Final report

Jarvis 'Stealth Box' Validation

Evaluation of the new Jarvis 'Stealth Box' in improving animal movement into restraint

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1.0 Executive summary

Improving animal welfare in abattoirs is crucial, particularly with increasing public concern about meat production. A key stage in the process is handling of animals from lairage pens, along the lead in race to the knocking box. The movement of livestock and subsequently stress reduction at abattoirs can be improved by using new and less noisy operational systems. Jarvis Australia have developed a restraint unit incorporating a variety of noise-reduction strategies (e.g., silencer manifolds on actuators and valve exhausts; nylon lining inside the box and on the roll out cradle; rubber matting on the floor). Known as the ‘Stealth Box’, the unit is also fitted with the latest models of the Jarvis pneumatic and electrical stunning systems, which include stun monitoring processes as well as the ability to control stun input variables.

The aim of the present study was to (1) validate the new Jarvis ‘Stealth Box’ in improving animal movement into restraint, in terms of animal behaviour, meat quality and acoustic measurements; and (2) evaluate the feasibility of automating monitoring of animal handling through identification of acoustic signatures of specific sounds from audio recordings. A real-time acoustic monitoring system would be a highly effective way to facilitate automated animal surveillance, allowing processors to receive rapid updates on important events occurring within the processing system. This would also ease the burden of auditing and legislative requirements by providing detailed reports on animal welfare indicators. Video and audio recordings were collected at eight beef processing plants and annotated offline. Pre- and post-installation data were available for four plants, while post-installation data was available for all eight. Pre- and post-installation pH declines were obtained from three processors, while samples of striploin and eye round were collected pre- and post- installation from one processor and analysed for objective meat quality parameters (shear force, water holding capacity, colour and lipid oxidation).

For animal handling and behaviour, two separate analyses were conducted: one to compare pre- and post-installation data to evaluate the impact of the ‘Stealth Box’, and another using only post-installation data to compare the processors in terms of operational performance using the ‘Stealth Box’. Individual scores for behavioural and handling (aid and noise) interventions were measured to assess cattle welfare. Overall, there was a noticeable reduction in almost all parameters, with the exceptions of 'Back up' and 'Rush'. Statistical analysis confirmed that cattle were 1.74 times less likely to receive a handling aid post-installation compared to pre-installation (60% vs. 80%, respectively; $P < 0.01$). However, there was no reduction in handler noise, which might suggest that using the ‘Stealth Box’ shifted animal handling from physical methods to less stressful options such as whistling. The post-installation analysis showed that handling interventions varied significantly among processors ($P < 0.05$) indicating the operational effects on animal behaviour and welfare in abattoirs.

For meat quality and pH declines, the results indicated that there might be a slight improvement post ‘Stealth Box’ installation, but the sample size was small and the outcomes would have been strongly influenced by animal and processing factors.

From the acoustic analysis, it is highly likely that the acoustic environment has changed in the knock box and lead in race, indicating that there have been changes to animal and/or personnel behaviour.

In terms of automating monitoring via acoustic recording, there is a high level of variability between animals within processing groups, and between groups. Individual animals display different responses to stimuli experienced within the processing environment and may require differing levels of staff handling. Groups of animals arrive at the processing site from disparate locations, and their behaviour is largely based on their temperament and previous experiences, such as the level of human contact and handling they have been exposed to. These groups may require a higher level of staff intervention to successfully navigate the processing system. Staff members have individual preferences pertaining to animal handling and intervention. For example, some staff may whistle to keep animals moving, while others may use calls. These differences in staff behaviour will produce dissimilar acoustic environments as the types of sound events will change. Staff members can change over time, adding further variability to long-term data collection. There are numerous external factors that can also affect the acoustic environment, such as weather, seasonal conditions, and natural phenomena. Future studies should aim to collect more contiguous data over a longer period, to reduce the effect of variability on acoustic analysis. By gathering data from a wider range of processing sessions, a more accurate representation of the acoustic environment can be obtained.

