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Infra-red thermography and radio frequency identification for detection of stress in lairage

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Abstract

Distressed, diseased and injured animals in abattoirs have the potential to jeopardise animal welfare and product yield and quality. This project aimed to develop automated systems for the detection of these animals, through remote measurement of body temperature using infrared thermography and auto-drafting systems. The project consisted of trials with cattle at research farms and commercial abattoirs. Trials at commercial abattoirs recorded animals prior to slaughter with associated body temperature and indicators of stress in blood and meat via eating quality. Body temperature measured with infrared cameras was significantly but weakly correlated to indicators of stress and thus further refinement of the technology is required to be useful under commercial conditions. A pilot automated system to identify, send an alarm and segregate sick and stressed animals with high body temperature was developed.

Executive summary

Distressed, diseased and injured animals in abattoirs have the potential to jeopardise animal welfare, product yield and quality, and public trust in the industry. Detection of these animals is currently done by visual inspection of animals by personnel and veterinary staff throughout the supply chain, including at abattoirs. However, this is time consuming and subjective and requires a degree of knowledge to achieve high accuracy. Automated methods to detect stressed or sick animals are required to improve the accuracy of detection and improve animal management.

The objective of this project was to develop an automated system for detection and segregation (drafting) of stressed animals based on remote measurements of body temperature using infrared thermography (IRT). The project consisted of 5 trials conducted at commercial abattoirs and research and commercial farms.

The trials conducted on farm aimed to; 1) Determine the suitability of the type of IRT device to measure body surface temperature (still images vs. video vs. smartphone sensor), 2) Understand factors affecting IRT in cattle including part of the body and coat colour, and 3) Develop a method to analyse IRT data from the different devices.

Maximum eye temperature with a handheld camera and still images was the closest indicator of rectal temperature ($P = 0.026$). However, video surveillance cameras were the most practical as they were not required to account for animal speed and movement compared with still images. Data from IRT videos required extensive processing due to it being very noisy. A preliminary filtering of the data deleted low IRT values when the eye of the animal was not within the region of interest, and high values due to hotspots created by artefacts from the surrounding environment. This was done using mixture distributions methodology and a 1-second rolling median. The final step for data analysis consisted of calculating a summary statistic for each animal using quantiles which also eliminated outliers. The 95 quantile and the maximum temperature obtained with the 1-second rolling median had the highest correlations with physiological indicators of stress, including rectal temperature, blood composition (acute phase proteins and aspartate transaminase) and meat characteristics (e.g. lactate concentration).

One trial recording animals at commercial slaughter speed, using IRT video cameras at a Victorian abattoir, did not find a significant correlation between IRT temperature and meat pH ($P > 0.05$). However, IRT temperature was correlated with meat colour and meat colour was correlated to meat pH ($P < 0.05$). Furthermore, animals with higher IRT showed lower marbling and lower Meat Standard Australia (MSA) index values.

The MSA mixing and transport stress trials, in Tasmania and King Island, consisted of measuring body temperature using IRT on animals sourced from a range of commercial farms and subjected to a series of mixing and transport stress treatments. Infrared thermography was used to remotely measure body temperature at the abattoir prior to slaughter (race leading to the knocking box) and at slaughter in the knocking box. Blood samples were taken upon exsanguination, carcase grading data was obtained and meat cuts were analysed for sensory attributes (eating quality) by untrained consumers.

IR body temperature was not found to be associated with meat pH however animals with higher body temperature showed associations with indicators of stress, including blood and meat composition and meat quality. These associations were observed only on those stress treatments which yielded the highest incidence of dark cutters. For the treatment groups of sea transport and no rest prior to slaughter, animals with higher IRT temperature at the race showed higher concentrations of haptoglobin ($P < 0.05$). This indicates that animals with higher IRT experienced more stress because haptoglobin is an acute phase protein which increases in blood as a result of tissue trauma and inflammation.

For the treatment groups of land transport with no rest prior to slaughter, animals with higher IRT temperature at the race showed higher concentrations of aspartate transaminase, which is an indicator of damage to internal organs including the liver and gluconeogenesis, and amino acid degradation and formation from muscle as well. For treatment groups of land transport with rest prior to slaughter, animals with higher IRT temperature at the race showed higher concentration of muscle lactate, which is a key compound coming from glycogen during the maturation of meat. In addition, animals with higher IRT temperature had lower eating quality, as measured with sensory analysis (cmq4). However, these relationships were stronger when the analysis was done with those animals that had IRT data for at least 5 seconds. It is important to acknowledge that these relationships and indicators of stress were significant however were too low to be accurate under commercial conditions.

A pilot automated system to identify, detect, send an alarm and segregate animals with high IRT temperature was developed. The system is composed of an RFID reader, an IRT video camera and an auto-drafter. The system was trialled with both beef cattle and sheep at research farms and has the capability of moving drafting gates according to thresholds on IRT, measured in real time. However, the application of this auto-drafting system under commercial conditions is hampered until the IRT temperature thresholds are more accurate to detect stressed animals. Otherwise, it would result in many false positives and false negatives when drafting animals.

The main challenge of the present project was on the algorithms to detect stressed animals due to the low correlation between IRT and indicators of stress in commercial abattoirs. This could be due to several factors including the lack of a gold standard to measure animal stress at a point in time, and the stage of development of the technology including appropriate data processing methods. Therefore, further refinements or different approaches are required for this technology to be applicable in practical commercial conditions. Recommended approaches for further investigation include; 1) Develop the IRT technology further to estimate other indicators of stress from IRT videos, such as respiration rate and heart rate in addition to body temperature, 2) Develop machine vision ‘software’ to automatically track the eyes of the animals, and 3) Develop algorithms to correct IRT temperature from environmental conditions (e.g. ambient temperature, humidity and solar radiation). These approaches were beyond the scope of the current project however have the potential to improve the accuracy of detecting stressed, sick or injured animals before slaughter.

The present project is the first study reporting the use of IRT videos at high frame rate (up to 60 Hz) to measure body temperature in cattle as they pass through a race in the field of view of the camera under commercial conditions. The project has developed a protocol to setup and configure the IRT cameras, a methodology and algorithms to analyse the data collected at high frequency, and an automated drafting system for animals with high body temperature.

Infrared thermography has the potential to improve the monitoring of animals, to detect those under stress, and improve the prediction of eating quality. However, extensive work is needed before this technology can be deployed at commercial abattoirs with reasonable accuracy.

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1 Background

Stress could potentially lead to detrimental effects for meat quality and yield (Ferguson & Warner, 2008; Hambrecht, et al. 2004; Lahucky et al. 1998; Warner et al. 2007). Dark, firm and dry (DFD) is the main type or poor-quality meat produced and occurs due to a depletion of glycogen stores in the muscle as a result of stress experienced by the animal pre-slaughter (Guàrdia et al., 2010; Lahucky et al., 1998). Dark cutting beef costs the Australian beef industry over $35 million dollars annually, with producers being penalised by up to $0.60 for every kilogram of carcase weight when this occurs (DPI, 2001; Ponnampalam et al., 2016). Detecting stress earlier and on a timely basis could help improve animal welfare, reduce cases of poor quality meat and improve the public perception around the red meat industry. An automatic remote monitoring system to detect individuals likely to be experiencing stress and result in DFD meat could have large benefits to the industry.

Previous methods to measure stress in livestock have been largely invasive, time consuming, and generally require restraint and handling of the animal, which can produce confounding results as handling elicits a stress response (Alam Dobson, 1986; Moberg, 2000; Stewart et al., 2007). Therefore, a non-invasive and remote measure of stress in livestock would significantly improve the ability to assess animal welfare across the supply chain and allow this to be performed automatically under commercial conditions (farms, transport, auction markets and abattoirs). Several studies have shown the potential of infrared thermography as a simple, non-invasive indicator of stress, disease and tissue trauma (Schaefer et al. 2002; Stewart et al., 2007; Moya et al., 2014). The relationship between stress and surface body temperature using infrared thermography (IRT) has been well documented in the literature (Sapolsky et al., 2000; Stewart et al., 2007). When an animal is stressed the sympathetic nervous system is activated and heat production increases through increased metabolic activity, which causes blood flow to be redistributed depending on the response (Fraser et al., 1975; Lepkova et al., 2007; Stewart et al., 2007). The amount of blood flow in the peripheral tissues will determine heat loss and therefore is potentially a useful tool to measure physiological stress (Stewart et al., 2007). IRT converts radiation emitted from a source into an image (thermogram) that displays pixel temperature (Clerc & Gonzalez, 2012; Franze et al., 2012; Hildebrands et al, 2010; Naas et al, 2014; Stewart et al., 2007). From this, a map of temperatures is provided with information regarding every pixel in the image. More specifically, surface temperature of the eye has been a good indicator of stress, particularly around the lachrymal gland, because it is an area rich with capillary beds (Cook et al. 2001; Schaefer et al. 2002; Stewart et al., 2007).

The objective of the study was to develop an automatic system for the detection of stress in livestock under commercial conditions based on infrared temperature, RFID and auto-drafting. To achieve this aim, the project consisted of 3 main sub-objectives: 1) To determine factors affecting IRT measurements including anatomical location (i.e. eye, back or nose) and infrared technology (i.e. video camera, still image handheld camera or smartphone sensor), 2) To develop data processing methods and algorithms to detect animal with high body temperature, and 3) To determine the suitability of IRT to detect remotely measured indicators of stress in cattle including meat pH, eating quality and blood parameters.

2 Project objectives

The objectives of the present project were:

2.1 To develop a system for automated identification of animals with stress, disease, injury or trauma (e.g. bruising and inflammation) particularly from transport and marketing stress.

2.2 To scope the potential of an automated drafting system of at-risk animals consisting of IRT technology, RFID readers, and data management and processing.

3 Methodology

All experimental procedures performed in this project were approved by The University of Sydney Animal Ethics Committee (approval #2015/869 and #2015/993) and Murdoch University Animal Ethics Committee (approval # R2839/16) in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

3.1 Infrared cameras

The cameras used in the present project were from one of the leading brands in the world (FLIR Systems, Boston, USA) with a wide range of products for a wide range of applications (Table 1). The following cameras and sensor were used in the present project.

a) FLIR A310 is an IR surveillance monitoring camera which can be installed almost anywhere in the supply chain to monitor body temperature of cattle and transmit the information in real time through the network. The camera operates only when connected to a computer (has no battery and no storage capabilities) and can be placed within a pan-tilt enclosure allowing automatic movement or remote control to capture different areas in an abattoir. The radiometric video recordings are at a frame rate of 9 to 16 Hz meaning that it can measure body temperature of animals within the field of view (FOV) at up to 16 times per second (57,600 frames per hour).

b) FLIR T420bx is a handheld camera with better pixel resolution than the A310 and a touchscreen, which is portable within an abattoir, to obtain still images. As a backup, the camera has an SD card slot and batteries. It also has the advantage of obtaining both IR and colour (RGB) still images. It can also obtain radiometric video footage (i.e. with temperature measured for each pixel) when connected to a computer however it cannot be deployed for long periods of time or transmit videos in real time. The maximum frame rate is 32 Hz which allows measuring body temperature of animals 32 times per second for fast-moving animals (e.g. in a race). The T420 can record radiometric videos at 60 photograms (frames) per second (216,000 frames per hour).

c) FLIR ONE is a small camera sensor that connects to a smartphone or a tablet to obtain still images and videos in IR format only (not colour images). It has a low pixel resolution but could potentially become a practical monitoring tool for personnel to walk around lairage pens monitoring body temperature of cattle. Videos recorded show only relative temperature in colours but cannot be processed to obtain temperatures from pixels unlike its still images.

d) *FLIR C2 or i40* are small, low cost handheld cameras which take still IR and colour images at low resolution and allow easy reporting on the spot.

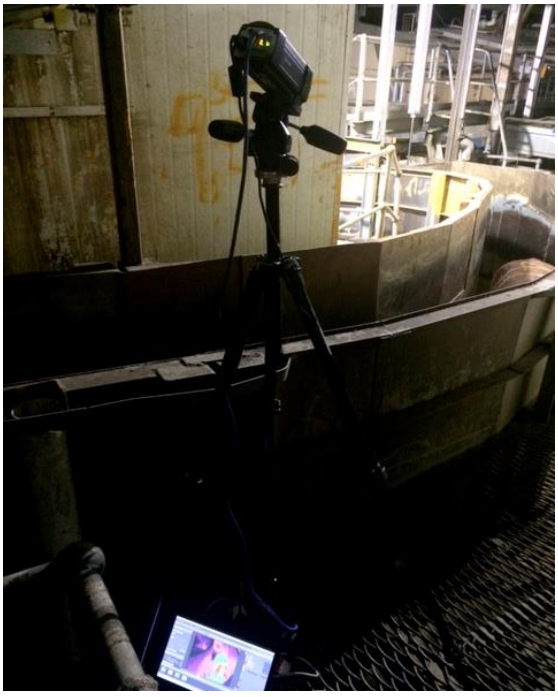
e) *FLIR A65.* This is a purpose-built surveillance camera designed as a standalone system with its own computer. It can be powered by a battery or main power and can record radiometric videos at 30 photograms (frames) per second (108,000 frames per hour).

Table 1. Specifications of FLIR System Infrared Cameras used in the project.

	IR Resolution (pixels)	Accuracy	Sensitivity	Temperature Range	Frame rate (Hz)
A310	320 x 240	±2◦C	0.05◦C at 30◦C	-20◦C to 120◦C	30
A65	650 x 512	±5◦C	0.05◦C at 30◦C	-20◦C to 500◦C	30
T420	320 x 240	±2◦C	0.045◦C at 30◦C	-20◦C to 650◦C	60
C2, i40 and One	80 x 60	±2◦C	0.05◦C at 25◦C	-10◦C to 150◦C	Still image

3.2 Examples of Infrared images

Cameras were setup at fixed locations at the abattoir and were also carried by personnel to scan animals throughout different locations. Fig. 1 shows an example of images obtained from IRT video cameras mounted on a tripod on the left-hand side of the race leading to the knocking box. The IR camera was set where animals pass in single file to allow identification of individual animals, e.g. race leading to knocking box. Video cameras with high frame rate are critical for rapidly moving animals which may be under the FOV of the camera for less than 1 second.



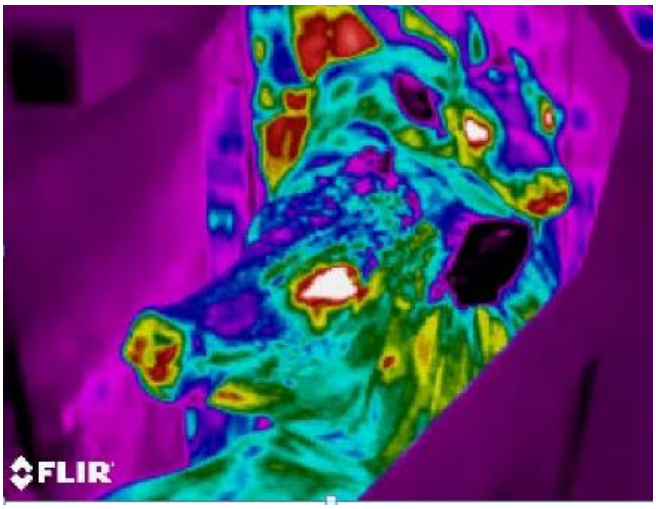


Fig. 1. Set up of infrared video cameras (left) and screenshot of video IR footage (right) in the race before the knocking box at a commercial abattoir. Different colours indicate temperature ranges with white being hottest and black being coldest. Images were taken with FLIR A310 video camera.

Handheld IRT cameras taking still images (Fig. 2) were also found useful for personnel at the abattoirs to monitor body temperature of animals in real time viewing data on the screen or recording images for later analysis.

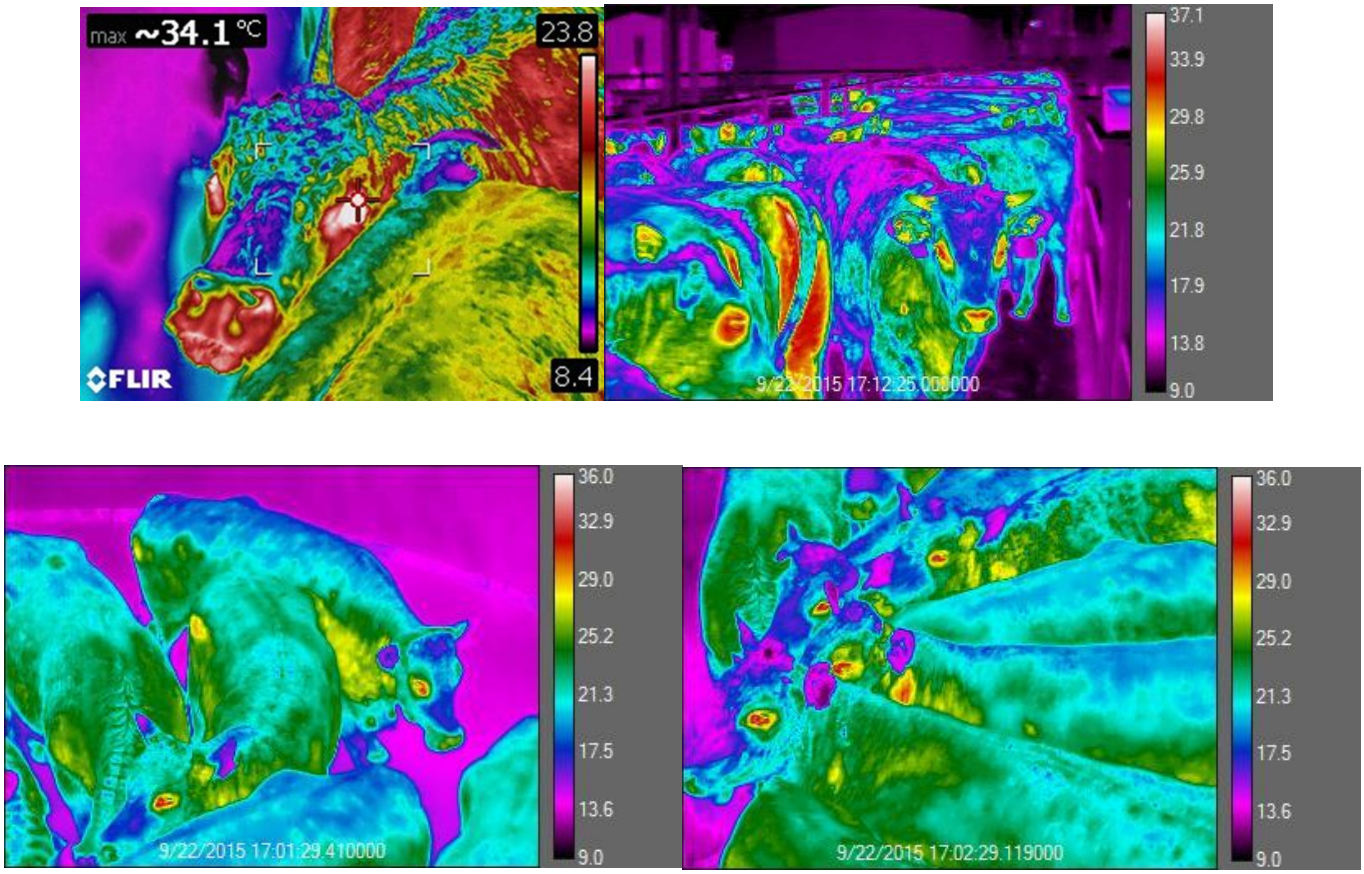


Fig. 2. Infrared still images of cattle in the race before the knocking box with crosshairs marking the pixel in the eye with maximum temperature (top left), and in lairage pens (top right) and round crowd pen (bottom) in a commercial abattoir.

