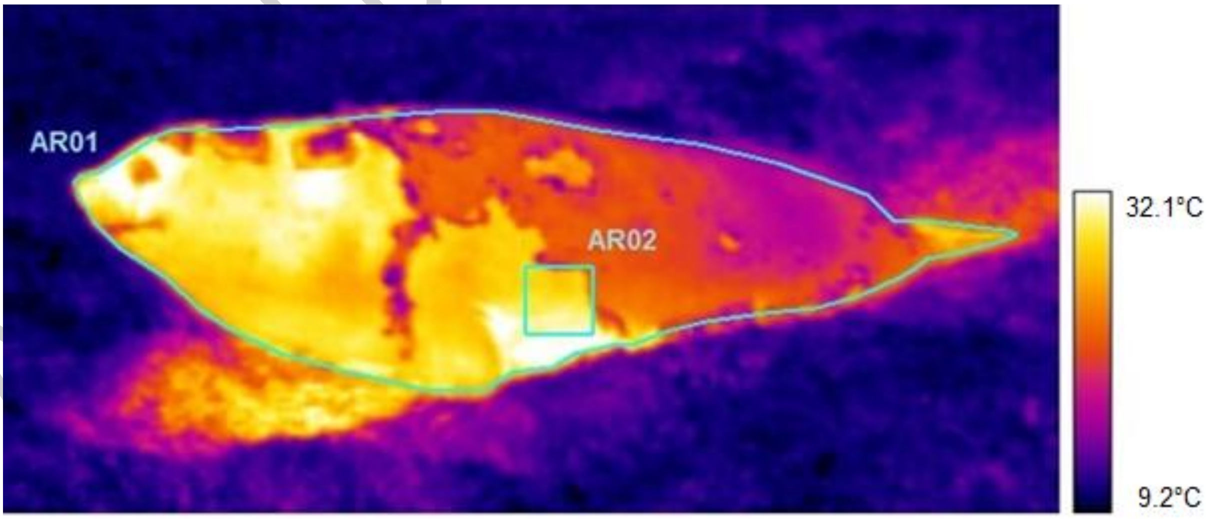
Electronic Supplementary Material

Online Resource 1 Two days post-surgery monitoring with digital (a) and thermal (b) images of the healing wound (2016). Minimum (Min), maximum (Max), average (Avg) and standard deviation (Stdev) of surface body temperature (AR01) and implantation site (AR02) are presented for comparison in the insert.

a)



b)



Label	Min (°C)	Max(°C)	Avg (°C)	Stdev (°C)
AR01	12.0	32.1	23.4	4.3
AR02	15.7	30.0	26.2	3.2