Based on the results obtained from the behavioural analysis, we conclude that, while the 'Stealth Box' has improved animal movement into the race, variations between processors indicate that other components of each system may need to be replaced or reviewed to achieve further improvements in animal welfare. Meat quality and pH decline data indicate that beneficial effects may be present, but these are confounded by animal differences and other electrical inputs during processing. The processors who provided pH decline data were using percussive stunning – if electrical stunning were implemented, this would increase the electrical inputs to the carcase and increase the rate of pH decline.

An AI-based system capable of detecting and interpreting sounds of interest that may occur in a processing site is most certainly feasible, but significant research is required.

2.0 Introduction

Improving animal welfare in abattoirs is crucial, particularly with increasing public concern about meat production. A key stage in the process is handling of animals from lairage pens, along the lead in race to the knocking box. Beef cattle are exposed to a variety of stressors during the pre-slaughter period, such as transportation, handling, unfamiliar environments, feed and water deprivation (Cockram and Corley, 1991, Ferguson and Warner, 2008, Gallo et al., 2003, Grandin, 2020, Warriss, 1990). In addition to these factors, the operational systems of a slaughter plant can significantly influence how animals respond to pre-slaughter conditions. For example, modern commercial slaughter of cattle involves the use of restraint boxes to present the head of the animal for accurate placement of the stunning device. Restraint boxes constructed predominantly of concrete and steel can produce loud and unfamiliar noises during operation. Such noise is known to cause stress in animals, leading to baulking and requiring additional coercion to move them into the restraint. Distractions such as noise, darkness, and seeing people, can significantly impact their welfare (Bourguet et al., 2011b).

The movement of livestock and subsequently stress reduction at abattoirs can be improved by using new and less noisy operational systems. Jarvis Australia have developed a restraint unit incorporating a variety of noise-reduction strategies (e.g., silencer manifolds on actuators and valve exhausts; nylon lining inside the box and on the roll out cradle; rubber matting on the floor). Known as the 'Stealth Box', the unit is also fitted with the latest models of the Jarvis pneumatic and electrical stunning systems, which include stun monitoring processes as well as the ability to control stun input variables.

This study aimed to investigate:

- The ability of the 'Stealth Box' unit to facilitate animal handling and movement;
- The effects of using the 'Stealth Box' on meat quality;
- The effects of using the 'Stealth Box' on overall acoustic parameters in the animal handling phase;
- The potential for using acoustic recordings to monitor animal handling and therefore by proxy, welfare;
- Impacts of animal attributes and stun parameters on outcomes in terms of halal compliance and incidence of ecchymoses (blood splash).

3.0 Project objectives

- To validate the new Jarvis ‘Stealth Box’ in improving animal movement into restraint, in terms of animal behaviour, meat quality and acoustic measurements.
- To evaluate the feasibility of automating identification of acoustic signatures of specific sounds from audio recordings collected at the focal processor.

4.0 Methodology, Results and Interpretation

The data presented in this report pertain to eight participating processors. Data collection was as follows:

- Four processors provided data both prior to and after ‘Stealth Box’ installation. Percussive Stunning was in use at all times during data collection
- Four processors, three of which were greenfield sites on which new facilities were construced, provided post-installation data only. With the exception of one processor, where Electrical Stunning was in use, Percussive Stunning was in use during data collection.

Video and acoustic recordings of animal flow through the force pen, lead in race, knock box, and roll out areas were obtained before (n=4) and after (n=8) ‘Stealth Box’ installation. At the first processor enrolled in the study, live observations of animal behaviour were also conducted, and the combination of offline annotation and live observation data from this processor was used to refine the data collection approach for the remaining installations.

Pre- and post- installation pH decline data were collected from three processors.

Samples of striploin and eye round were collected from a single processor, before and after ‘Stealth Box’ installation and assessed for objective meat quality parameters.

Full details of the methods associated with each component of the study are given in the following sections.

4.1 Ethical Review

Data were collected under the authority of the CSIRO Armidale Animal Ethics Committee (AEC), reference ARA 22/05 (NSW, QLD and SA), and under the authority of the CSIRO Wildlife and Captive Large Animal AEC, reference 2022-44 (VIC), in accordance with the provisions of the Australian Code for the care and use of animals for scientific purposes (National Health and Medical Research Council, 2013). Human Ethics Approval (related to video capture) was granted by the CSIRO Social and Interdisciplinary Science Human Research Ethics Committee (CSSHREC) reference 159-22.