The IRT video cameras were also setup at the knocking box of commercial abattoirs as shown in Fig. 3 for a steer after being stunned (hot temperature in the forehead with yellow colour indicating high temperature) using the FLIR A65 camera.

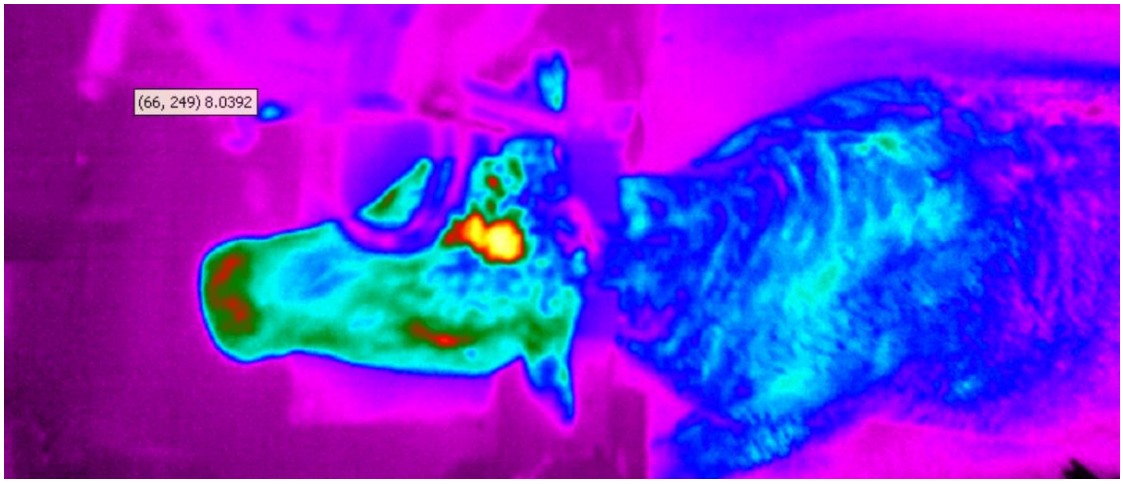
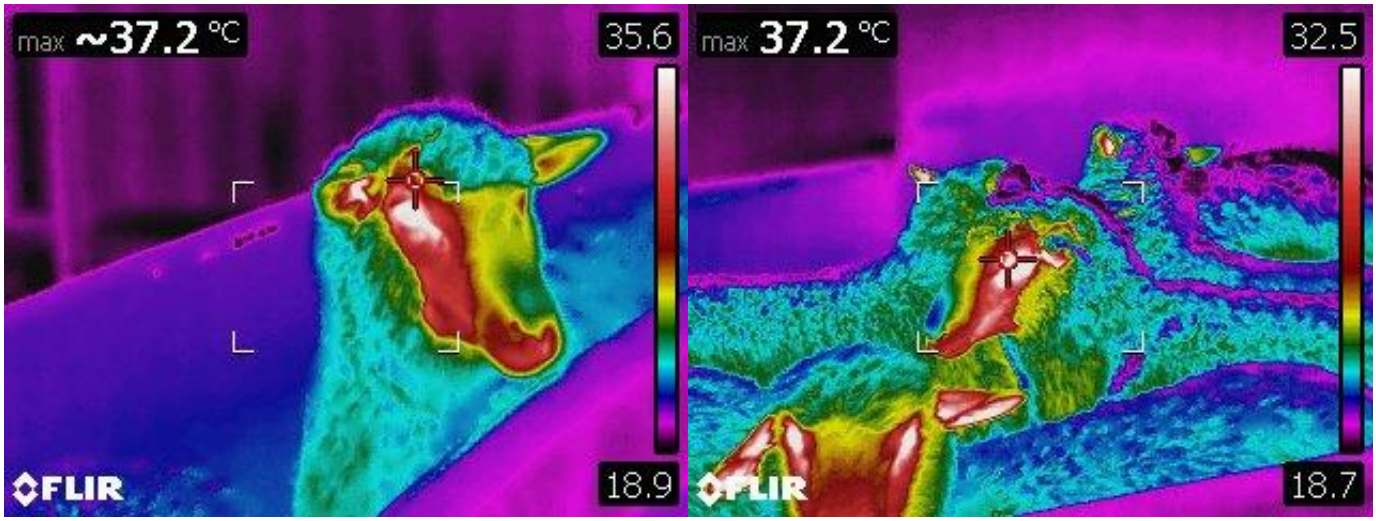


Fig. 3. Infrared image of a steer after being stunned in the knocking box with the yellow spot on the forehead indicating the place where the stunning captive bolt was applied.

Examples of IR images from sheep at an abattoir are shown in Fig. 4 with the crosshair function marking the hottest pixel in the image which could help identifying those animals with the highest body temperature amongst a group.



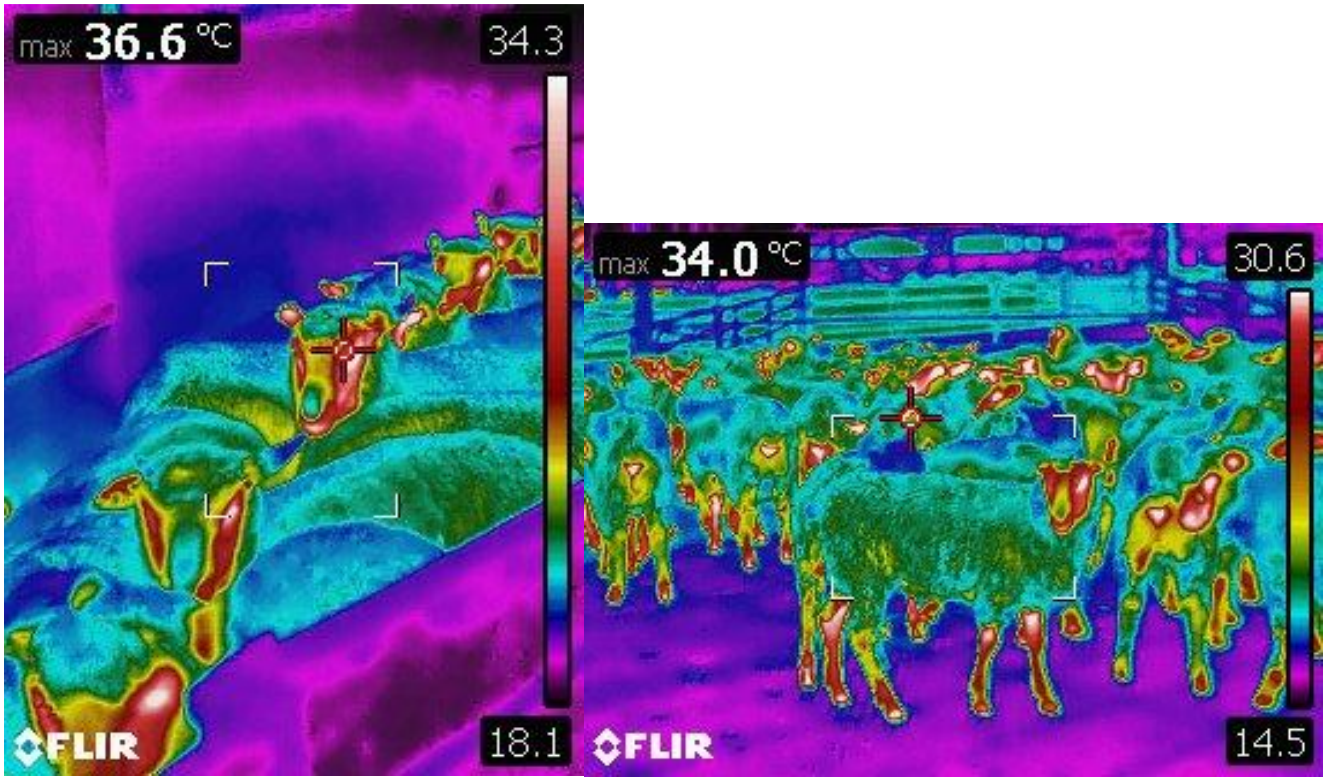


Fig. 4. Infrared images of sheep in the race prior to stunning and in lairage pens at the abattoir. The crosshairs in the images indicate the pixel (or animals in this case) with maximum temperature. Images taken with T420 handheld camera.

3.3 Factors affecting IR body temperature

3.3.1 Objectives

The first trial was carried out at a research property, J.B. Pye farm (Camden) from The University of Sydney as a preliminary trial recording infrared (IR) imagery from animals in a cattle race and crush.

The objectives of this trial were:

a) To determine which anatomical locations on animals offer the optimal means of recording temperature using IR cameras
b) To compare results amongst different devices for body temperature measurements
c) To understand the advantages and disadvantages of different IR devices before starting data collection under commercial conditions

3.3.2 Animals and experimental design

Trial 1. Thirty-seven Angus cattle (170 to 430 kg of LW; 2-3 years old) of both sexes were used. On the day of the trial, cattle were mustered from the paddock to the holding yards, a distance of approximately 1 km. From the yards, animals were moved through a race into an automatic weighing box to measure live weight (LW; Tru-Test XR500 model). Upon release from the weighing box cattle moved through a 3 m race into the cattle crush, where they were restrained for measurement of rectal temperature, obtaining infrared images and registering coat colour. A digital thermometer (Liberty

model DT-K01a) was used to record rectal temperature. Animals were not exposed to direct sunlight during these procedures as the work was performed under a large roof.

3.3.3 Infrared image acquisition

Infrared Still Images. For every animal, a minimum of 2 images for 3 different body parts were taken using the handheld infrared camera (FLIR T420; Fig. 5) and the smartphone sensor (FLIR ONE; Fig. 6) attached to an iPad (IOS 8, Apple INC, California, USA). Both images were taken from the eye (0.5 m), nose (0.5 m) and side of the body (4 m) however the lower resolution of the smartphone sensor can be observed in the images below.

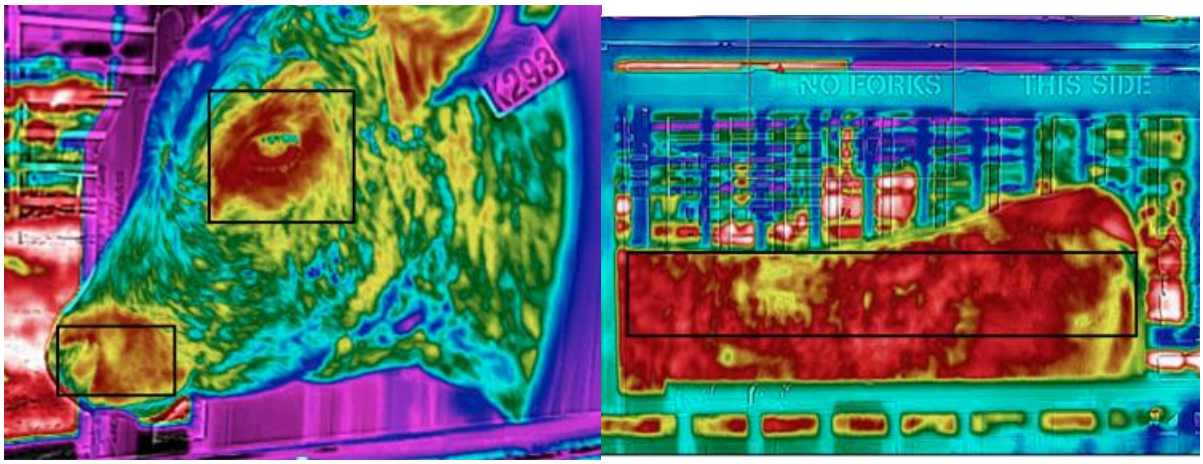


Fig. 5. Still images of the head (left) and the back (right) using the FLIR T420 handheld camera. Solid rectangles show the region of interest such as eye and muzzle within which maximum and average temperature is measured.

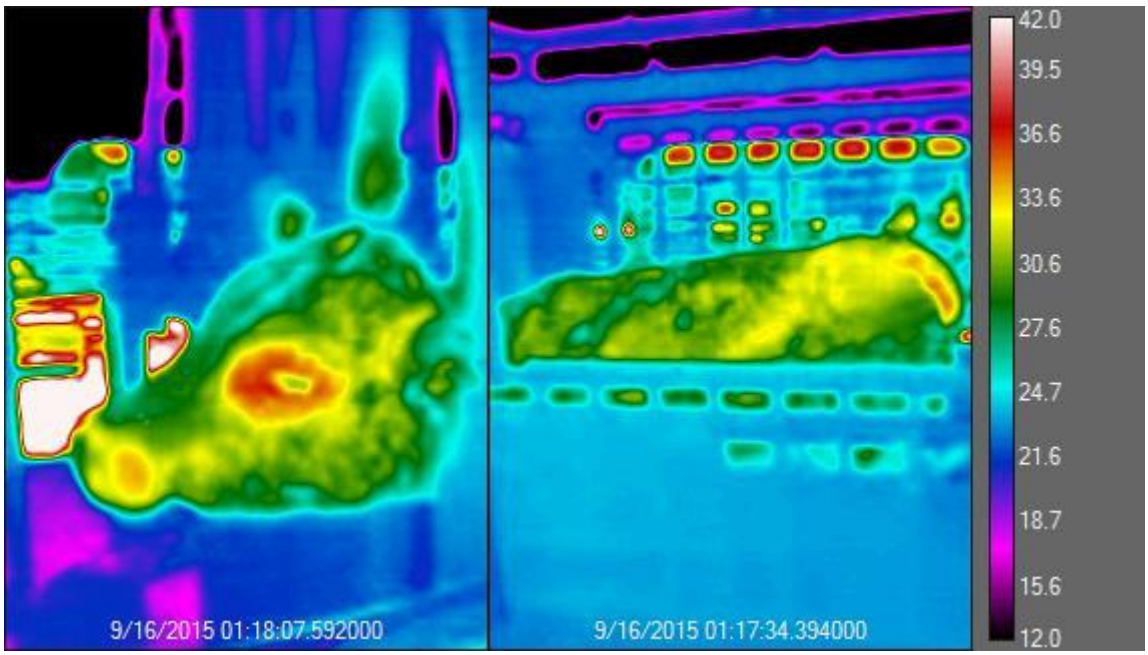


Fig. 6. Still images of the head (left) and the back (right) using the IR smartphone sensor FLIR ONE.

Infrared video footage. The FLIR A310 video camera was placed at a distance of 2 m from the race where the animals walked between the weighing box and the crush. The camera was placed on the left-hand side of the animal, on a 45˚ angle from the race. This setup was used to allow for the greatest opportunity to capture body parts of interest whilst cattle move through at different speeds. Technical parameters, such as the camera emissivity (0.98) and the distance between the IRT camera and the animal (3 m) were set.

3.3.1 IRT image analysis and statistics

Selection of Images and Frames. Still images and frames from videos were discarded before analysis in the following circumstances; 1) the area of interest was not entirely visible (e.g. the eye was closed or nose hidden by steel bars in race), 2) the focus was poor, or 3) there was a presence of 'cold spots' occupying the cow's body because of being wet as a result of urination or defecation from another animal while in the race. In cases where the image was suitable for one location (e.g. the eye) but not suitable for the other (e.g. the skin on the back), the image was used for the suitable area only.

Image Analysis. The area of interest to calculate IR temperature from the surface of the eye was a rectangle (24 x 16 pixels) enclosing it in totality (Fig. 1). For the nose, the area of interest was defined as a rectangle (21 x 14 pixels) enclosing the nostrils (Fig. 1) and for the images from the side of the animal's body consisted of the topside of the animal. The frontal limit of the area of interest for images from the side was defined as a rectangle (245 x 23 pixels) at the elbow and in the back by the thigh (Fig. 1). Average and maximum temperatures of all pixels within the area of interest were calculated from all images and frames.

Statistical analysis: Data was analyzed in SAS (version 9.4, SAS Inst. Inc, Cary, NC, USA) to determine descriptive statistics using the MEANS procedure and simple linear regression were used to determine the relationship between any 2 variables such as rectal and IR temperatures. The effect of Coat colour, Camera, and Body Part on IR temperature was analysed using Restricted Maximum Likelihood (REML) in Genstat (17th edition, VSNi, UK). Animal ID was included as a random effect. Two different measures were considered for the dependent variable; 1) difference in temperatures between maximum IRT temperature and rectal temperature, and 2) difference between average IRT temperature and rectal temperature. A probability level of $P < 0.05$ was chosen as the limit for statistical significance in all tests, whereas probability levels of $P \leq 0.10$ were considered as a tendency. Means were separated using least significant differences (LSD) obtained from the REML output upon pairwise comparisons. A simple t-test was used to test if maximum IRT and average IRT were significantly different.

3.4 Development of a methodology for data processing and analysis of infrared thermography used to measure cattle temperature in real time

The aim of this study was to evaluate how video infrared thermography (VIRT) can be used to detect core body temperature in cattle. Most IRT studies use the maximum or a combination of maximum values from IRT measurements for analysis (Schaefer et al., 2012). The temperature of the pixel with the maximum value is most commonly used because of the ease to calculate this within a region of interest (ROI). An animals' IRT eye temperature is generally lower than the rectal temperature of that animal (Clerc and Gonzalez, 2012). This methodology works well when there is only one animal

in the field of view (FOV) at a time, there is no background interference and/or environmental effects i.e. direct sunlight. Under circumstances where that cannot be guaranteed, outliers are likely to be present, and the maximum value may no longer be as accurate.

In addition, most of the previous animal studies using IRT cameras have used only single still images to measure temperature but using VIRT at high rate has not been used under commercial conditions and a methodology to analyse such data has not been published. The objective this study within the larger project was 1) To obtain VIRT imagery from cattle in commercial abattoirs to determine the characteristics of the data collected (Trial 2 at abattoir), 2) To develop a method to process and analyse VIRT which is closest to rectal temperature.

3.4.1 Animal Management and camera setup

There were two trials conducted to achieve the aim of this part of the project, one being done at the handling facilities of a research farm and another being done at a commercial abattoir.

3.4.1.1 Trial 2 (research farm)

Animals used in this trial were Angus yearling beef steers (n=52) videorecorded at the cattle crush after weighing. On the day of sampling, animals were mustered to the holding yards one hour before the trial commenced. Animals were moved through the race, into a weighing box and then each animal was held in the crush using a head bail for approximately 3 minutes. During this time, rectal temperature and IRT were obtained. Thermal cameras used for this experiment included FLIR A310, A65, T420 and C2 (FLIR Systems Inc.). IRT videos were taken at a distance of 2 m from the animals and at a 90° angle to the crush. The C2 IR camera captured radiometric still images manually operated, with photos taken from the same location, angle and distance as the video IR cameras. A minimum of two still images were captured for each animal.

3.4.1.2 Trial 3 (abattoir)

Infrared cameras were set up at an Australian abattoir to record cattle moving through the race prior to the knocking box. Animals were then slaughtered as per routine practice. Two different infrared cameras (FLIR A310 and T420; FLIR Inc.) were placed at an angle of 45° and a distance of 2-3 m from the animal. No IR still images were obtained at the abattoir because of the difficulty of obtain them in animals moving at high speed and head movement in the race.

3.4.2 Infrared Thermography Recording, Processing and Analysis

FLIR's Research IR software (Research IR Version 4.30.0) was used to process the IR data correcting for ambient temperature, distance to object and emissivity (0.98; Schaefer et al., 2012). The FOV of all IR cameras contained the animal's head, with a clear focus on the eye however these were unrestrained animals under commercial conditions and therefore there were periods of time when no animals were present. The eye region was selected because it has consistently shown to be the best anatomical location for detection of stress and disease (Clerc and Gonzalez, 2012; Schaefer et al., 2012) and it is the part of the body least affected by environmental conditions. The ROI was set to ensure that only one animal was within it at the time and that there was no presence of background interference resulting in temperature higher than the animals (Fig. 7 and 8).

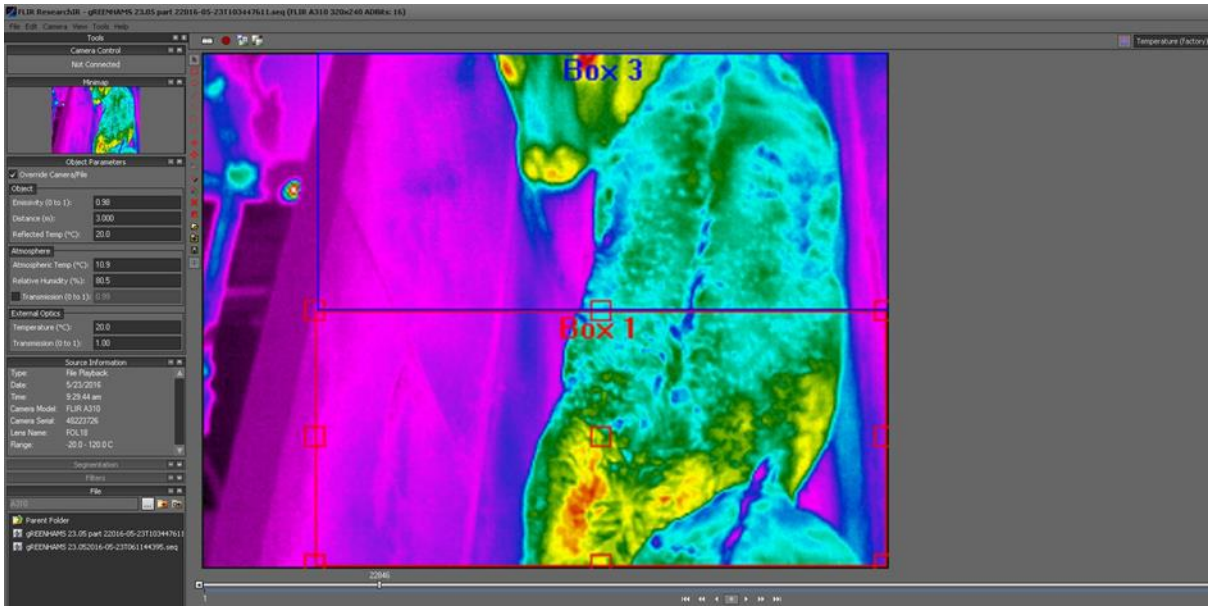


Fig. 7. Example of the regions of interest (Box1 and Box 3) from an IRT camera setup above the animals along a race. The value of the pixels with maximum temperature within the boxes is extracted for analysis.

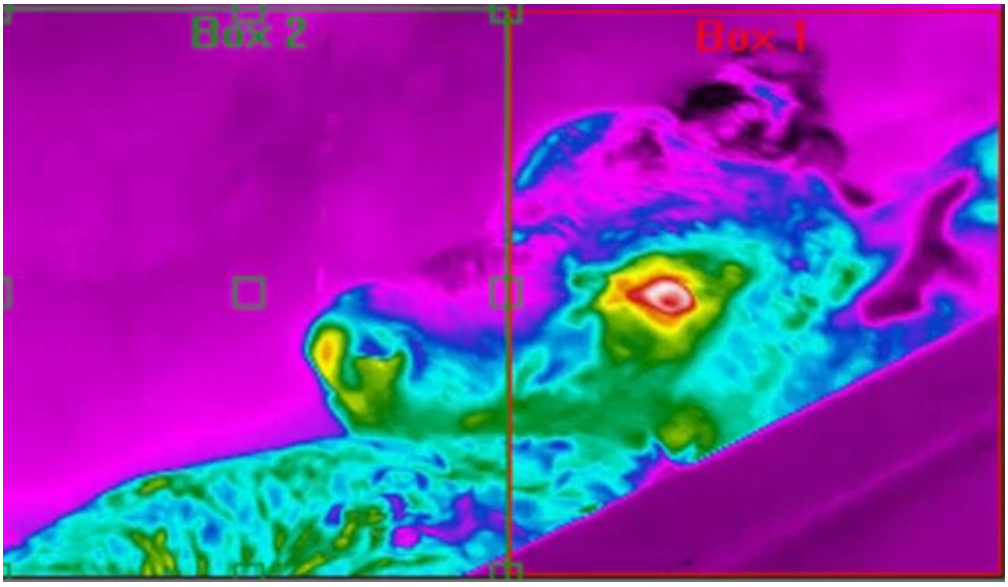


Fig. 8. Example of the regions of interest (Box1 and Box 2) from IRT cameras set up at 45˚ from the race. The value of the pixel with maximum temperature is extracted for analysis within the boxes.

The videos were viewed manually annotating the animal ID within the ROI for each frame. For *trial 3*, the part of the body (eye or skin) and the quality of each frame was recorded on a subset of 20 animals. The quality of the frame was considered very good if the lacrimal caruncle of the eye was within the ROI and the image was focussed. Otherwise the image was classified as poor quality because the eye was not clearly open, or the angle did not allow a clear view of the caruncle, the body was not within the ROI or out of focus. Therefore, many frames were unsuitable for analysis and removed, e.g. the animal was entering or leaving the ROI, the camera was refocussing or the animal had abrupt head movements. This step reduced the amount of background interference that may be present from the surrounding environment. The video was run through the FLIR software to

extract maximum, minimum and mean temperatures within the ROI. However, only the maximum IRT measurements within the ROI were used for statistical analysis (Stewart et al., 2008; Salles, 2016).

3.4.3 Statistical Analysis

Trial 2 (on-farm) explored the minimum amount of time needed for an accurate IR temperature reading using non-overlapping temperature data chunks of 5, 10, 15 and 20 seconds for each animal and compared with another 5, 10, 15 and 20 second chunk from that same animal to determine the effects of video length on the measured IR temperature and the repeatability of the measures. Maximum IRT measurements at the original frame rate were first processed calculating a 1-second rolling median to reduce the effect of sudden and unrealistic measurements (spikes) in the dataset due to camera auto-calibration and troughs over periods when the animals moved the head out of the ROI. This 'smoothed' data was then processed to calculate the following summary statistics for each animal: 50^{th}, 60^{th}, 70^{th}, 75^{th}, 80^{th}, 90^{th}, $95^{th}$and 99^{th} quantiles (q) for each of the three video cameras. Quantiles for each animal were used to remove potential outliers encountered with high-frequency data (e.g. any hot objects that entered the ROI). The value used for analysis from still images (C2 camera) was calculated as the average of the maximum values across the two images for each animal. These IRT statistics were then used to calculate Pearson correlation coefficients with rectal temperature (considered as the gold standard). Correlations were considered small between ±0.1-0.3, moderate ±0.3-0.7 and strong > 0.7. A probability level of $P<0.05$ was chosen as the limit for statistical significance in all tests.

Trail 3 used only the maximum IRT within the ROI at the original frame rate as animals moved throughout the race. This IRT data was then plotted over time to visualise changes as animals moved through the race annotating what part of the body was within the ROI for each frame. Frequency distribution histograms were obtained for the whole data independently of what part of the body was within the ROI (entire dataset) and also overlaying histograms for data points containing the eye or other parts of the body. The latter was done for a subset of data where the part of the body (eye or body) within the ROI was registered for each frame.

3.5 Using IRT to detect animal stress at commercial abattoirs

The ability of IRT to assess stress in beef cattle was studied in two different commercial abattoirs in southern Australia (Victoria and Tasmania). IRT data was collected prior to slaughter along with indicators of stress including carcase data (e.g. meat pH for both trials), meat eating quality (Tasmanian trial) and physiology (e.g. blood composition for the Tasmanian trial). A description of the materials and methods of these two trials is included in this section.

3.5.1 Commercial cattle abattoir trial (Victoria)

The objective of this trial was to determine the relationships between IRT temperature and meat quality with a focus on meat pH as an indicator of stress in commercial cattle slaughtered at a commercial abattoir where no background information about the animals was known. The data of this project was also used to develop the methodology for data analysis described in 3.4 above (*Trial 3*).

Infrared video cameras were setup along the race leading to the knocking box at approximately 3 metres from the head of the animals to record animals slaughtered at commercial speed. All animals recorded in this trial were slaughtered during the afternoon between approximately 2PM till 11PM for a period of 3 days. Carcase grading data (Aus-Meat and Meat Standards Australia (MSA) grading data and index) was collected from the abattoir. Pearson correlation coefficients were calculated to estimate the relationship between IRT and linear variables from carcase grading such as meat pH, marbling, and carcase weight. ANOVA was used to determine average IRT values for those variables which were not linear, such as meat colour as a categorical variable.

3.5.2 Mixing and stress trials (Tasmania)

The objective of this trial was to utilise a combination of stress factors (shipping, cattle mixing and recovery periods before slaughter) to evaluate the potential of infrared thermography (IRT) as an indicator of stress and eating quality. If successful, IRT pre or at slaughter could offer opportunities to improve existing MSA cattle delivery conditions while providing further information to better manage cattle stress on farm, during transit and at slaughter.

There were 2 trials designed to increase the stress level of animals, one in King Island and another in Tasmania, assessing sea transport and the saleyard pathway, respectively. Additional stress was placed on some groups to ensure changes in animal physiology and to observe any increase in dark cutting.

The King Island Trial 1 project utilised either a large or small shipping vessel to transport 243 animals for slaughter in Tasmania, together with an evaluation of no recovery period (slaughter 1 day after arrival to Tasmania), or a post transport 14-day recovery period on pasture.

Tasmania Trial 2 (237 animals) dealt with replicated truck loads within Tasmania which were subjected to either saleyard stress (2 saleyards) before slaughter or controlled direct consignment to slaughter from farm. Four farms supplied the animals for each of the 2 trials. The number of animals within each treatment is shown in Fig. 9.

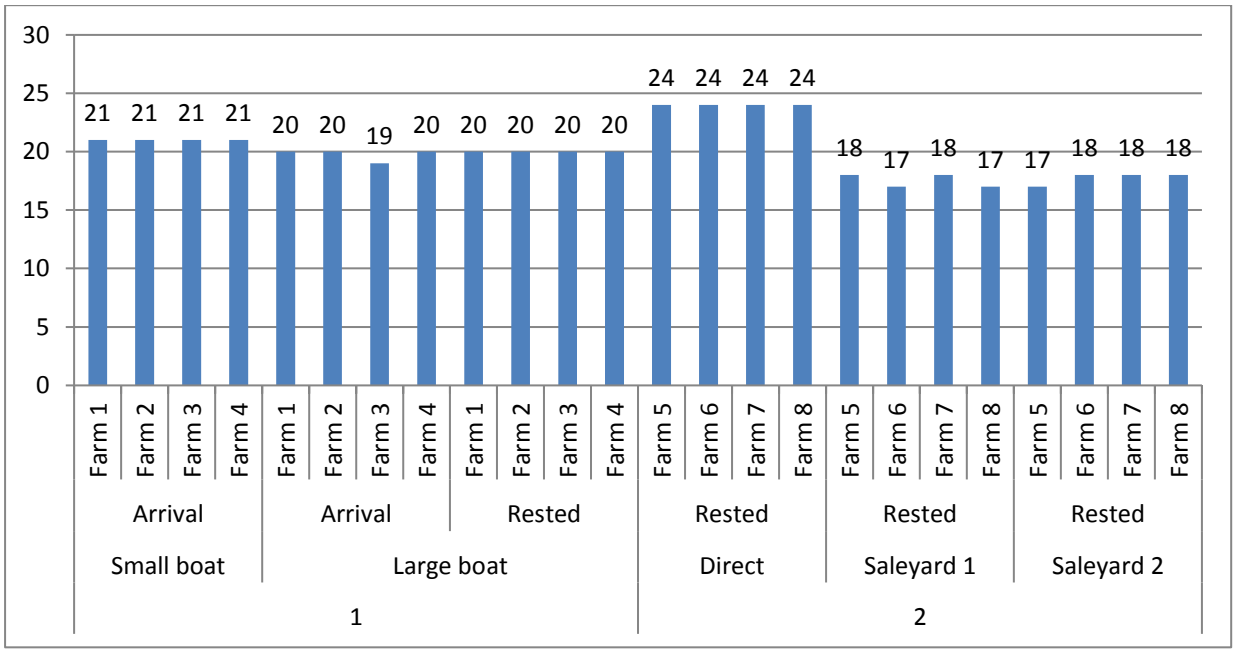


Fig. 9. *Number of animals supplied by each farm for each treatment. X-axis labels: first row from the bottom is trial number, second row is boat size for trial 1 and saleyard name for trial 2, third row is resting treatment before slaughter, and row 4 is farm supplying the animals.*

Other stress treatments were imposed on the animals by mixing unfamiliar animals from different farms upon loading to the trucks and mixing different sexes. Several mixing treatments were designed by co-mingling animals from different farms within a compartment of the truck as shown in Fig. 10.

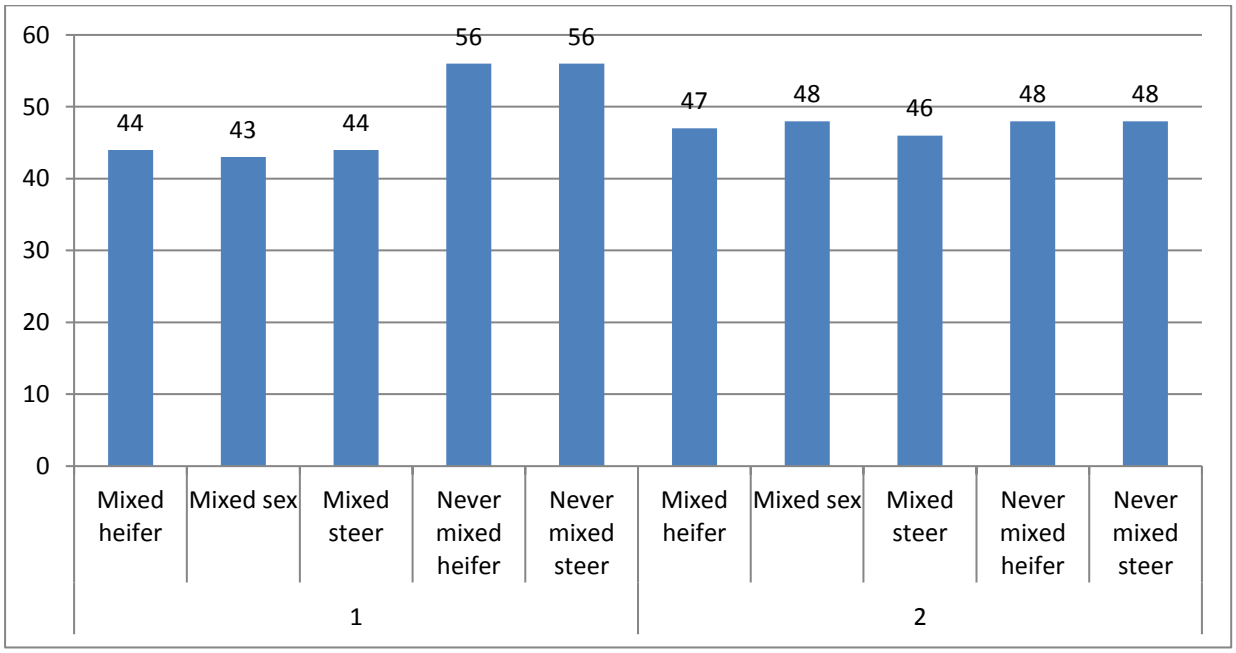


Fig. 10. *Number of animals for each mixing treatment which consisted of mixing unfamiliar heifers, mixing unfamiliar steers or mixing unfamiliar sexes, or never mixed.*

3.5.2.1 Trial 1 (boat transport from King Island to Tasmania)

Baseline stress measures were obtained two-three weeks prior to shipment and included IRT and flight speed. Forty-six cattle from each farm were shipped on the small boat from King Island to Stanley (Tasmania) with the remaining 14 cattle per farm loaded on a common double deck crate and shipped on the large boat to Devonport. Both voyages were on the same dates within each replicate. Half of each sea consignment was slaughtered on the day of arrival and the balance slaughtered 14 days later after rest on pasture on a paddock adjacent to the abattoir.

On the day of shipment, the cattle were mustered and sequentially drafted quietly into three groups based on randomised ear tags to avoid all the head or tail being in one mob. Two trucks were used: the first a single double deck that progressively loaded 14 head in a defined penning arrangement at each of the four farms which sailed on the large ship; and the second truck was a typical single deck truck that carried the remaining 46 animals (in three groups of 18, 18 and 10) to the King Island wharf. Mixing of animals (when required) was done in the truck compartment upon loading.

During transport on the boat, eight shipboard pens of 18 head each and two of 20 head each were used, with the pens randomly distributed across the ship. Upon arrival to the abattoir, cattle were sorted to separate those that were killed upon arrival from those that were rested for 2 weeks in a paddock next to the abattoir before slaughter. The pasture rest groups were maintained in seven separate groups for the 14-day rest period. All cattle on the double deck from Devonport were trucked to Smithton and slaughtered on day 1.

At slaughter a range of stress measures were taken including collection of blood plus IRT imagery. Independent MSA graders measured representative pH and temperature declines and collected MSA grading data pre-boning the day after slaughter. Selected cuts were collected at boning with laminated ID tags placed on the cut prior to vacuum packing. Both objective tests and untrained consumer evaluation were conducted on the collected cuts.

3.5.2.2 Trial 2 (Land transport and Saleyard Trial in Tasmania)

Four properties located in Tasmania supplied the cattle for this trial. Sixty head were selected at each property, weighed, tagged with trial ear tags and flight speed and IRT were recorded (June 6th, 7th and 8th).

Animals were loaded onto trucks and then sent to 2 saleyards before going to the abattoir (June 27th). Animals were weighed and IRT imagery recorded on the day of transport to the saleyards. Thirty-six head from each property were loaded at approximately 4 PM to achieve a constant time from property to slaughter. Control cattle remained on the properties for direct consignment. Slaughter was performed either on June 30th or July 14th. The 3 treatment groups were drafted off and the control groups returned to the paddock(s) as these left the farm on the day of slaughter.

Each of the saleyard groups (18 head each per farm) were then drafted into three pens of 6. Each group were then penned separately on the trucks. Four pens per deck (double deck trailers) were used for all but one of the properties where a 3 pen per deck trailer was used due to the heavier animal weight. Twelve of the 24 head that remained on each farm were loaded on the afternoon of June 29th.

3.5.2.3 Animal measurements (both trials)

Temperament

Temperament was assessed by calculating flight speed (Petherick, Holroyd et al. 2002) and measuring crush score (Cafe, Robinson et al. 2011) for each individual animal. The first of these metrics was recorded as cattle exited the crush, operated by the managers of each farm. This was measured as the time (s) it took cattle to interrupt two beams of infrared (IR) light crossing the exit route from the crush. The first infrared laser was located approximately 1.8 metres in front of the crush. Distances separating the two lasers differed between properties and therefore flight speed (m/s) was calculated to provide a comparable measure across all farms using flight speed per unit distance to account for the inter-farm variation (Coombes, Gardner et al. 2014).

Crush agitation scores were measured while cattle were confined within a crush for IRT measurements. All crush scores were assigned to cattle by the same MSA assessor on all properties

using a five-point scoring system. This scoring system was based on behavioural observations of the animals and associated docility scores set out by the International Beef Recording Scheme (Table 1).