4.2 Behavioural analysis

4.2.1 Data

First Processor

Video recordings of animal flow through the force pen, lead in race, knock box, and roll out areas were obtained before, and on two occasions after ‘Stealth Box’ installation. On each occasion, data were collected over a 2-day period, with the target of 100 animals videoed on each day. Actual numbers videoed was influenced by processing flow on the day.

Live behaviour observations were carried out in the forcing pen and lead in race. Due to the rate of processing, it was not possible to follow individual animals, so behaviour of animals was scored on a group basis. Groups varied in size, depending on the flow of animals to the forcing pen and from the forcing pen into the race.

Data on pH and temperature declines were also collected, to represent the normal processing at this abattoir.

Other processors

Video recordings of animal flow through the force pen, lead in race, knock box, and roll out areas were obtained before and after 'Stealth Box' installation (3 processors), or after installation only (4 processors). On each occasion, data were collected over a 2-day period, with the target of 100 animals videoed on each day. Actual numbers videoed on each processing day was influenced by kill schedules.

The design of the force pen to roll out areas (Figure 1) differed at each site. At 3 processors, the walls of the pens and races were constructed of steel, in the others, concrete was used. One processor had wire mesh flooring; the others had grooved concrete. One had a straight lead in race, the others had curves of between 90 and 180 degrees on the race leading up to the entry to the box. All had a single-animal pen immediately before entry to the box itself. All processors needed to make some modifications to the lead in race and roll out areas to accommodate the 'Stealth Box', which was larger than their existing box. In some processors, this modification was reasonably minor, e.g. removal of a wall, or relocating a door or gate in the lead in race; in other processors, the entire lead in race was replaced and reconfigured. In those which undertook major reconfiguration of the lead in area, it is difficult to assign any changes in behaviour or handling to the 'Stealth Box' alone.

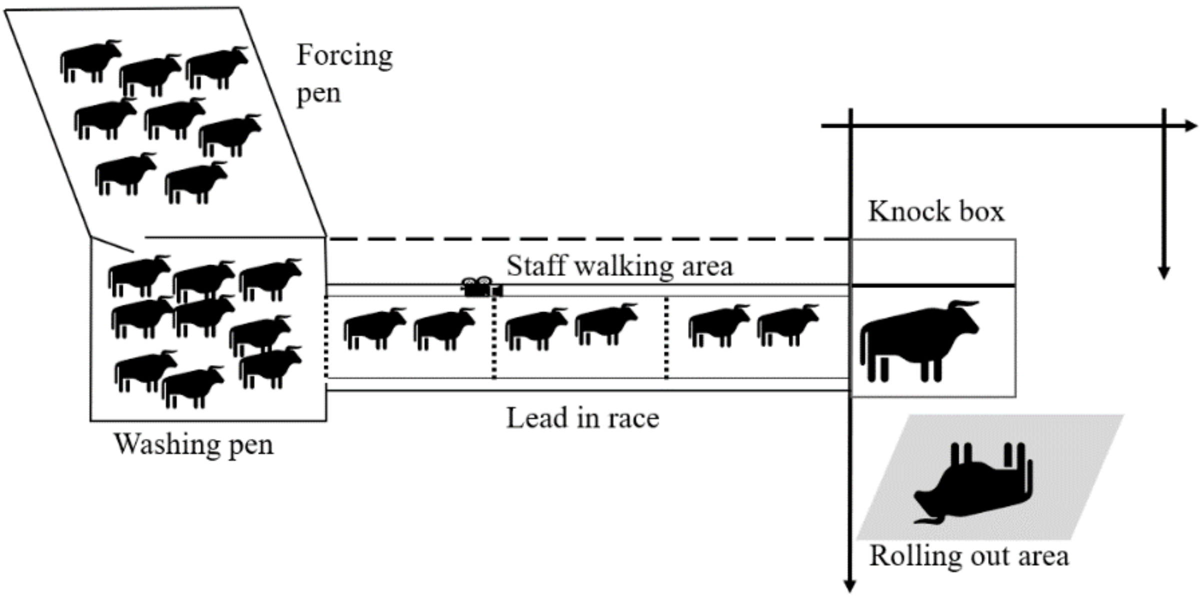


Figure 1: Schematic diagram of the force pen to roll out areas in a beef abattoir