Slaughter and Carcase Measurements

At the time of slaughter, visual ID and RFID were recorded against carcase number in the abattoir. Slaughter order of treatment groups was completely randomised and dual blood samples were collected from every animal directly following exsanguination, before being immediately centrifuged then frozen until analysis.

All carcases were graded the day after slaughter by qualified independent MSA graders with all measurements taken (Polkinghorne, Philpott et al. 2008, Watson, Polkinghorne et al. 2008, Hocquette, Botreau et al. 2012). PH was measured using a TPS MC-80 or TPS WP-80M pH meter (TPS Pty Ltd, Springwood, Brisbane, QLD, 4127, Australia) inserted into the rib eye muscle face at approximately 20 hours post-mortem.

Blood Sampling and Analyses

Once the blood had been centrifuged, then plasma collected and frozen, plasma aliquots were then thawed and analysed for physiological measures of stress. All assays were designed for the Olympus AU400 Automated Chemistry Analyser (Olympus Optical CO, Ltd, Melville, NY) and the following Reagent kits were used: glucose (Ref. OSR6121), lactate (OSR1693), magnesium (OSR6189), Creatine Kinase (CK) (OSR6279) and AST (OSR6209). Non-esterified fatty acids (NEFA) was analysed using a separate kit (C Kit Wako Pure Chemical Ind. Osaka, Japan) and the Roche Cobas Integra 400plus Reagent kit (0738085) was used for sodium and chloride. Batch sampling by enzymatic methods using an Olympus AU400 Clinical Chemistry Analyser (Olympus Optical CO. Ltd, Melville, NY, USA) was used to analyse beta-hydroxbutyrate (BHB). In house methods were used for haptoglobin (**HAP**; NTM-62 as per Eckersall et al, 1999) and ceruloplasmin (**CP**; NTR-23 as per Siotto et al., 2014).

3.5.3 IRT image analysis and statistical analysis

IRT videos were processed as described in the previous section. Briefly, the maximum temperature within the ROI was extracted from the videos after adjusting for ambient conditions. This data was then smoothed using a 1-second rolling mean and finally the quantiles for each animal were calculated. The start and end frame of each animal was registered by visually watching the videos, and data from each frame assigned an animal ID with which to match blood, carcass and meat quality data. IRT was affected by environmental conditions and therefore slaughter date was considered as batch effects due to ambient temperature amongst other factors. Hence, the within-date eye temperature data were robustly normalised by subtracting the median temperature.

To reduce the skewness, four physiology measurements (HP, CK, AST and CP) were log-transformed. The pH values were used to create DFD groupings intended to explore any differences between high pH carcasses (pH > 5.7) and carcasses failed on meat colour > 3. A probability level of $P < 0.05$ was chosen as the limit for statistical significance in all tests.

Linear regression and Pearson correlation were used to assess the relationship between IRT and the variable of interest (carcass traits, blood composition and eating quality).

3.6 Pilot automated system for detecting and drafting sick and stressed animals

A prototype for both beef cattle and sheep was developed to automatically detect, identify and segregate animals with high body temperature indicative of stress and disease in abattoirs. The prototype measures body temperature in real time, can send an alarm via the internet and sends commands in real time to move the drafting gates of an auto-drafter so animals with high body temperature can be separated automatically. The automatic detection system is composed of 3 main components: the RFID system, an infrared camera system and auto-drafter. Full integration and automation of all the components to work autonomously will require extra funding. An image of the system drafting cattle according to body temperature is shown in Fig. 11.

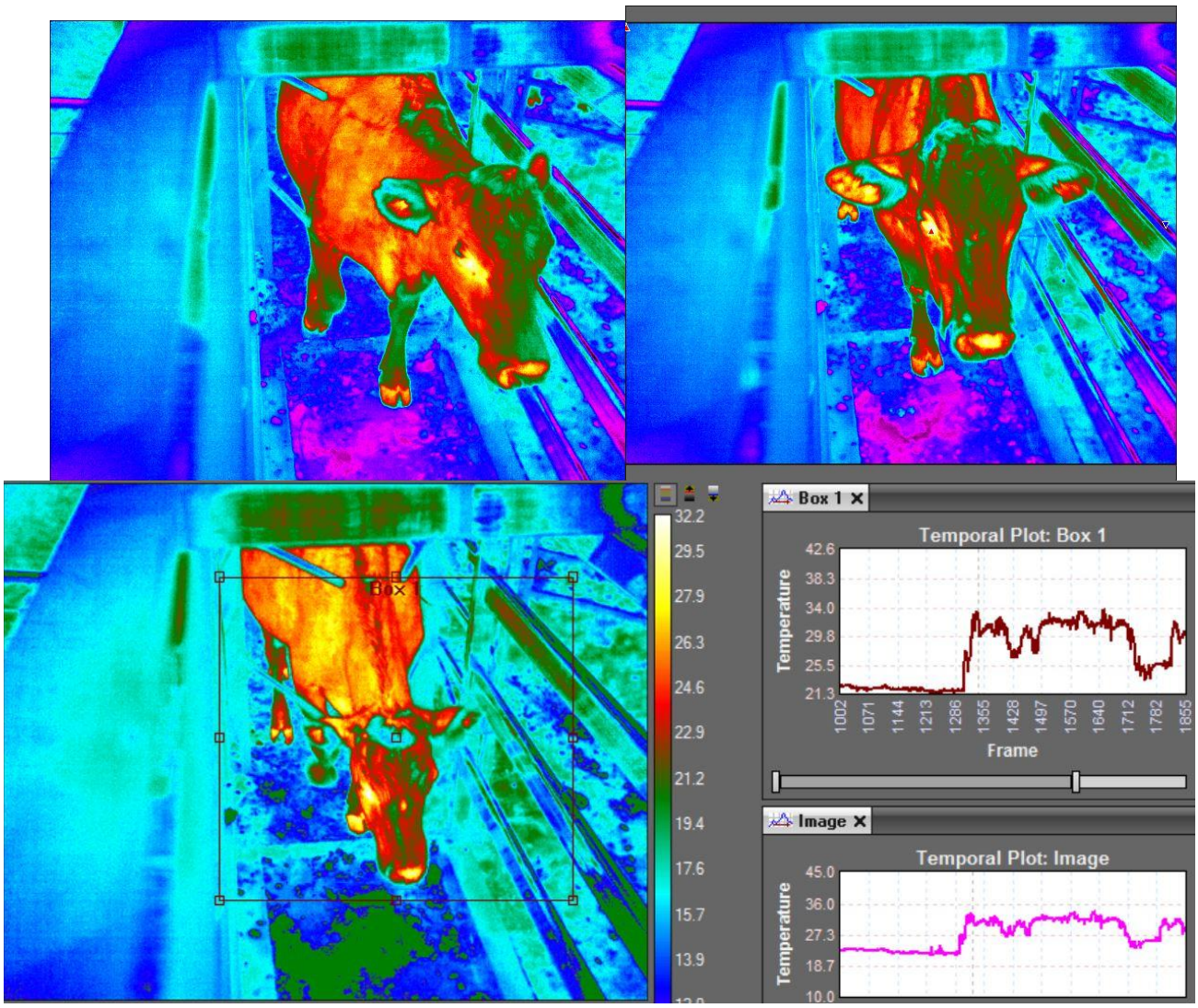


Fig. 11. *Imagery from an infrared camera setup in a race where animals walk through (top row), with real time analysis of IRT data (middle row) and drafting of animals in 1 of 3 ways depending on their body temperature (bottom row).*

The 3 main components of the auto-drafting system can potentially be used independently or integrated together as follows:

a. **Infrared thermography (IRT) system only**. A camera could be installed in an abattoir to analyse the data in real time as the animals are passing through the field of view of the camera. An alarm can be triggered when the temperature reaches a predefined threshold as a result of animals showing high temperature because of stress or sickness. This option could be suitable for all animal species but particularly for those where RFID is not used such as in sheep and goats.

b. **Integrated IRT and RFID systems.** This option could be suitable for animals that use RFID as standard practice in the industry such as cattle. The objective is to link RFID and body temperature which in this project was conducted by merging both independent databases.

c. **Integrated IRT and auto-drafting systems.** This option could be suitable for all animal species as the IRT camera will measure body temperature of animals under the field of view in real time and send the command to the drafter according to temperature thresholds. These temperature thresholds could be drafting animals with high temperature.

d. **Integrated RFID, IRT and auto-drafting systems.** This option would be suitable for animals carrying an Electronic Identification Device (EID) as standard practice such as cattle.

The other components could also be used independently, however for different applications beyond the scope of this project. For instance, RFID system could be deployed as a standalone system to measure the flow of animals through the abattoir in real time. Similarly, the auto-drafter could also be deployed either as a standalone system to draft animals automatically throughout the abattoir (e.g. to lairage pens) or in combination with RFID where animals are drafted and segregated according to specified characteristics linked to the RFID. This latter option could, for example, be used to draft mixed animal types in an arriving mob to the abattoir according to vendor, sex, class, breed, etc. for differentiated management. The concept could also be applied to automatically identify and segregate carcasses and meat cuts which show abnormal temperature as a result of the

lack of cooling down properly. The system was trialled only at research farms of The University of Sydney (not in abattoirs) due to the system being a prototype which requires further development.

3.6.1. RFID system

This is an electronic identification system (RFID) is similar to those used for beef cattle at abattoirs and farms being NLIS compliant. The system consists of an EID reader panel that can be setup in a race or unloading ramp where animals walk through in a single file, a controller of the reader panel and data logging system. The information can be stored in the system and sent wirelessly to another device. We have used Aleis components for cattle and Gallagher components for sheep.

3.6.2. Infrared camera with computer system

The IR camera used in this system was the FLIR A65 with Ethernet capabilities able to stream data in real-time to the computer and the network (Fig. 12). The collected data could be analysed in real time to obtain statistics such as average or maximum temperature in the entire frame or in a region or pixel of interest. The infrared videos were recorded to a solid state drive for later retrieval and analysis. The camera system also had a Wi-Fi modem so any device with Wi-Fi (e.g. mobile phone or tablet) can be connected to the camera system to configure it and watch the IR video in real time on the device using a webpage interface. The system also has an RS232 connection to interface with the auto-drafter or an RFID system.

3.6.3. Auto-drafting system

A 3-way auto-drafter was used which can receive a command to move 2 gates so individual animals can be segregated into 3 different groups according to measurements performed on them in real-time or drafting according to a list of RFIDs previously loaded.

Fig. 12. *Components of the automated prototype to detect and draft animals with high body temperature. A) RFID system, b) IRT camera system.*

3.6.4. Web interface of the system

A webpage application was developed to manage the IRT auto-drafting system from any device via Wifi (Fig. 13). The web interface has multiple tabs to visualise and configure the system (top of the image). The 'Stream' tab is used to watch the IRT videos in real-time with corresponding temperature for each pixel. The 'Area' tab is to set the region of interest where statistics can be calculated from for triggering alarms and the auto-drafter, and the 'Sp1' is the temperature of the pixel that could trigger the alarm or send the command to the auto-drafter. The alarm is triggered, or the command is sent to the auto-drafter in real-time when a predefined temperature threshold is measured within the Area or in the Sp1.

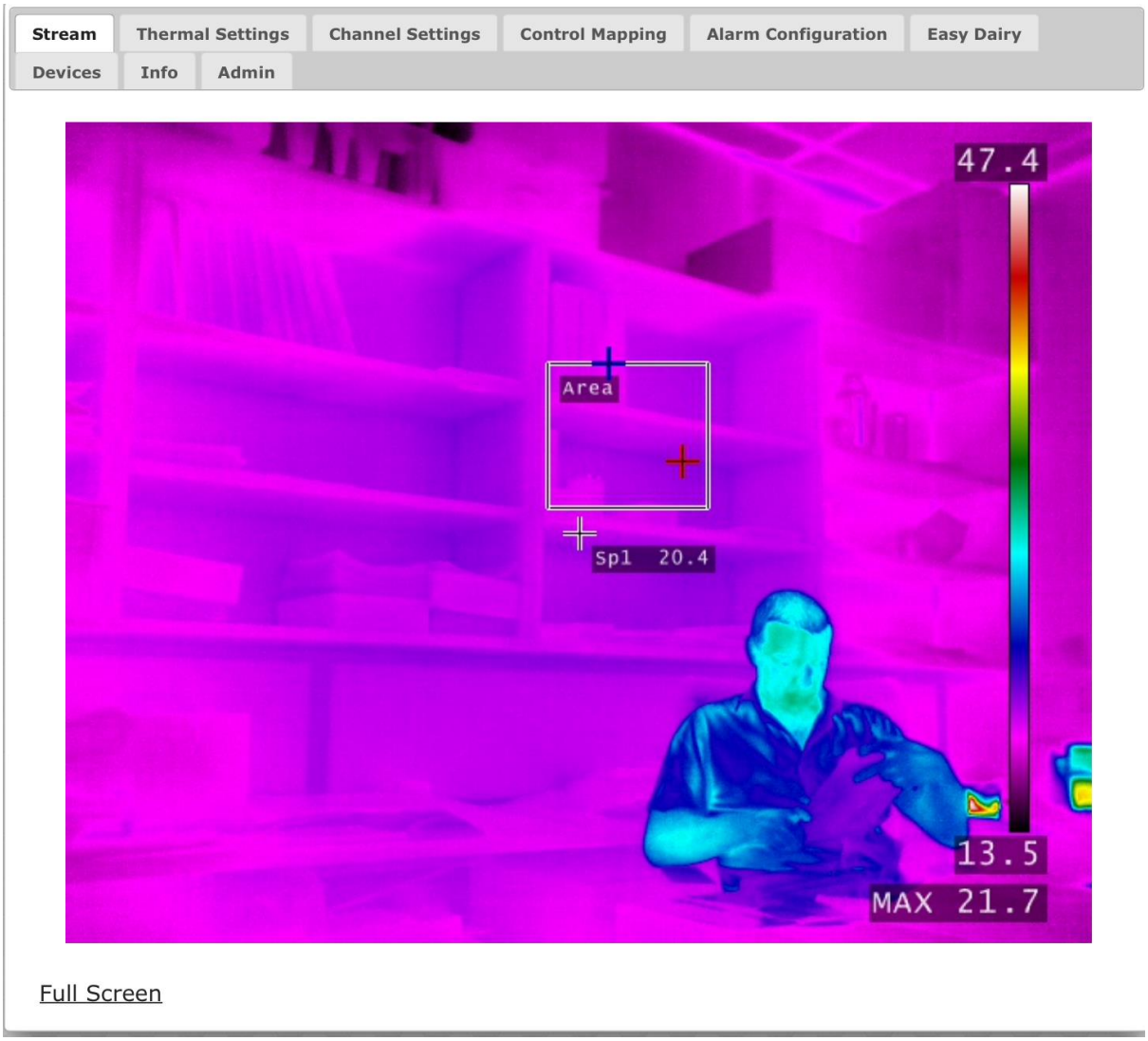


Fig. 13. *Web interface to change the configuration of the automated prototype to detect and draft animals with high body temperature.*

The temperature threshold to move the gates of the auto-drafter is set in the Easy Dairy tab. The 'Temperature Threshold' allows setting a **Lower** temperature threshold below which an alarm or command will be sent whereas the **Upper** temperature will send an alarm or a different command when this temperature value is reached or exceeded. The 'Off Delay' is the minimum number of seconds that need to pass before a command or alarm is sent. This was done to avoid commands being sent too quickly while the same animal was still in the region of interest. This value will depend of the speed at which the animals walk through the region of interest or frame. Further testing should be done in abattoirs to find out this Off Delay value which will likely depend on of the location within the abattoirs (e.g. animals are likely to pass through faster in the unloading rump compared to the race leading to the knocking box).

4 Results

4.1 Factors affecting IR body temperature in cattle

Results of factors affecting the IRT measurement are presented as the difference between rectal and IR temperatures because the objective was to determine which body part or device could be the best indicator of rectal temperature. The average IRT measured within the ROI revealed that coat colour ($P = 0.005$), IR device ($P < 0.001$) and body part ($P < 0.001$) affected IR temperature measurements. In contrast, maximum IR temperature within the ROI deemed coat colour to be non-significant ($P > 0.05$) whereas camera ($P < 0.001$) and body part ($P < 0.001$) were significant.

Camera Type. Both handheld camera and smartphone sensor (iPad) recorded the smallest difference between average IR and rectal temperature (Fig 14a). However, the difference between maximum IR and rectal temperatures were lowest with the handheld camera, medium with the sensor and greatest with the video camera ($P < 0.05$; Fig. 14b). The video recordings with the A310 showed the largest difference between IR and rectal temperatures for both average and maximum IR measures ($P < 0.05$; Fig. 14).

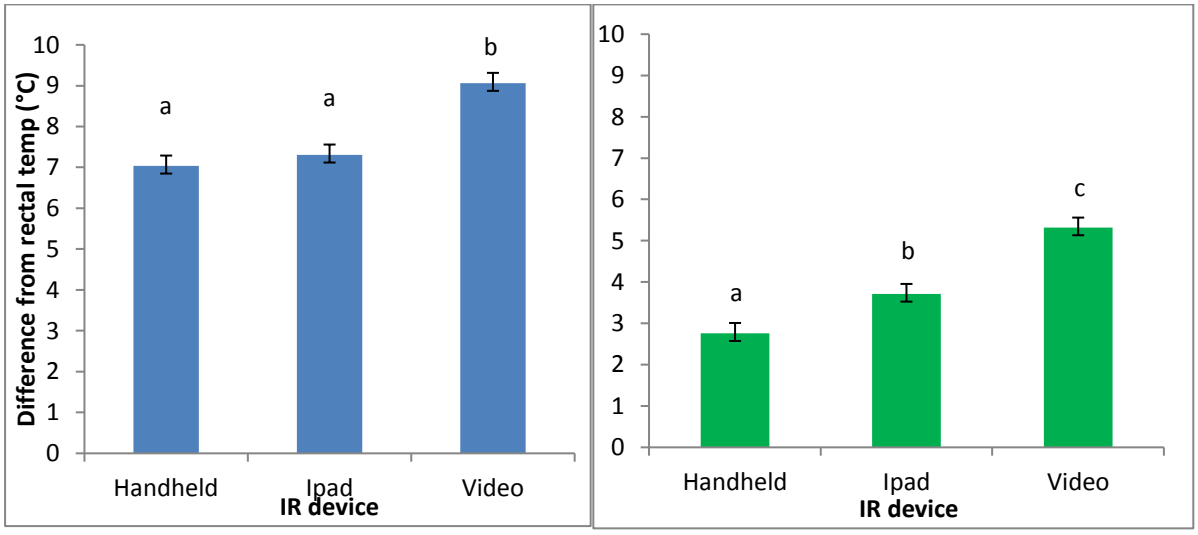


Fig. 14. *Effect of IR device ($P < 0.001$) and data processing method on the difference between IR and rectal temperatures in cattle. Left panel shows the difference between rectal and average IRT within the region of interest, whereas the right-hand panel shows difference between rectal and maximum IR temperatures within the region of interest.*

Body Part. Temperatures of all three body parts were significantly different between them with the eye showing the smallest difference between IR and rectal temperature (Fig. 15).