4.2.2 Analysis

Analysis focused on the lead in race, with other footage being used to cross check against any unexpected noise or event, e.g. breaks in process flow, to assist in identification and classification of outliers. Matching video footage and audio recordings were imported into Noldus Observer XT12 (Noldus, Wageningen, the Netherlands, www.noldus.com), and handling events, animal behaviours and facility noises were annotated to a defined ethogram (Tables 1, 2 and 3). Events were annotated for a single focal animal from the point it entered the lead in race until it entered the stunning box, before beginning observations on a second animal. Annotations were as counts of each behaviour/handling intervention, with prolonged instances counted as a single bout (e.g., a continuous series of whistles without a break).

Table 1: Ethogram for behavioural annotation on video footage

Behaviour	Descriptor
Slip	<u>Loss of balance</u> with the shoulders or rump dropping, but the animal <u>stays on its feet</u> and the body doesn't touch the ground.
Rushes	The animal <u>suddenly moves forward rapidly</u> into an open space, usually from standing.
Mounts other animal	The focal animal jumps onto another animal's back, with its <u>forelimbs off the floor</u>. Forelimbs may be onto the back of the second animal, or clasping the second animal, with the brisket or base of the neck on the second animal's rump or back.
Mounted	Another animal jumps onto the focal animal's back, such that the <u>second animal has its forelimbs off the floor</u>. The second animal's forelimbs may be onto the back of the focal animal, or clasping the focal animal, with the brisket or base of the neck on the focal animal's rump or back.
Baulk	The animal stops suddenly and <u>refuses to move forward when there is space</u> (greater than 2 m) ahead of it.
Vocalisations	The animal performs <u>loud bellowing noises.</u> Subcategorised into: 'bellow in response to handling aid or restraint'; 'bouts of continuous mooing'; and 'low level murmuring while in restraint'.
Back up	The animal <u>reverses</u> in the race.
Turn attempt	The animal <u>turns its head</u> and <u>attempts to turn its shoulders</u> to face the opposite direction. Full 180 degree turn usually prevented by the walls of the race or box.

Table 2: Ethogram for handling intervention annotation on video footage

Handling intervention	Descriptor
Handling aid	The handler <u>uses a tool</u> to encourage the animal to move forward. Subcategorised into tool type: 'flapper', 'hand', 'cattle pole' or 'goad' (combined into a single metric 'stick electric jigger'); Each further subcategorised into: 'poke' – with the end of a stick or flapper; 'tap' – a light tap of the tool on the animal, with no pressure other than the weight of the tool, the tool is removed immediately; 'hold on animal' – the tool is laid on the animal with no pressure, but remains there; 'whack' – the tool is applied onto the animal with some force, usually audible; 'push' – prolonged pressure on the animal with the flat of the hand; and 'slap' – the flat of the hand is applied abruptly with some pressure to the animal and released immediately. May be audible.
Handling noise	The handler <u>makes a noise</u> to encourage the animal to move forward. Subcategorised into: 'whistle' – a high pitched sound formed using exhalation, lips and teeth; 'shout' – a loud, lower pitched sound formed using exhalation and the vocal cords; 'speak' – a lower pitched sound at conversational level, formed using exhalation and the vocal cords; 'cluck' – a short, sharp sound formed using the tongue and teeth, with no exhalation; 'scrape' – the handling tool is rubbed along the sides of the race or pen; and 'hit race' – the handling tool is tapped sharply on the sides of the race or pen.

Table 3: Ethogram for equipment or facility noise annotation from acoustic recordings

Noise	Descriptor
Air line hiss	A high-pitched susurration associated with air escaping as an air-driven actuator is released.
Gate door slam	A loud metallic 'clang' associated with a metal door closing abruptly and coming into contact with the frame or support.