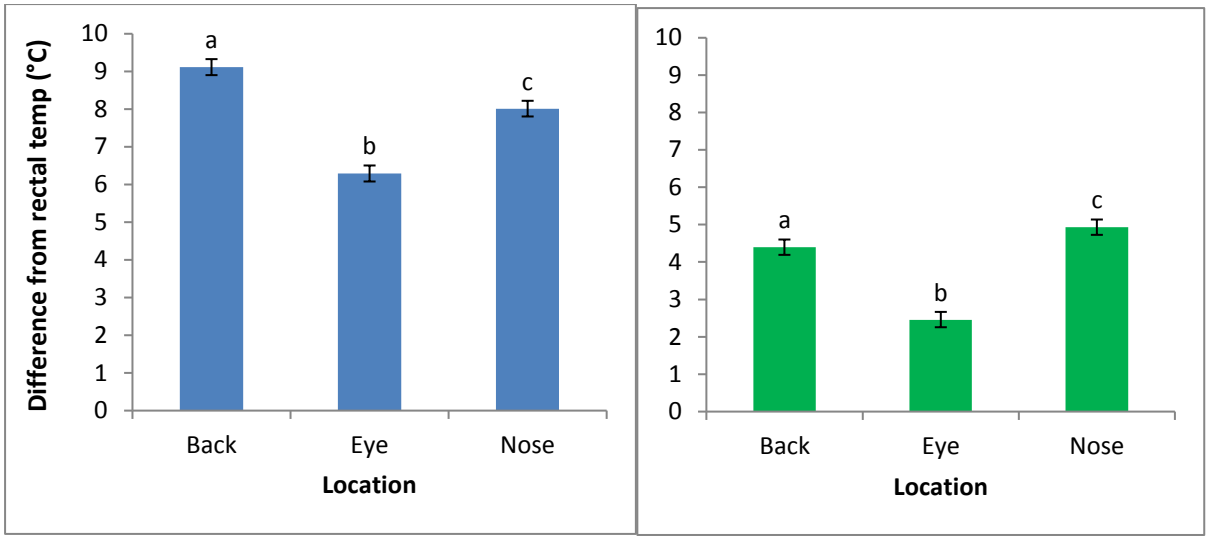


Fig. 15. *Effect of body part (P < 0.001) on the difference between IR and rectal temperatures in cattle. Left panel shows the difference between average IR and rectal temperature whereas the right-hand panel shows the difference between maximum IR and rectal temperatures.*

Data processing. Maximum IRT temperatures measured the smallest difference from rectal temperature in comparison to average IRT temperatures across all devices. A simple t-test revealed that maximum IR temperature of the eye using the handheld camera was significantly closer to rectal temperature in comparison to average eye temperature with the handheld camera (P < 0.001; data not shown).

Coat colour. Light brown coat colour showed larger (P < 0.05) difference with rectal temperature than all coat colours except speckled brown and white. IR temperatures in light brown and speckled cattle had the largest differences from rectal temperature at 8.6 and 8.3°C lower, respectively, compared to the rest of the coat colours (Fig. 16).

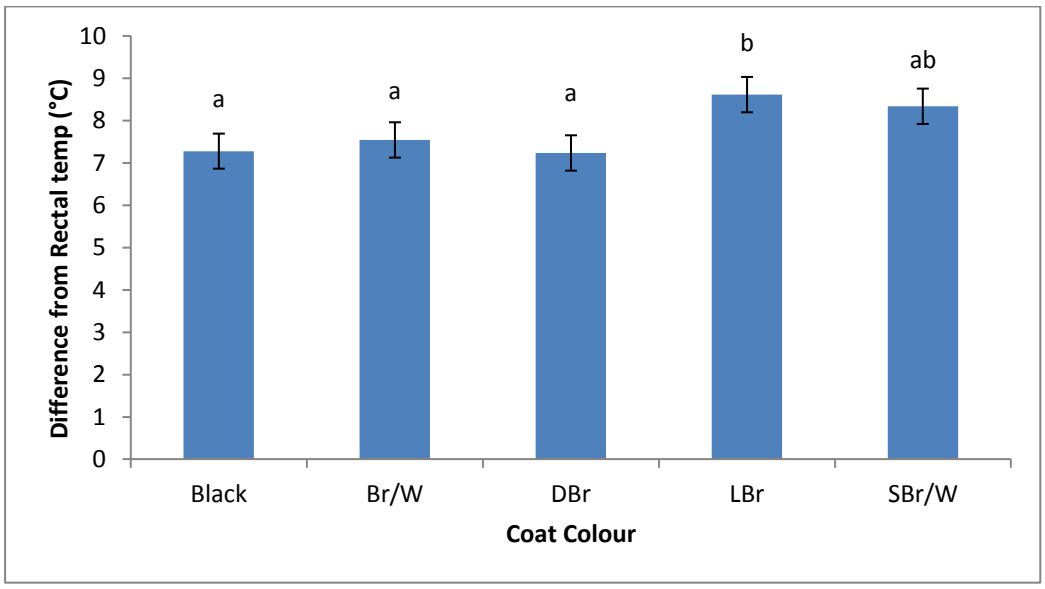


Fig. 16. *Effect of coat colour (P < 0.005) on the difference between mean IR temperature and rectal temperature. Coat colours are Br/W= brown white, DBr= dark brown, LBr= light brown, SBr/W= speckled brown white.*

Body temperatures measured with IR devices in all body parts were lower than rectal temperature ($P < 0.001$; Table 2). There was however a significant positive relationship between maximum IR eye temperature and rectal temperature with the handheld camera ($P = 0.026$), and a positive tendency between maximum IR eye temperature and rectal temperature with the IR video camera ($P = 0.089$; data not shown).

Table 2. *Summary statistics of infrared temperature (IRT) of cattle on different body parts measured using a video IR camera (VIR), a handheld IR camera (CAM) or a smartphone sensor.*

Body Part	Camera	Mean	SD	Minimum	Maximum	Observations
Average IRT (˚C)						
Eye	VIR	32.60	0.834	30.1	33.9	37
Eye	CAM	33.55	0.878	32.0	35.9	37
Eye	Sensor	33.63	1.104	31.3	35.3	19
Nose	VIR	30.05	1.469	25.7	32.4	36
Nose	CAM	32.42	1.140	28.2	34.2	36
Nose	Sensor	32.40	1.609	28.1	35.0	17
Back	VIR	28.96	1.716	26.0	32.7	37
Back	CAM	31.72	1.707	26.0	34.6	37
Back	Sensor	29.37	4.475	21.1	34.1	10
Maximum IRT (˚C)						
Eye	VIR	35.56	0.629	33.7	36.4	37
Eye	CAM	37.87	0.674	36.0	39.1	37
Eye	Sensor	37.15	0.933	35.6	38.5	19
Nose	VIR	32.81	1.027	30.6	34.8	36
Nose	CAM	35.36	0.815	33.2	36.8	36
Nose	Sensor	35.28	1.913	31.2	38.4	17
Back	VIR	33.63	2.127	28.6	38.1	37
Back	CAM	36.42	1.659	33.7	39.8	37
Back	Sensor	33.24	2.802	26.0	35.9	10
Rectal Temperature (˚C)		39.32	0.255	39.0	39.9	37
Animal Characteristics						
Body condition score		1.804	0.344	1.25	2.50	37
Temperament score		1.412	0.501	1	3	37
Weight (kg)		309.7	60.23	171.5	423	37

4.2 Development of a methodology for data processing and analysis of IRT videos

IRT data was first plotted to visualise and describe the data obtained from IR video cameras and processing methods and algorithms were developed to objectively analyse that data.

4.2.1 Description of data obtained from IRT videos at high rate

IRT videos were processed to obtain the temperature of the pixel with maximum temperature within the region of interest (ROI) with the results of a short section of the video shown in Fig. 17. When no animals were in the race, the temperature of the background infrastructure was approximately 19 °C. Temperature increased to approximately 27 °C when the skin (e.g. back) of the animals are within the ROI, and temperatures increased further to 34 to 36 °C when the eyes of the animals are within the ROI because the eye is the hottest body part of the animal. Fig. 17 also shows spikes of short duration in temperature when animals run fast through the race (less than 1 second in the ROI), or the high temperature is maintained when animals stay for longer periods of time within the ROI.

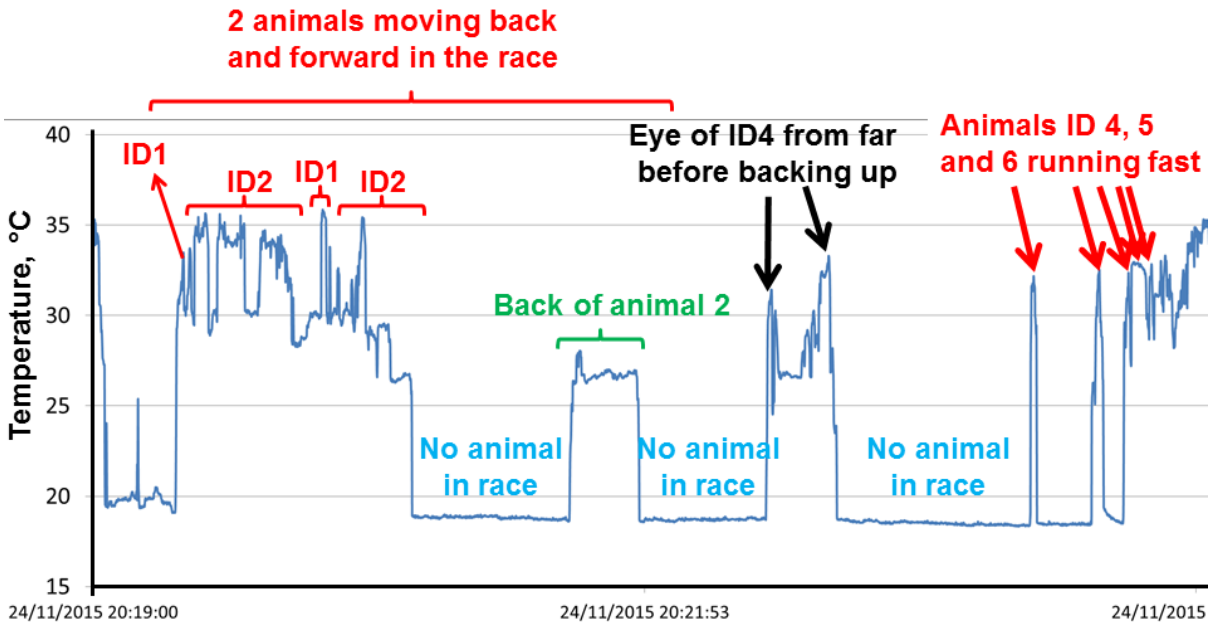


Fig. 17. *Example of automation of measurements of body temperature of cattle in the race of an abattoir. Maximum temperature of each frame in the video was measured 32 times per second.*

4.2.2 Quality of the images and data processing of raw data

Maximum IR temperature within the ROI of each frame is shown in Fig. 18. The figure shows examples of data obtained from animals walking through the FOV of the camera with the head down where the eye is not visible, poor image quality where the eye is not in the best position and very good image quality with the eye fully opened under the FOV. Poor quality of images can arise from multiple factors including position of the head, focus of the camera and part of the body under the FOV. Animals with the eye within the ROI result in higher IRT compared to animals walking through the head down thus the camera only captures the skin or body (Fig. 19).

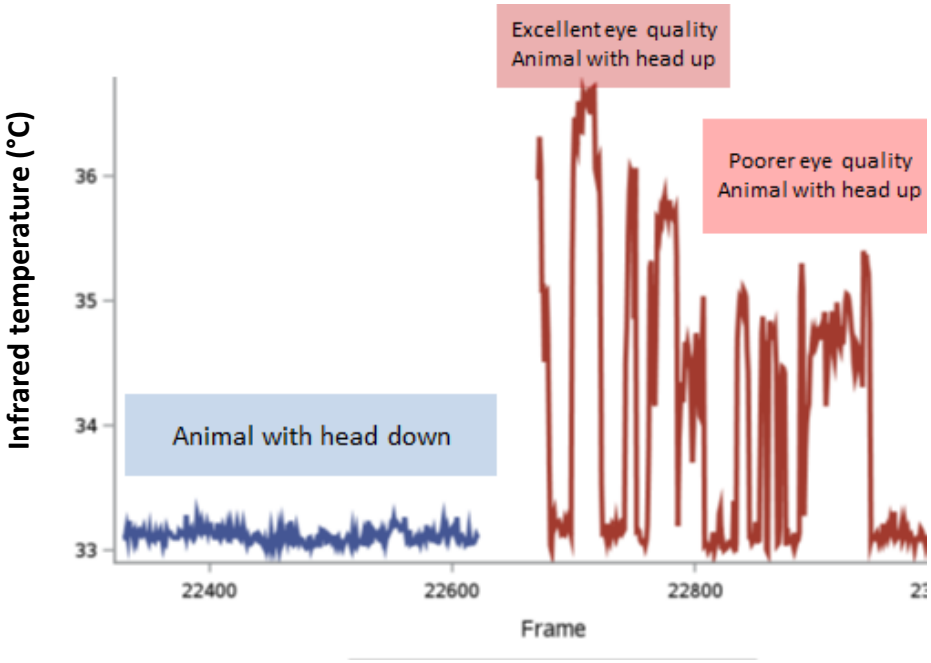


Fig. 18. Infrared temperature of cattle with poor (eye not within the region of interest) and good quality videos walking through a race at a commercial abattoir.

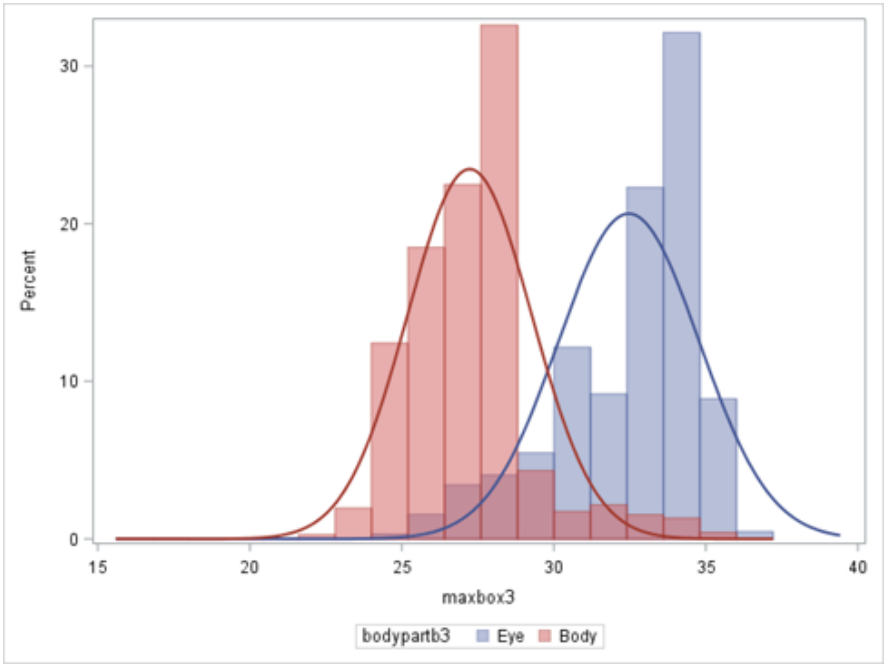


Fig. 19. Frequency distribution of the infrared temperature of cattle with poor and good quality videos where the eye is within the region of interest walking through a race at a commercial abattoir. Poor image quality results from videos where the body of the animal is within the region of interest but not the eye. Good image quality results from videos where the eye is within the region of interest for large proportion of the data.

The image above shows that both populations of data points, i.e. eye and skin, have different means and frequency distributions which compose mixed distributions. Mixed distributions are present

when different populations of data points are observed in a dataset, with each population representing particular characteristics such as part of the body where the measurement was obtained or quality of the image. This finding is important as fitting probability density functions allow determining threshold points that assign a data point to a particular population with the minimum miss assignment rate. This methodology has previously been used with success on cattle biological data for unsupervised classification of data points (Gonzalez et al., 2015; Tolkamp et al., 2000). A similar methodology could be applied to IRT data to define threshold values in IRT that allow deleting data points which do not contain good quality images of the eye or those where the eye is not in the ROI. The frequency distribution of whole IRT dataset at the abattoir shows 3 populations of data points representing populations of data points that contained the eye, those that contained the skin of the body of animals and those without an animal present (Fig. 20).

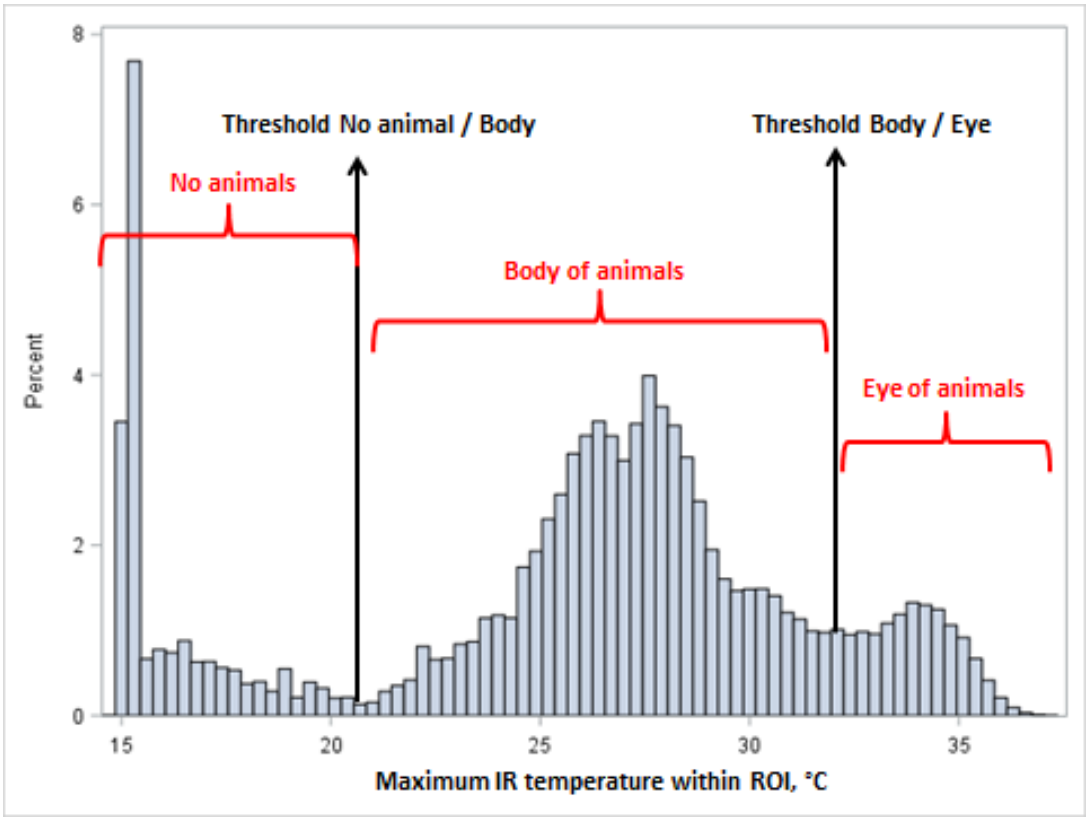


Fig. 20. *Histogram of infrared temperature obtained from a camera setup in the race of a commercial abattoir (n = 137,788).*

Measuring the maximum temperature within the ROI could be the most practical and objective way to analyse IRT data from videos. However, this approach did not prove to be accurate when there were 'hotspots' with temperature higher than the animals in the background (e.g. facilities) or when the camera was auto-calibrating and focussing. The first approach taken to reduce the noise in the IR data was a smoothing method to effectively smooth out any random spikes or declines due to camera glitches or artefacts. The IRT data was smoothed calculating a rolling median over one second as shown in Fig. 21.

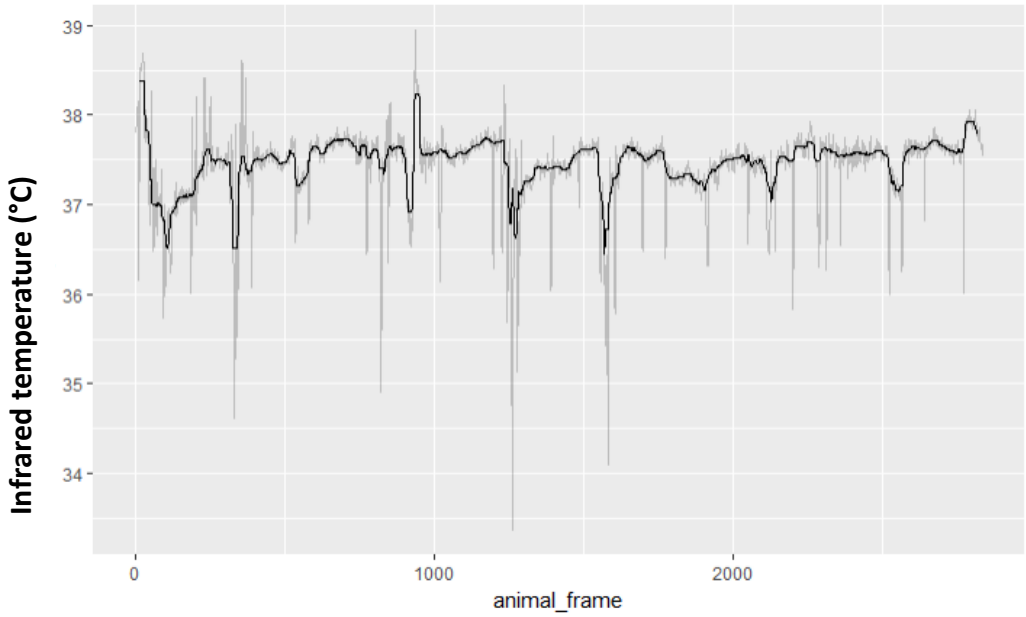


Fig. 21. *Rolling median (thick black line) smoothing technique to remove random spikes from the raw maximum temperature measured in each frame (grey thin line).*

Examples of hotspots which would not be reflecting core body temperature are shown in Fig. 22 with the left panel showing a hotspot on the neck of an animal due to rubbing with the head bail and the right-hand panel with a hotspot from machinery on the back of the animal. These factors can sometimes be eliminated by proper setup of the ROI as shown in the images.

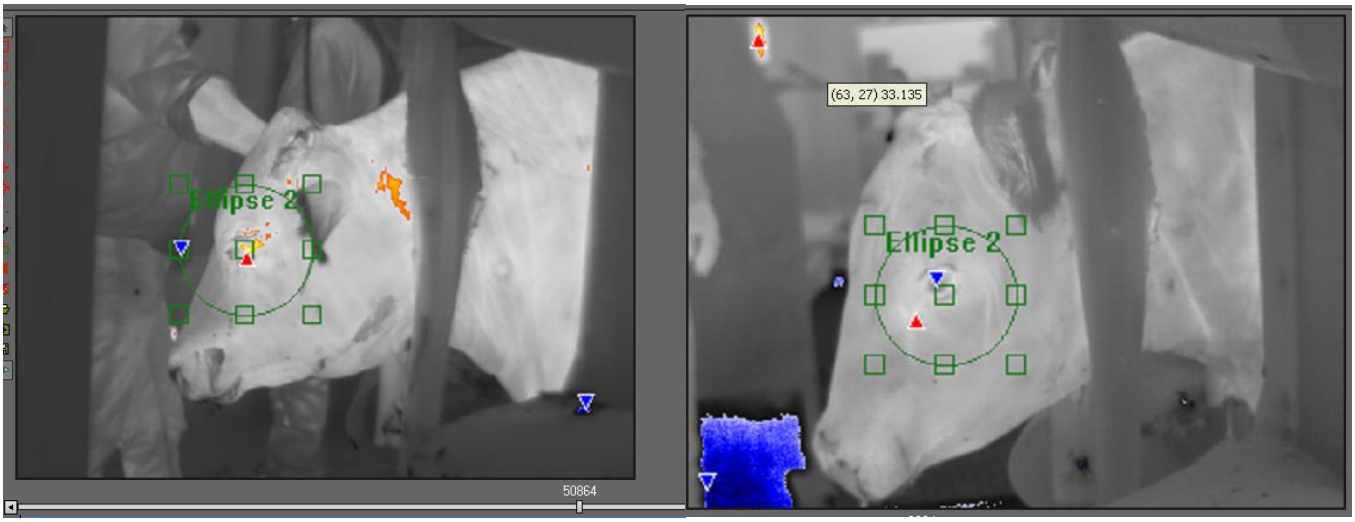


Fig. 22. *Hotspots on IRT images of cattle that may reduce the correlation between IRT and rectal temperature due to rubbing of the neck with the head bail (right hand panel) and hot machinery on the back (right panel).*

After the data was smoothed using the rolling 1-second media, a second step to remove any residual outliers in the data involved the calculation of quantiles for each animal. Examples of 2 animals with outliers removed due to hotspots in the background are presented in Fig. 23. The figures show the maximum temperature within the region of interest of each frame in a video from animals walking

through a race. The animal on the top panel has 2 episodes of outliers which are removed when quantiles are calculated.

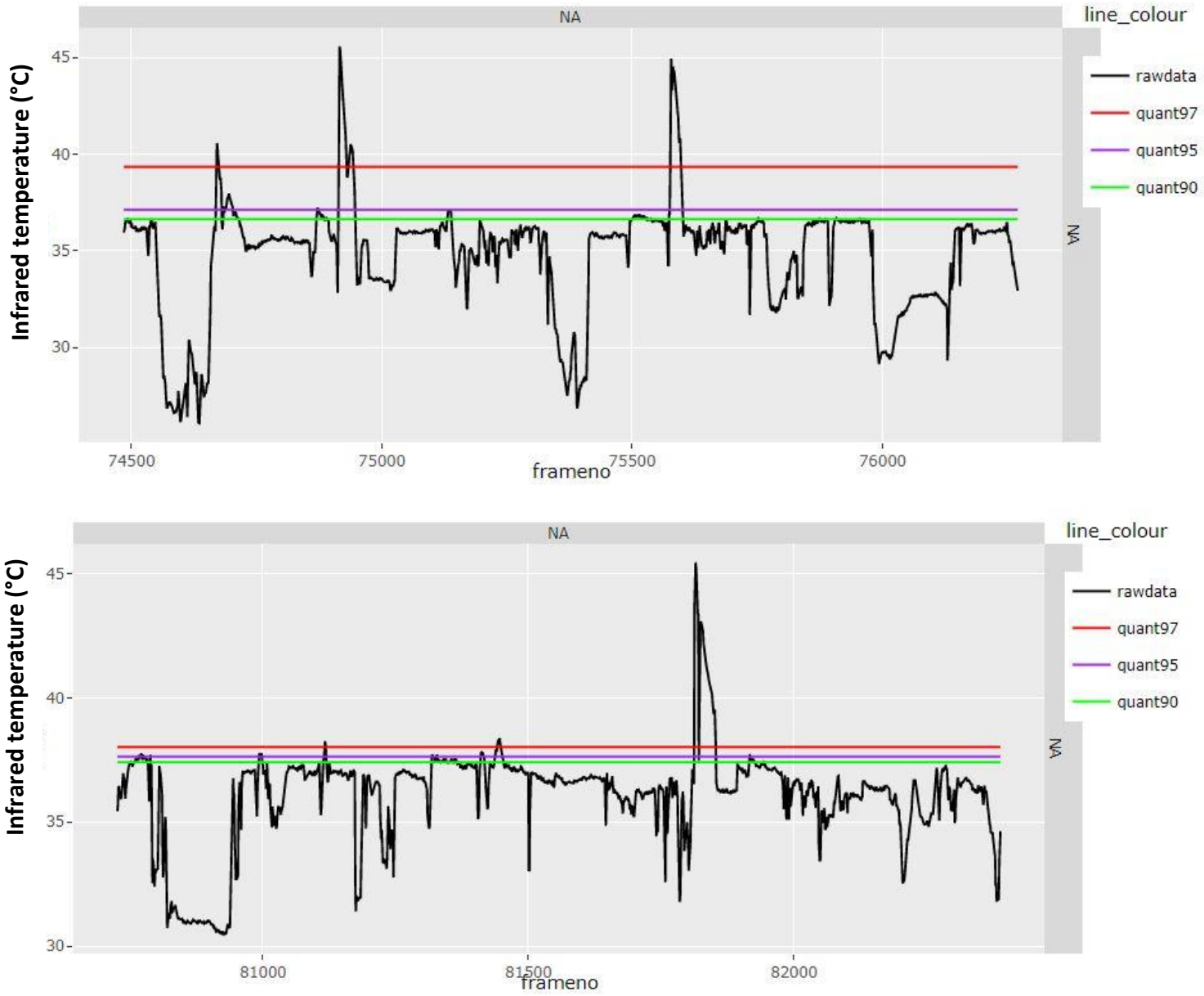


Fig. 23. *Maximum IRT within the region of interest (black line; rawdata) measured on 2 animals and algorithm developed to reduce the effect of the artificial spikes of high temperature calculating 90, 95 and 97% quantiles on the maximum temperature (quant90, quant95, quant97).*

4.2.3 Determining the best data processing method

All IRT statistics described in this section were calculated for data collected at the University of Sydney's research farm (Trial 3) and the correlation between IRT statistics and rectal temperature were calculated (Table 3). All three IR video cameras revealed positive correlations with rectal temperature ($P < 0.05$). The C2 camera, which only took radiometric still images, resulted in the lowest correlation coefficient. The A310 and A65 cameras had the highest correlations with rectal temperature across all summary statistics. The summary statistics that had the highest correlation

with rectal temperature was q99 and q97 from the A310, followed by the q90, q95, q97 from the A65 camera. Overall, the 95 quantile seemed to yield the highest correlation with rectal temperature.

Table 3. *Pearson correlation between different IRT and rectal temperature of cattle measured using 4 different cameras (A65, T420, A310 and C2) and different summary statistics (Q50 = 50% quantile, and so on).*

	Q50	**Q60**	**Q70**	**Q75**	**Q80**	**Q90**	**Q95**	**Q97**	**Q99**	**Max**
A65	0.34	0.35	0.40	0.39	0.39	0.42	0.43	0.41	0.39	0.37
T420	0.37	0.37	0.37	0.36	0.36	0.36	0.36	0.37	0.35	0.36
A310	0.35	0.35	0.36	0.36	0.37	0.37	0.41	0.45	0.45	0.49
C2 [A]	-	-	-	-	-	-	-	-	-	0.22

[A] C2 only took single still images and calculation of quantiles is not possible.

The length of time to estimate the IRT of an animal from videos seemed to have affected the strength of the correlation between IRT and rectal temperature (Table 4). The A65 and A310 had the strongest correlation with IRT when all data available was used whereas the T420 showed the highest correlation when analysed for those animals which had at least 10 seconds of IRT data.

Table 4. *Pearson correlation between different IRT and rectal temperature of cattle measured using 3 different cameras (A65, T420 and A310) and different summary statistics (Q50 = 50% quantile, and so on) with all data available, or only when using animals with 5 seconds (5s) or 10 seconds of data (10s).*

	Q90/all	**Q90/5s**	**Q90/10s**	**Q95/all**	**Q95/5s**	**Q95/10s**
A65	0.42	0.29	0.33	0.43	0.29	0.34
T420	0.36	0.40	0.43	0.36	0.40	0.42
A310	0.37	0.28	0.32	0.41	0.30	0.31

4.3 IRT to detect animals under stress at the abattoir

This section of the project used previously developed analytical methods to assess the relationship between IRT and different indicators of stress at abattoirs (Trial 3 in Victoria and MSA trials in Tasmania). Results are presented first for the associations between IRT and carcass traits including meat pH and colour, and then with physiological indicators including blood composition and eating quality.

4.3.1 Relationship between IRT and carcass traits (Victoria abattoir)

Summary of statistics for maximum eye temperature and carcase traits recorded during the abattoir trial are presented in Table 5 for the Victorian abattoir trial. Calculated values of IRT showed large variability across animals with a difference of 10° C between the animal showing the lowest and the animal showing the highest IRT.

The number of IRT measurements available (number of frames) to calculate the mean temperature for each animal ranged from 2 to 9,034 data points demonstrating that the speed at which the animals went through the FOV of the camera was highly variable under commercial abattoir conditions. Most of the variables showed also large variability amongst aniamls including carcase weight, tropical breed content, marbling and MSA index which also demonstrate the variable characteristics of commercial slaughter cattle. Meat pH ranged from 5.3 to 6.5 with a low proportion of dark cutters at 4.21% (pH > 5.7; Table 5 and Fig. 24). It is important to highlight that all animals in this dataset were grass fed cattle.

Table 5. *Summary statistics of IR body temperature and carcass traits of cattle at a commercial abattoir (N = 594).*

Variable	Minimum	Mean	Maximum	Std Dev
Maximum IRT,° C	28.9	34.5	38.9	1.7
Nro. IRT measures per animal	2.0	118.7	9034.0	407.7
Total HSCW, kg	213.0	316.6	451.0	46.1
Hump Height Cold	15.0	46.6	85.0	15.2
Eye Muscle Area, cm^2	48.0	70.1	96.0	7.1
Ossification Cold	100.0	154.8	280.0	24.0
Aus Marbling score	0.0	1.2	5.0	1.1
MSA Marbling score	120.0	347.8	770.0	110.3
Fat Colour score	0.0	1.4	4.0	0.9
Rib fat Cold, mm	2.0	7.7	24.0	3.4
Meat pH	5.3	5.5	6.5	0.1
Loin Temperature, °C	1.3	6.3	8.9	0.9
Hide Puller Damage	0.0	0.0	3.0	0.3
MSA Index	56.1	60.9	67.9	2.1

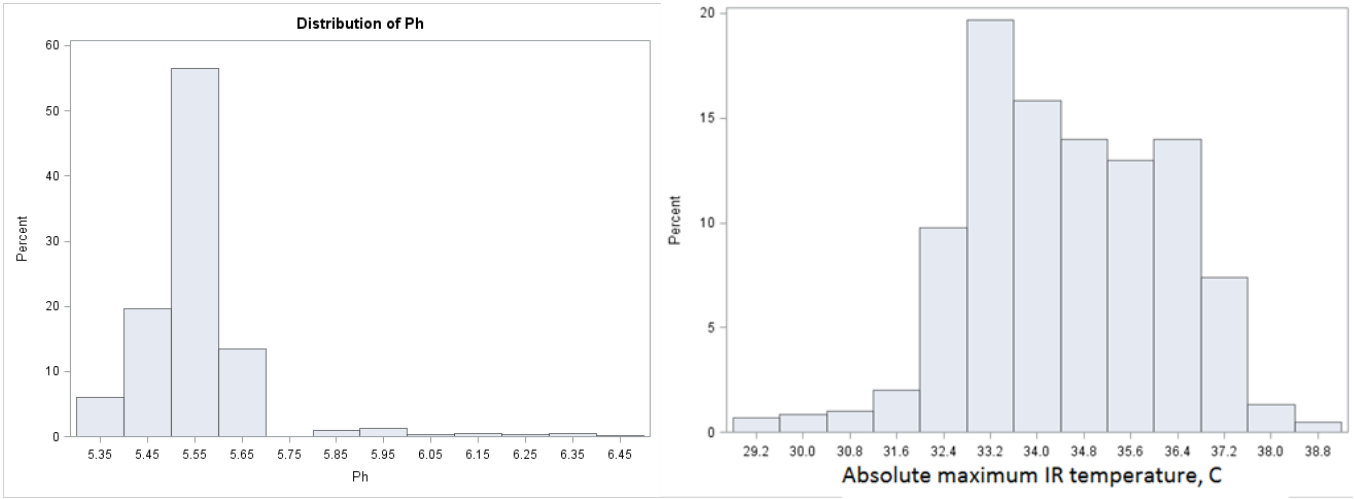


Fig. 24. Frequency distribution of meat pH and IRT temperature from animals at a commercial abattoir.

Pearson correlation coefficients amongst continuous variables are shown in *Table 6*. IRT temperature was not correlated ($P > 0.05$) with meat pH however IRT was negatively correlated with marbling and MSA index ($P < 0.05$) indicating that animals with higher IR body temperature had lower MSA index and marble scores. The strongest positive correlations were reported between HSCW, hump height, eye muscle area and rib fat ($P < 0.05$), and between MSA index and marbling score ($P < 0.01$; Table 6).

Table 6. *Pearson correlation coefficients (above diagonal) between IR body temperature and carcass traits at a commercial abattoir (N = 594). Respective p-values are shown below the diagonal.*

	Max IRT(°C)	Nro. IRT per animal	Total HSCW (kg)	Hump Height Cold	Eye Muscle Area	Ossification Cold	Aus Marbling	MSA Marbling	Fat Colour	Rib fat Cold, mm	pH	MSA Index
Maximum IRT (°C)		0.27	-0.06	-0.07	0.07	-0.01	-0.18	-0.16	0.08	0.00	0.00	-0.15
Nro. IRT per animal	<0.001		0.01	-0.04	-0.04	0.02	-0.02	0.00	-0.01	0.03	-0.05	-0.02
Total HSCW (kg)	0.121	0.788		0.60	0.30	0.12	0.15	0.14	-0.18	0.29	-0.07	0.17
Hump Height Cold	0.095	0.368	<0.001		0.42	-0.18	0.21	0.18	-0.20	0.19	-0.01	0.31
Eye Muscle Area	0.113	0.389	<.0001	<0.001		-0.10	0.21	0.20	0.08	-0.08	0.08	0.23
Ossification Cold	0.857	0.549	0.003	<0.001	0.013		0.06	0.10	0.11	0.23	0.08	-0.44
Aus Marbling score	<0.001	0.554	<0.001	<0.001	<0.001	0.152		0.97	-0.03	0.22	0.03	0.79
MSA Marbling score	<0.001	0.974	0.001	<0.001	<0.001	0.014	<0.001		0.00	0.22	0.03	0.80
Fat Colour score	0.066	0.860	<0.001	<.0001	0.041	0.006	0.429	0.938		-0.09	0.18	-0.08
Rib fat Cold (mm)	0.921	0.440	<.0001	<.0001	0.041	<.0001	<0.001	<0.001	0.037		-0.05	0.26
Meat pH	0.987	0.239	0.105	0.723	0.047	0.068	0.538	0.465	<0.001	0.251		0.10
MSA Index	0.001	0.640	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.048	<0.001	0.015	

[A] Hot standard carcass weight

There was a relationship between meat colour and IRT body temperature of cattle ($P < 0.05$; Fig. 25) with animals resulting in darker meat showing higher body temperature particularly for colour 4 and above.