Statistical analysis was performed in the R environment (R Core Team, 2021) to compare animals' behaviour, use of handling aids and use of handler noise for: 1) pre- and post-installation of the 'Stealth Box', and 2) post-installation of the 'Stealth Box' between processors. For both analyses, the data were edited to remove: 1) records with incomplete information (e.g., the animal was not visible for much of the time spent in the race), and 2) records with outliers. According to the nature of our data, we only removed extreme outliers based on a box plot and histogram plot for statistical analyses but retained them for descriptive statistics to ensure the integrity and representativeness of our data.

Four abattoirs provided complete data for pre- and post-installation of the ‘Stealth Box’, with data collected over two days (except for one processor, which collected data for one day post-installation). Most parameters for behavioural annotation were at low level both before and after the installation. Consequently, the statistical analysis was primarily focused on handling interventions- handling aid and handling noise. We conducted two separate analyses: one to compare animal handling in the new system versus the old system (Pre-post installation, four processors), and another to investigate differences between processors post-installation, focusing on the operational effect (Post installation- operational effect, eight processors).

Pre-post installation: The initial dataset for pre-post installation included 1,490 records; 791 for pre-installation and 699 for post-installation from four processors. For each intervention trait, the data was edited to remove the extreme outliers. For instance, four observations were removed for handling aid used (1 pre, 3 post), and three for handler noise (1 pre, 2 post).

A chi-square test was used to evaluate whether there was an association between phases (pre- and post-installation) and the animal’s behaviour and/or handling interventions. For this purpose, it was considered that the observed focal animal performed that behaviour, or received that handling intervention, while being moved along the race towards the knocking box. The “associationTest” function of “lsr” package in R was used to compare the observed with expected results.

Based on the nature of the data as count data, a Poisson regression model often fits. However, there were two challenges: 1) zero inflation, which might require a zero-inflated model, and 2) overdispersion, where the variance was greater than the mean and didn’t meet the Poisson regression standards. According to Lee et al. (2023), an outcome is defined as zero-inflated if more than 60% of counts are 0 and the outcome is overdispersed, which refers to any data in which the variance exceeds the mean. The former was not true with our data, with a proportion of zeros of 29% and 53% for handling aid and handling noise for pre- and post-installation data, respectively.

To address the overdispersion, we used a negative binomial regression model (“glm.nb” function of the "MASS" package) which had also a lower AIC compared to a generalized linear model with Poisson distribution (“glm” function in R) (8511.62 vs. 6293.38 for handling aid). The included effects in the model were treatment (pre- and post-installation), processors (1, …,4), and their interaction. Comparisons are presented based on the original scale, i.e., frequency for chi-square and means ± standard deviation for the glm model. An acceptable significant difference in the negative binomial regression was assumed when $P < 0.05$.

To estimate the correlation coefficient between handling aid used and handler noise, Spearman's rank correlation was used. This non-parametric test does not carry any assumptions about the distribution of the data and is appropriate for correlation analysis when the variables are measured on a scale that is at least ordinal. The “spearman_corr” function in R was used to estimate the correlation, and “ggplot” was used to plot the association between the variables. The correlation was estimated separately for pre- and post-installation, as well as for the combined pre-post installation data.

Post installation- operational effect: For the post-installation analysis, data from eight processors were available, but one processor was excluded (due to unrelated events in the lairage leading to abnormal data post-installation - see Figure 15 below). The total records were initially 1,100. After removing outliers, 1,091 records remained for handling aid, and 1,089 records for handling noise. A negative binomial regression model (“glm.nb” function of the "MASS" package) was applied to analyze the data, with the only effect included in the model being the processor effect (1, ...,7). A post-hoc Tukey test (“emmeans” function of "emmeans" package) was conducted to compare contrasts between processors. An acceptable significant difference was assumed when $P < 0.05$.