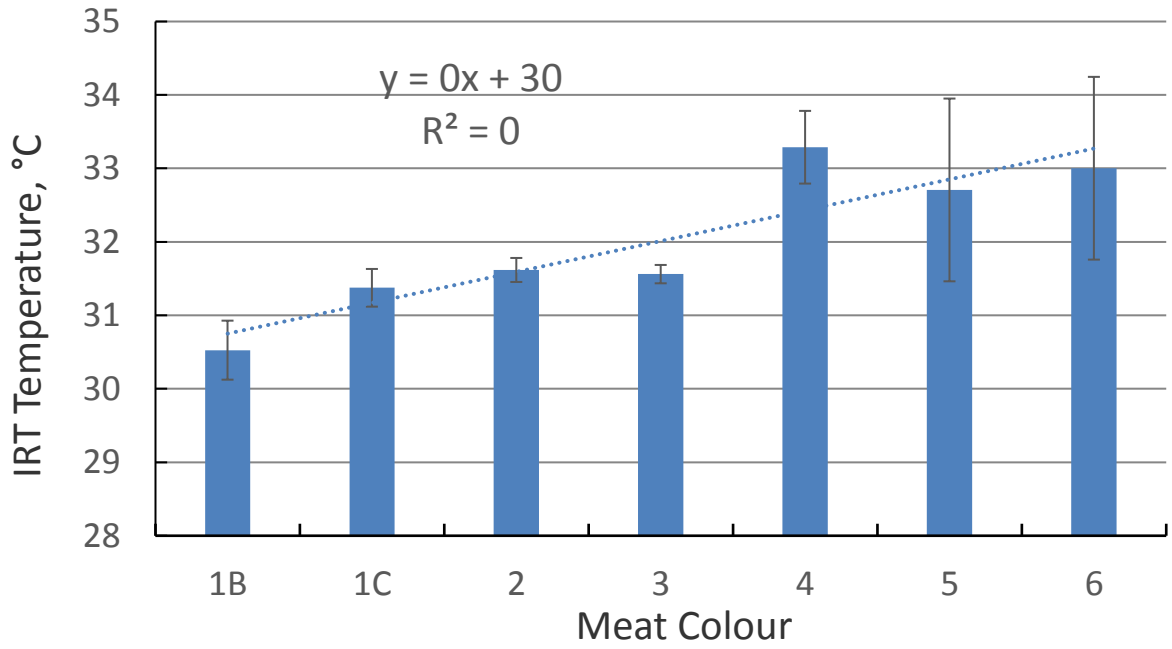


Figure 25. Infrared body temperature for each meat colour score of cattle at a commercial abattoir (n = 594).

In addition to the relationship between body temperature and meat colour, animals with darker meat have also resulted in higher meat pH as expected ($P < 0.05$; Fig. 26) also markedly higher for colour of 4 and above.

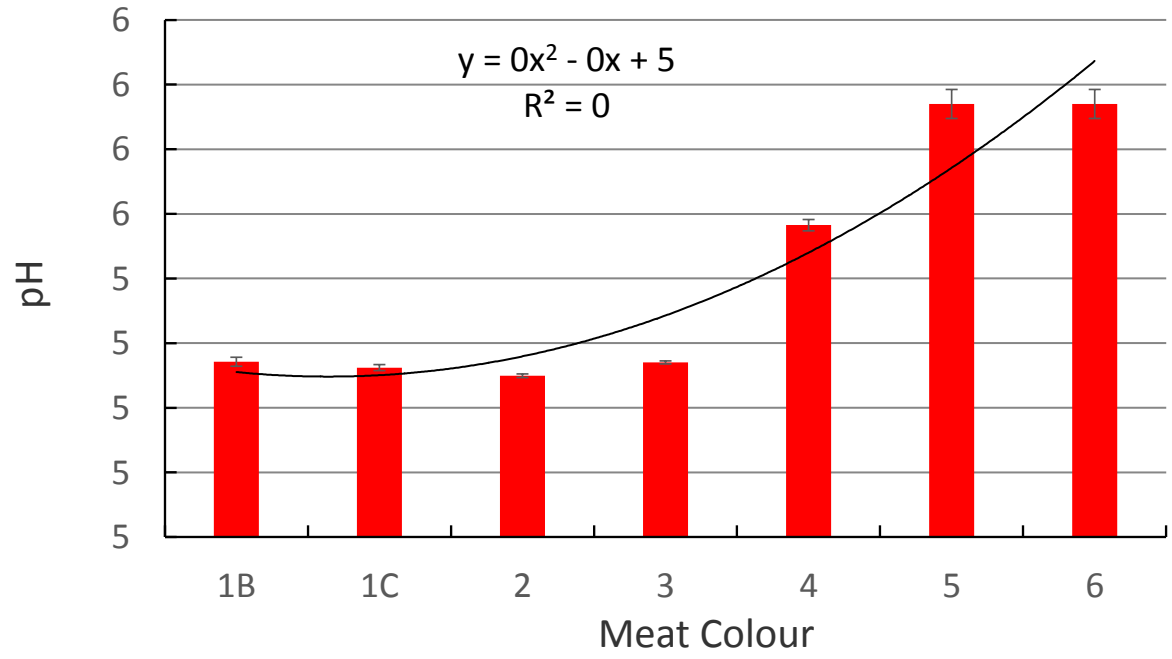


Fig. 26. Average meat pH for each meat colour score of cattle video recorded with IRT cameras at a commercial abattoir.

Marbling score was negatively correlated with body temperature ($P < 0.05$) because animals with higher marbling showed lower IRT temperature (Fig. 27).

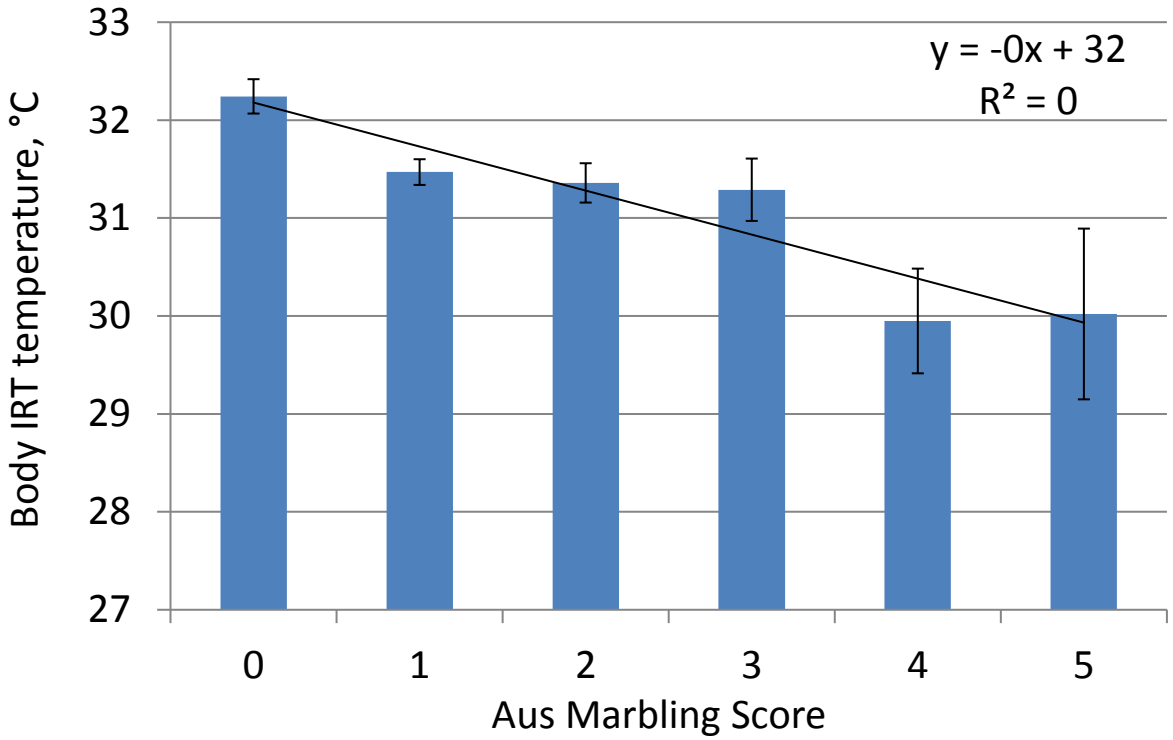


Fig. 27. Average IR body temperature of cattle that graded each marbling score slaughtered in a commercial abattoir.

4.3.2 Mixing and stress trial (King Island and Tasmania)

Pearson correlation coefficients between IRT and blood and meat parameters indicators of stress are presented for animals which had IRT measured at the race (Table 7) and at the knocking box (Table 8) prior to slaughter during the mixing and stress trial. These analyses were done with animals which had a minimum of 10 seconds of IRT video as this was found in the previous section to be the most accurate method of analysis. In general, correlations between IRT and indicators of stress were weak with the highest correlation being negative between IRT and muscle st-lactate at -0.48 ($P < 0.05$).

For animals transported by boat and not rested prior to slaughter, higher IRT at the race was linked to higher concentration of haptoglobin ($P < 0.05$). However, for animals transported by boat and rested for 14 days before slaughter, higher IRT was associated with lower concentrations of magnesium (Mg) in blood ($P < 0.05$; Table 7). For animals transported by land within Tasmania and not rested before slaughter, higher IRT temperature was associated with higher Aspartate Transminase (AST; $P < 0.05$). For animals transported by land and rested before slaughter, higher IRT body temperature was associated with lower St-lactate in muscle ($P < 0.05$; Table 7).

At the knocking box, for animals transported by boat from King Island to Tasmania and not rested before slaughter, higher IR body temperature was correlated with higher concentration of NEFA ($P < 0.05$; Table 8). For animals transported by land within Tasmania and not rested before slaughter, higher IRT resulted in higher concentration of glucose in blood at the time of slaughter ($P < 0.05$). In contrast, for animals transported by land and rested before slaughter, higher IRT was associated with lower concentration of BHB ($P < 0.05$; Table 8). For animals transported by boat and rested

before slaughter, higher IRT was associated with higher concentration of lactate in blood ($P < 0.05$). For animals transported by land and not rested before slaughter, a positive correlation existed between IRT and glucose ($P < 0.05$) whereas if these animals were rested then a negative correlation existed between IRT and BHB. No other significant correlations existed between IRT and blood or meat indicators of stress ($P > 0.05$).

Table 9 shows the regression coefficients between IRT and meat quality from sensory analysis for each of the slaughters performed during the mixing and stress trial. Eating quality as measured by the clipped meat quality score (cmq4) parameter was affected by IRT body temperature however the direction of effect depended on the location where IRT was measured (race or knocking box). In addition, the relationship between IRT and eating quality was dependent on the data processing method, i.e. whether all animals independently of the number of data points available to calculate IRT data was used for analysis, or only those animals with at least 5 or 10 second of IRT data.

Animals with higher IRT at the knocking box had lower eating quality particularly for those animals slaughtered after land transport and those transported by boat and slaughtered without resting ($P < 0.05$; Table 9). The regression coefficient was greater when the dataset contained only animals with 10 seconds of IRT or all data available. In contrast to animals scanned at the knocking box, animals with higher IRT at the race had better eating quality ($p < 0.05$), especially for those not rested before slaughter and rested if transported by land (Table 8).

Table 7. *Pearson correlation coefficients between IR body temperature measured at the race and indicators of stress measured of cattle killed on four different dates which were subjected to stress treatments of transport (sea or land) and resting (not rested or rested for 14 days before slaughter).*

	Glucose	Lactate	NEFA	Mg	BHB	Hapto	CK	AST	CP	St-lactat	Ld-lactat	St-Glycogen	Ld-Glycogen	DM
Sea transport Not rested cattle (Kill 1)														
IRT_q90	0.18	0.10	-0.13	-0.19	-0.05	0.21*	0.09	0.17	0.10	- [A]	0.02	-	-0.02	-0.04
IRT_q95	0.16	0.10	-0.13	-0.18	-0.03	0.21*	0.05	0.14	0.12	-	0.04	-	0.01	-0.04
IRT_q97	0.16	0.09	-0.12	-0.18	-0.03	0.21*	0.05	0.16	0.12	-	0.04	-	0.00	-0.04
Max	0.15	0.09	-0.12	-0.18	-0.03	0.22*	0.06	0.16	0.11	-	0.04	-	0.01	-0.04
Sea transport Rested cattle (Kill 2)														
IRT_q90	0.11	-0.16	-0.08	-0.31*	-0.22	-0.12	-0.13	0.14	-0.09	-	0.19	-	0.00	0.15
IRT_q95	0.10	-0.17	-0.05	-0.34*	-0.20	-0.10	-0.14	0.13	-0.09	-	0.20	-	-0.02	0.13
IRT_q97	0.10	-0.17	-0.05	-0.34*	-0.20	-0.10	-0.14	0.13	-0.09	-	0.20	-	-0.02	0.13
Max	0.10	-0.18	-0.04	-0.33*	-0.19	-0.09	-0.15	0.12	-0.09	-	0.15	-	-0.04	0.16
Land transport Not rested cattle (Kill 3)														
IRT_q90	0.16	0.06	-0.09	-0.02	0.05	0.17	0.24	0.34*	0.02	-0.10	0.08	-0.05	-0.01	-0.22
IRT_q95	0.14	0.07	-0.06	0.03	0.07	0.18	0.26	0.31*	0.04	-0.08	0.05	-0.08	-0.01	-0.28
IRT_q97	0.14	0.07	-0.05	0.03	0.07	0.18	0.25	0.31*	0.04	-0.08	0.05	-0.07	0.00	-0.29
Max	0.14	0.07	-0.05	0.03	0.07	0.17	0.25	0.31*	0.04	-0.08	0.05	-0.07	0.00	-0.29
Land transport Rested cattle (Kill 4)														
IRT_q90	-0.22	0.17	-0.36	-0.27	-0.15	0.12	0.07	0.20	-0.33	-0.40*	0.12	0.16	0.16	-0.25
IRT_q95	-0.16	0.05	-0.33	-0.25	-0.07	0.12	0.05	0.12	-0.37	-0.48*	0.12	0.04	0.19	-0.23
IRT_q97	-0.16	0.08	-0.37	-0.25	-0.09	0.15	0.04	0.14	-0.35	-0.47*	0.02	0.05	0.17	-0.20
Max	-0.18	0.08	-0.40	-0.26	-0.08	0.19	0.02	0.15	-0.32	-0.46*	-0.06	0.04	0.14	-0.17

*, **, *** $P < 0.05$, $P < 0.01$, $P < 0.001$

Analysis was done for animals which had 10 seconds of IRT data only

NEFA: Non-esterified Fatty Acids; Mg: Magnesium; BHB: Beta-hydroxybutyrate; Hapto: Haptoglobin; CK: Creatine Kinase (CK); AST: Aspartate Transminase; CP: Ceruloplasmin.

[A] not measured

Table 8. *Pearson correlation coefficients between IR body temperature measured at the knocking box and indicators of stress of cattle killed on four different dates which were subjected to stress treatments of transport (sea or land) and resting (not rested or rested for 14 days before slaughter).*

	Glucose	Lactate	NEFA	Mg	BHB	Hapto	CK	AST	CP	St-lactat	Ld-lactat	St-Glycogen	Ld-Glycogen	DM
Sea transport Not rested cattle (Kill 1)														
IRT_q90	-0.08	-0.13	0.25*	-0.04	-0.12	-0.13	0.01	-0.10	-0.14	- [A]	-0.16	-	-0.18	-0.17
IRT_q95	-0.07	-0.12	0.28*	-0.01	-0.15	-0.12	-0.01	-0.10	-0.12	-	-0.16	-	-0.18	-0.17
IRT_q97	-0.08	-0.12	0.29*	0.00	-0.16	-0.12	-0.02	-0.10	-0.11	-	-0.16	-	-0.17	-0.17
Max	-0.08	-0.12	0.29*	0.00	-0.15	-0.12	-0.02	-0.09	-0.11	-	-0.16	-	-0.17	-0.16
Sea transport Rested cattle (Kill 2)														
IRT_q90	0.46	0.30*	-0.11	0.07	-0.08	-0.07	-0.17	-0.23	0.05	-	0.14	-	0.08	0.20
IRT_q95	0.46	0.29	-0.11	0.05	-0.11	-0.03	-0.17	-0.22	0.05	-	0.06	-	0.11	0.30
IRT_q97	0.46	0.29	-0.11	0.05	-0.12	-0.03	-0.17	-0.22	0.06	-	0.06	-	0.12	0.29
Max	0.48	0.31*	-0.13	0.04	-0.11	-0.06	-0.15	-0.22	0.04	-	0.06	-	0.13	0.29
Land transport Not rested cattle (Kill 3)														
IRT_q90	0.31*	0.01	-0.17	-0.14	-0.13	0.17	0.12	0.05	-0.02	-0.05	0.02	-0.15	0.05	-0.05
IRT_q95	0.31*	0.06	-0.16	-0.10	-0.13	0.18	0.13	0.09	-0.03	-0.01	0.02	-0.12	0.05	-0.03
IRT_q97	0.31*	0.05	-0.17	-0.09	-0.14	0.18	0.13	0.1	-0.01	-0.01	0.01	-0.13	0.05	-0.03
Max	0.30*	0.04	-0.13	-0.09	-0.12	0.18	0.14	0.09	-0.01	-0.02	-0.01	-0.15	0.03	-0.04
Land transport Rested cattle (Kill 4)														
IRT_q90	0.15	0.18	-0.17	-0.04	-0.36*	0.25	-0.03	-0.16	0.01	0.08	0.02	0.24	0.05	-0.25
IRT_q95	0.15	0.17	-0.17	-0.03	-0.36*	0.24	-0.03	-0.13	-0.02	0.10	-0.01	0.26	0.06	-0.20
IRT_q97	0.15	0.17	-0.18	-0.03	-0.36*	0.24	-0.03	-0.13	-0.02	0.10	0.00	0.26	0.06	-0.20
Max	0.15	0.18	-0.19	-0.06	-0.38*	0.23	-0.03	-0.13	-0.01	0.10	-0.01	0.25	0.07	-0.19

*, **, *** $P < 0.05$, $P < 0.01$, $P < 0.001$

Analysis was done for animals which had 10 seconds of IRT data only

NEFA: Non-esterified Fatty Acids; Mg: Magnesium; BHB: Beta-hydroxybutyrate; Hapto: Haptoglobin; CK: Creatine Kinase; AST: Aspartate Transminase; CP: Ceruloplasmin.