4.2.3 Results

Combined analysis of all processors with both pre- and post-installation data

Behavioural annotation of video footage in the lead in race

When the data from all four processors were combined, there were 1490 records for pre- (791 records) and post-installation (699 records). For each parameter, the data was edited to remove the extreme outliers. Four observations were removed for handling aid used (1 pre, 3 post), and three for handler noise (1 pre, 2 post) (Figure 2).

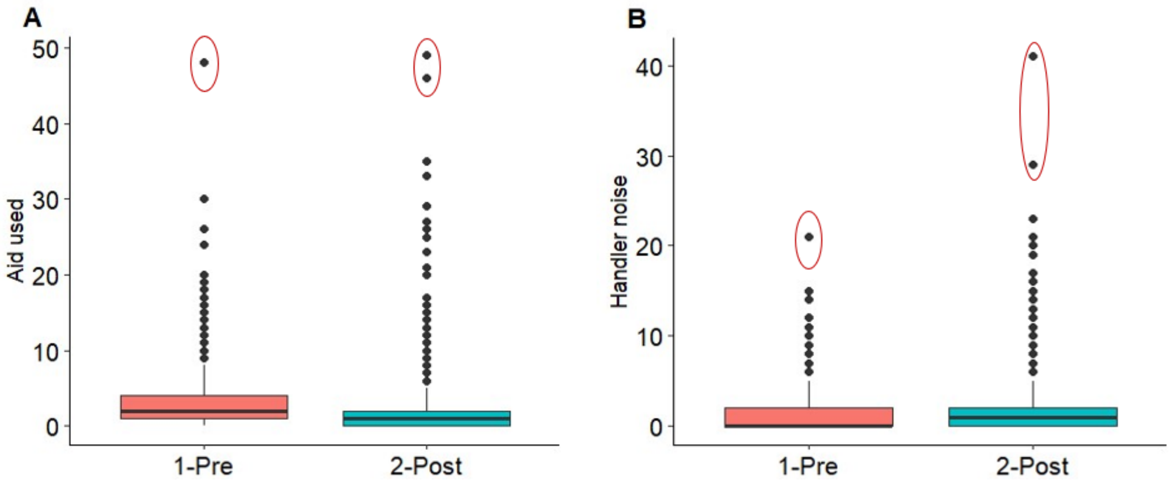


Figure 2: Data distributions for A) Handling aid used and B) Handler noise, pre- and post-installation of the 'Stealth Box' (all processors combined). Outliers that were removed for analysis are circled.

The differences between each observed parameter pre- and post-installation are shown in Figure 3. In general, there was an overall reduction in all parameters scored, with the exception of 'Hand push', 'Back up' and 'Whistle'. Animal behaviours 'Rush', 'Mount', 'Slip' 'Baulk', 'Vocalisation' and 'Check' were at a low level both pre- and post-installation, as were 'handler noise Cluck', 'Air-line Hiss', 'Gate door slam' and 'Scraping aid on race walls'.