[A] not measured

Table 9. *Regression coefficients of IR body temperature (measured at the race or knocking box) against eating quality from sensory analysis (cmq4) in cattle killed on four different dates which were subjected to stress treatments of transport (sea or land) and resting (not rested or rested for 14 days before slaughter).*

	cmq4 knocking box			cmq4 race		
	All data	5 sec	10 sec	All data	5 sec	10 sec
Sea transport Not rested cattle (Kill 1)						
IRT_q90	-0.205 (0.093) **	0.046 (0.085)	-0.099 (0.170)	-0.041 (0.058)	0.214 (0.085) **	0.142 (0.090)
IRT_q95	-0.208 (0.098) **	0.025 (0.086)	-0.075 (0.179)	-0.043 (0.056)	0.195 (0.085) **	0.139 (0.090)
IRT_q97	-0.201 (0.100) **	0.020 (0.086)	-0.073 (0.179)	-0.042 (0.055)	0.191 (0.085) **	0.125 (0.090)
Max	-0.200 (0.100) **	0.012 (0.086)	-0.080 (0.179)	-0.040 (0.053)	0.179 (0.086) **	0.120 (0.091)
Sea transport Rested cattle (Kill 2)						
IRT_q90	-0.065 (0.165)	-0.281 (0.144) *	-0.076 (0.220)	-0.025 (0.111)	0.075 (0.230)	-0.178 (0.219)
IRT_q95	-0.025 (0.173)	-0.263 (0.145) *	-0.076 (0.234)	-0.007 (0.108)	0.060 (0.233)	-0.126 (0.222)
IRT_q97	-0.037 (0.177)	-0.225 (0.144)	-0.075 (0.236)	-0.005 (0.107)	0.063 (0.233)	-0.122 (0.222)
Max	-0.043 (0.181)	-0.217 (0.143)	-0.057 (0.243)	-0.007 (0.106)	0.077 (0.234)	-0.098 (0.226)
Land transport Not rested cattle (Kill 3)						
IRT_q90	-0.241 (0.165)	-0.034 (0.158)	-0.530 (0.221) **	-0.072 (0.116)	0.820 (0.301) **	1.137 (0.355) ***
IRT_q95	-0.244 (0.180)	-0.073 (0.165)	-0.552 (0.249) **	-0.083 (0.113)	0.792 (0.306) **	1.027 (0.375) ***
IRT_q97	-0.228 (0.185)	-0.118 (0.167)	-0.560 (0.251) **	-0.079 (0.112)	0.789 (0.330) **	1.016 (0.376) ***
Max	-0.178 (0.189)	-0.170 (0.171)	-0.645 (0.263) **	-0.081 (0.111)	0.784 (0.335) **	1.021 (0.378) ***
Land transport Rested cattle (Kill 4)						
IRT_q90	-0.378 (0.164) **	-0.206 (0.139)	-0.931 (0.313) ***	0.177 (0.180)	-0.639 (0.580)	1.455 (0.767) *
IRT_q95	-0.387 (0.168) **	-0.213 (0.141)	-0.925 (0.323) ***	0.216 (0.175)	-0.603 (0.573)	1.751 (0.761) **
IRT_q97	-0.382 (0.169) **	-0.218 (0.142)	-0.920 (0.326) ***	0.216 (0.172)	-0.564 (0.568)	1.911 (0.780) **
Max	-0.385 (0.170) **	-0.191 (0.144)	-0.932 (0.333) ***	0.229 (0.170)	-0.529 (0.563)	2.026 (0.775) ***

5 Discussion

The present project recorded animals using IR video cameras at commercial abattoirs and research farms with the objective of developing an automated system for the detection and segregation of stressed animals. As far as the authors are aware, there are no scientific publications or reports using a similar approach with IR video cameras in commercial conditions addressing the detection of stressed cattle or sheep. However, a patent exists to detect animals with low meat quality in beef including DFD (Tong et al., 1997) and research has been published with pigs (Gariepy et al., 1989). Thus, this is one of the first reports working in challenging conditions where many factors cannot be controlled as it occurred in other published studies.

The project used IRT video cameras because of the need to capture animals which may be under the field of view for a very short time period often less than 1 second. These data at high frequency, at up to 60 frames per second, required the development of a methodology for the application of this data for the desired purpose. There was no published literature suggesting approaches for the analysis of this data in commercial conditions as in this study. The project started gaining basic knowledge of the latest technological developments and trialling the most promising ones for different practical applications. Once the main characteristics of the technologies and the factors affecting IRT measurements were investigated, a methodology for analysis was developed. Finally, trials were performed with a large number of animals under commercial conditions to determine if IRT was able to detect animals under stress using several physiological indicators. The discussion below follows that process.

5.1 Factors affecting IR body temperature

Body temperature of cattle using IRT has shown to be affected by multiple factors (McManus et al., 2016) however data presented in this report indicates that the type of device, the part of the body where temperature is measured and the data processing methods can be controlled and have a large influence on the values obtained. These factors need to be considered to develop an automated system for practical applications. A detailed discussion is provided below.

5.1.1 Body part where IRT temperature is measured

The eye was the closest indicator of rectal temperature and therefore the preferred body part to scan animals under commercial conditions. This may be due to the fact that the eye is more protected from the external environment and it has a dense number of capillary beds supplying blood to the surface (Church et al., 2014; Stewart et al., 2008; Weschenfelder et al., 2013; Gloster et al. 2011). Maximum eye temperature was also able to detect changes in response to pain and stress (Hsieh Chan et al. 1990; Stewart et al. 2010; Stewart et al. 2008). Schaffer (2003 and 2007) analysed the effects of diseases on maximum eye temperature and reported that maximum eye temperature mimicked rectal temperature showing a strong correlation between the two measures. In those studies, eye temperature was 2°C (Stewart et al., 2007) and 4°C (Schaefer et al. 2003) lower than rectal temperature, which is similar to the present study although animals in the cited study were sick and not treated with a stressing factor other than handling at the yards. It has been suggested that IR temperature of the eye could successfully detect changes earlier in response to acute stress in cattle (Clerc & Gonzalez, 2012; Stewart et al., 2008) with this level of sensitivity in the eye proven to be useful in the early detection of diseases where changes in rectal temperature were more difficult to detect and generally appear later (Schaefer et al. 2003; Stewart et al., 2007). Therefore,

IRT of the eye could have both advantages and disadvantages compared to rectal temperature because it can detect effects not detected by rectal temperature but it can be affected by many factors. Ambient conditions have a large influence on the temperature measured (Clerc & Gonzalez, 2012) however we have not studied such effect in the present project. Instead, we have accounted for the effects of ambient conditions using date as a factor of analysis and normalising data by date using the median temperature. However, an operational system would require the development of methodologies or algorithms to account for environmental factors, particularly ambient temperature and perhaps solar radiation.

5.1.2 Effect of IR devices

There are a plethora of IR devices from different manufacturers in the market which are suitable for multiple applications having different characteristics and prices. The IR handheld camera C2 and i40 (~$2,000) and smartphone sensor (~$350) were the cheapest however it was difficult to obtain good still images due to the time required to save the image (~30 sec) and the movement of the head of the animal to capture images containing the eye open. In addition, these devices had low pixel resolution and lower thermal accuracy. In contrast, the FLIR T420 had the highest accuracy and image resolution (pixels) however it also had similar limitations when used as a handheld camera. Obtaining images using the smartphone IR sensor was easier compared to the handheld camera as it was faster to save image, even though it had a lower pixel resolution. Thus, the handheld camera may not be practical in some commercial settings, such as abattoirs, as it could cause hindrance to the abattoir staff or the animals due to the difficulty to obtain images for loose and fast moving animals. However, they could be suitable for rapid scanning of a group of animals where there is interest to pick the 'warmest' animal or determine if an animal with signs of disease or stress is hotter than group mates. Nevertheless, the FLIR T420 handheld camera was the closest to rectal temperature and therefore seems the most accurate indicator. There are several reasons to explain these results: 1) the handheld IR camera resulted in better focus and still images were of a superior quality in comparison to the video footage, 2) still images using the handheld camera were taken at a close distance of approximately 0.5 meters whilst the animal was stationary and immobilised using the head bail allowing for better focus and resolution.

In contrast, the video cameras were located at a longer distance (~3 meters) from the animal and set up on a 45° angle to allow for the whole body of the animals to be measured while animals walked through at varying speeds. The video footage in trial 1 at the farm had background interference (very hot surfaces due to sunlight on fixtures) that could have reduced the ability of the camera auto-focus to obtain clear images. However, this challenge could be easily solved with eye tracking software to automatically detect and track the eye while obtaining the radiometric information inside the eye. On the positive side, extracting still images from videos was easy because videos recorded many frames (e.g. 16 frames) per second ensuring that good frames can be obtained for analysis. In the second trial comparing IR devices to develop a method of data analysis, the C2 resulted in the lowest correlation with rectal temperature whereas the 3 video cameras used performed similarly with no real advantage of one over another.

In conclusion, video recordings are preferable from a practical and operational point of view in abattoirs while the smartphone IR sensor could potentially be of value as a low-cost handheld scanning system which could be used for personnel walking around lairage pens or other places in the abattoir. However, the IR sensor and the C2 still camera have a lower image pixel resolution and thermal accuracy, and therefore may not have the accuracy required to detect subtle differences in temperature from a large distance. However, more research is needed in this area to determine the optimal device for different applications and environmental conditions. All cameras have alarm systems that can be setup to notify (e.g. on-site veterinarian) when an animal's temperature has recorded above or below the normal threshold.

5.1.3 Effect of coat colour on IR temperature

Coat colour did not show a significant effect on IR temperature however all IR imagery in trial 1 were taken under the shade. The absorption of sunlight by darker coats could increase body surface temperature and this should eventually be accounted for because coat colour can affect the absorption rate of UV rays as a result of skin pigments of animals (Decampos, et al. 2013). Animals with black, dark brown and brown coats showed IRT to be the closest to rectal temperature as these colours are attributed to higher solar absorption (Gebremedhin, et al. 2008). However, there is no scientific determining if eye temperature is also affected by coat colour interacting with ambient conditions (temperature, humidity and solar radiation). McCafferty (2007) pointed out that humidity in the animal's coat can also modify surface emissivity and directly affect the temperature measured by IRT. Animals at commercial conditions are often wet (and washed) and this could affect emissivity values. Nevertheless, it is important to note that maximum IR temperature in that trial was not affected by coat colour in contrast to average temperature. This is an important outcome from a potential application point of view as this can allow the use of automated monitoring systems without the need to account for coat colour.

5.2 Data processing methods

The present project is the first study to use IRT video at high frequency to measure body temperature in cattle under commercial abattoir conditions. It has been demonstrated in the present project that the data is very noisy and needs to be processed before it can be used for analysis. The method developed in the present project consists of 1) extracting the maximum IRT within the region of interest, 2) calculating a rolling 1-second median to eliminate outliers of short duration within the region of interest, and 3) calculating quantiles or the maximum IRT temperature after smoothing with the rolling media method. Using these statistics has yielded a moderate correlation with rectal temperature, considering that rectal and surface temperatures may not always go together.

The large difference in IRT observed between poor and good quality images also demonstrate the need for extensive processing of the data. Therefore, one of the main challenges under commercial conditions is to obtain IRT imagery containing the eye of the animal for a few seconds and then to develop a method of analysis that can reduce the effect of the variability in temperature due to the animals moving the head in and out of the ROI.

5.2.1 Image processing method

Comparison between average and maximum IR temperatures within a region of interest (e.g. the eye) revealed that maximum temperature was significantly closer to rectal temperature compared to average IR temperature, in agreement with previous research (Clerc & Gonzalez, 2012; Hoffmann et al., 2013; Naas et al. 2014). Therefore, maximum IR temperature within a region of interest is preferred although others have used averages within the ROI or the proportion of pixels with a temperature above a pre-determined threshold (Tong et al., 1997). Calculating average IRT has the disadvantage that the ROI where temperature is measured has to be carefully and consistently defined (size and location) which could be a challenge when using this approach under different conditions and different cameras with different pixel resolution or size. However, average temperature may be of value for some applications such as for detecting injuries or tissue trauma in

parts of the body of the animal because it may allow obtaining a more accurate thermogram of large areas and detecting abnormal 'hotspots'. Anecdotal evidence was presented in the present report where rubbing of the neck with the headbail increased IR temperature of the skin in the neck, or with images after stunning the animals in the knocking box.

In addition, noise on the IRT data appears as 'troughs' of low temperature when the eye is not within the ROI which means that either the skin of the animal or the fixtures are being measured. These values with low temperature also need to be removed to calculate quantiles because these will affect the values obtained. The methodologies developed in this project using the 1-second rolling median and mixture distributions allows these noises to be removed. Then, the data can be processed to calculate the statistics for each animal such as the quantiles or absolute maximum temperatures.

In terms of deciding which quantiles perform the best, the 95% quantile seemed to be on average slightly better however, for the A310 the maximum 1-second rolling median temperature outperformed the others. It is important to note that on an operational system the quantiles would require to determine when an animal has the eye within the ROI (e.g. start and end points using the mixture distribution methodology) whereas the maximum 1-sec rolling median would not need this estimation. Therefore, there is trade-off between accuracy (95% quantile) and ease of application (maximum IRT).

Finally, the amount of time to measure IRT also affected the strength of the relationship between IRT and rectal temperature. The correlation coefficients increased when only those animals with at least 10 seconds of IRT data were used in the farm trials. Approximately half of the animals were lost when this threshold of a minimum of 10 seconds with the eye within the ROI was applied to the abattoir data in the mixing and stress trails under commercial conditions due to many animals staying within the ROI for shorter periods of time. Having animals within the ROI for 10 seconds or more at commercial abattoirs could prove difficult and reduce the applicability of the technology unless eye tracking software is developed and the VIR cameras are placed at a far distance from the animals. Nevertheless, maximum IRT resulted in better correlations with rectal temperature compared to 95 quantile and 10 seconds of data. The importance of the effect of time to obtain reliable IRT measurements was also confirmed in the Victorian trial where there was a significant relationship between IRT and the number of measurements per animal. In conclusion, the 95 quantile with a minimum of 10 seconds may be the most accurate method of data processing however the maximum is the most practical and applicable.

5.3 Using IRT to detect stress

The indicators of stress used in the present project include meat pH (dark cutters), blood composition and eating quality (cmq4). The large trail at the Victorian abattoir resulted in a low proportion of dark cutters (4.21%) making it more difficult to assess the relationship between IRT and meat pH whereas the second large mixing and stress trial resulted in 39.3, 3.8, 17.2 and 22.2% of dark cutters for animals transported by sea and not rested, transported by sea and rested, and transported by land and not rested, and transported by land and rested, respectively (kill 1, 2, 3 and 4, respectively for an overall incidence of 23%). Therefore, assessing the suitability of IRT to detect stress using meat pH may not be the best approach for some of these datasets where the incidence of dark cutters was low.

The incidence of dark cutting meat varies in Australia and depends on nutrition, location, animal handling, processing conditions and season (DPI, 2001). It is estimated that incidence of DFD on MSA cattle is 7%, however ranges from 1-16% in processing plants dependent of the standards in place and the feed type of the cattle presented (grass vs grain). Results from the trial in the Victorian

abattoir demonstrated that cattle were managed with high standards showing low levels of stress and reflected by the low proportion of individuals displaying cases of DFD. On site veterinarians advised that a maximum of 5 animals are identified each week for assessment prior to slaughter. This is a very small percentage to make a sound analysis of the suitability of IRT to detect animals under stress. In contrast, the mixing and stress trails in Tasmania showed incidence rates that can be considered high for cattle. The objective of these trails was to create increasing levels of stress to increase the incidence of dark cutters which seemed to have been achieved.

In the Victorian trial, there was no relationship between meat pH and IR surface temperature yet there was a statistical tendency that indicated females recorded higher eye temperatures than males (data not shown). This could be due to the fact that generally males are more resistant to stress than females (Van der Wal et al., 1999) and that their energetic metabolisms are slightly different (Guàrdia et al., 2010). However, this wasn't amplified in a difference in meat characteristics with no significant differences between sex in meat pH or colour, or fat colour. IR temperature was negatively correlated with marbling and MSA index. Furthermore, data from Victoria indicated that animals with darker meat had higher IR temperatures and higher meat pH. This indicates that IRT may have applications to predict meat quality attributes and perhaps to add value to or increase the accuracy of the MSA system.

In the Tasmanian mixing and stress trial, IR temperature was not significantly associated with higher meat pH ($r = 0.3$; $p = 0.20$). However, IRT measured at the race was positively associated with the acute phase protein haptoglobin on animals transported by sea and not rested before slaughter and with AST on animals transported by land and not rested before slaughter. Both of these blood constituents are known indicators of stress, disease, and tissue trauma and inflammation and therefore demonstrate that IRT can have a predictive value of animal stress (Sattler and Fürll, 2004; González et al., 2008; Earley et al., 2012). Interestingly, the largest correlation was found between IRT and muscle lactate ($r = -0.48$) which comes from the conversion of muscle glycogen post-mortem (Coombes et al., 2014). The fact that animals with higher IRT had lower muscle lactate may support the hypothesis that animals with higher IRT experienced more stress during transport which depleted muscle glycogen reducing the formation of lactate during meat maturation. However, IRT was not correlated to muscle glycogen and therefore this hypothesis should be taken with caution. At the knocking box, animals transported by sea and not rested before slaughter with higher IRT had higher concentration of NEFA which is another indicator of stress because it comes from the mobilisation of body fat reserves during periods of high energy demands as a result of stress (González et al., 2008). Finally, animals transported by land and not rested before slaughter showed a positive correlation between IRT and plasma glucose. Glucose concentrations increase as a result of the release of catecholamines during acute stress (Apple, Dikeman et al. 1995, Gerrard and Grant 2003, Knowles 2007), which has also been linked to increased body temperature (Pighin, Brown et al. 2014). In conclusion, IRT thermography at the time of slaughter did not seem to reflect the prolonged stress response that characterises animals with high meat pH post-mortem however it seemed to detect animals with indication of stress as measured by blood and muscle indicators such as haptoglobin, AST, NEFA and glucose. It is important to note that IRT showed significant correlations in those groups of animals that had the highest incidence of dark cutters.

The lack of a significant relationship between IR body temperature and meat pH could be due to several reasons which deserve further explorations. The main hypothesis is that meat pH may not be the best indicator of stress at a particular point in time when IRT is measured but an indicator of long-term stress. Animals may show low meat pH as a result of stress experienced on farm or during transport which disappears by the time body temperature is measured in the abattoir, although muscle glycogen may have already been depleted. In addition, body temperature is but one of the many indicators of stress yet is unlikely to be the sole gold standard. Using other indicators of stress together with IRT may increase the accuracy of the temperature measurement. For example, IRT has also been used to measure heart rate and respiration rate in humans and animals (Lewis et al.,

2011). Therefore, an IR camera could be used to extract 3 different indicators of stress, i.e. temperature, respiration rate and heart rate.

One of the most important findings of this project was the negative associations between IRT and eating quality (cmq4) and carcase traits (meat colour, marbling and MSA index) which indicate that IRT may have value to improve the prediction of eating quality of live animals. However, it is important to point out that these relationships are weak at present and improvements would have to be made for IRT to be of value in this regard. As previously mentioned, tracking software that can measure eye temperature for longer time periods, and respiration rate and heart rate could offer great value.

Results from the abattoir trials are encouraging because they show the potential for the IRT technology to measure temperature at very high speeds (up to 32 Hz) increasing data throughput for improved accuracy which is critical for animals moving fast in the race or unloading ramp. Video IR cameras with fast frame rates may be needed for these applications under commercial conditions.

6 Conclusions/recommendations

The present project was unique in the literature in that it used video infrared thermography to measure body temperature of cattle under commercial conditions for the detection of stress at abattoirs. The project developed a methodology for data analysis, an auto-drafter to segregate animals with high body temperature and assessed the relationships between IR surface temperature, meat quality and carcase traits. The methodology uses ocular thermography and extensive data processing to eliminate outliers and calculate statistics for each animal measured at high frequency from IR surveillance cameras. The data shows the potential for IRT to aid in detecting animals under stress as well as determining those animals with reduced eating quality under commercial conditions. However, the relationships between IR and indicators of stress were weak and improvements need to be made before this technology can be used under commercial conditions with high accuracy. Thermal measurements could become important in the prediction and early detection of ill-thrift animals and application of this would be employed widely across many different livestock enterprises.

The selection of the IRT device (video camera, photo camera or smartphone sensor) has a large influence on the measured temperature, with still images being closer to rectal temperature and requiring the animal to be still (e.g. using head bail). IR video cameras are more practical because the very high rate of data capture make them suitable for their use under commercial abattoir conditions with large number of animals which could result in low cost of monitoring per animal. The prototype automated system for the identification, detection and segregation of stressed or sick animals in the abattoir is unique in its kind and was proven to be feasible for operating at abattoirs.

This could lead to developing a tool to predict meat quality and automatically detect stressed or sick animals under commercial conditions. However, further development of video capture and processing techniques are necessary as well as refinement of techniques and hardware to increase the strength of the relationship between IRT and stress indicators. Approaches to achieve this include software for tracking the eyes of animals, adding value to IRT developing appropriate software to measure other indicators of stress from the same camera such as respiration rate and heart rate, and correcting for environmental factors such as ambient temperature and solar radiation. Overcoming these limitations can improve the prediction and early detection of compromised animals for applications across the livestock supply chain.

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