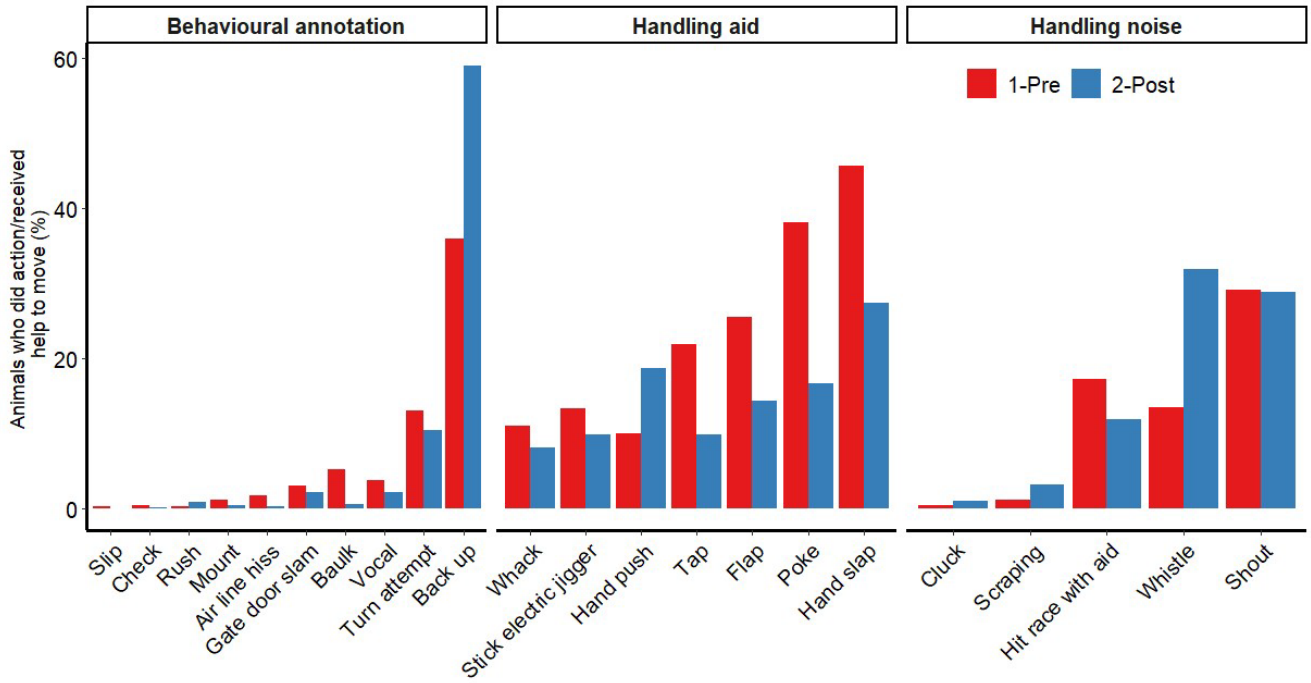


Figure 3: Differences in each observed parameter pre- and post-installation of the 'Stealth Box' (all processors combined).

Animal behaviour parameters

As shown in Figure 3, nearly all parameters for animal behaviour had values close to zero. Due to the lack of variation in the data, conducting meaningful statistical analysis was not feasible, as it would not yield reliable or interpretable results. Therefore, we restricted the statistical analysis to the 'Back up' category only. For this purpose, a negative binomial regression model was used. In total there were 1490 records from which 4 records (2- pre, and 2 for post-installation) were detected as outliers and removed from the dataset. The included effects in the model were treatment (pre- and post-installation), processors (1,…,4), and their interaction.

Figure 4 presents the results for back up behaviour of pre-post installation for all four processors. The analysis showed that back up significantly ($P < 0.001$) increased post-installation compared with pre-installation (Figure 4A). In addition, back up varied significantly ($P < 0.001$) between different processors with increased post-installation in three processors, but not in processor 2 in which back-up was decreased (Figure 4B).

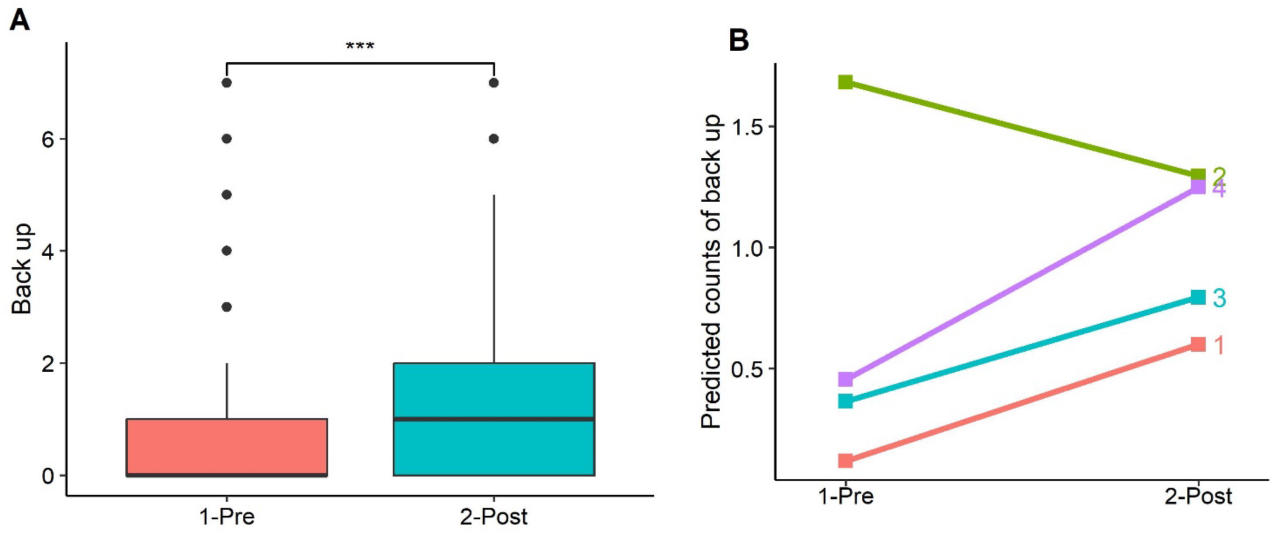


Figure 4: Comparison of the effect of A) trait (pre- and post-installation), and B) individual processor results of back up.

Handling interventions

Figure 5 shows the distribution of records with handling interventions (handler noise or handling aid) – the vast majority of animals received less than 2 interventions.

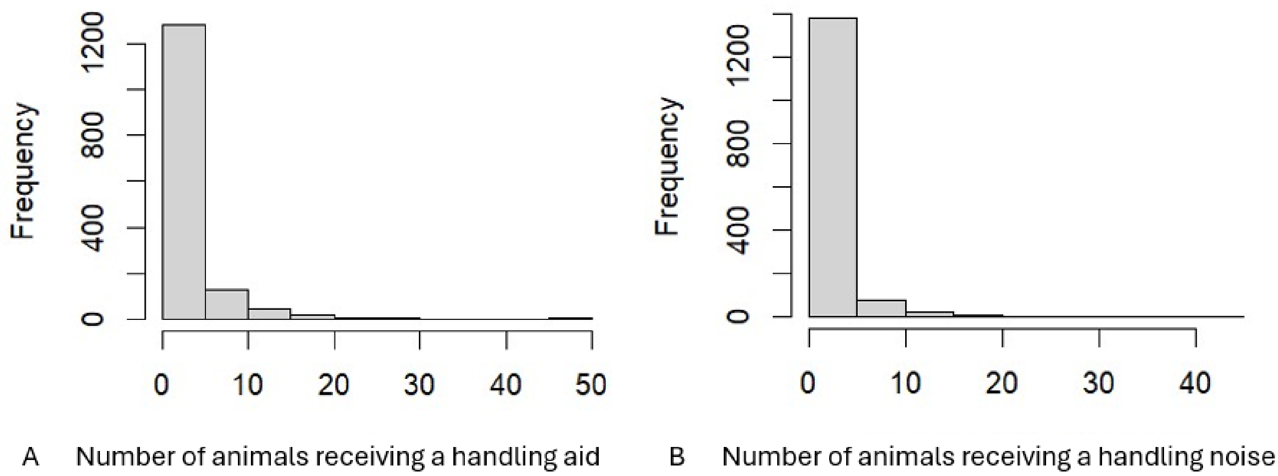


Figure 5: Distribution plots of A) Handling aid used and B) Handler noise in the lead in race to the knocking box (all processors combined).

A Chi-square test was used to evaluate whether there was an association between pre- and post-installation and the proportion of animals receiving each handling intervention. The null hypothesis tested was that variables are independent of one another. According to the Chi-square test, there was a significant reduction in the proportion of animals that experienced use of handling aid (P < 0.001), and also in the proportion of animals that experienced handler noise (P = 0.002) (Table 4).

Table 4: Results of Chi-square test for aid used and handler noise (all processors combined).

Phase	Aid used			Handler noise		
	Number of animals that did not receive a handling aid	Number of animals on which a handling aid was used	P-value	Number of animals that did not receive a handler noise	Number of animals on which handler noise was used	P-value
Pre-installation	157	633	<0.001	450	340	0.002
Post-installation	284	412		341	356	

A Poisson regression using glm function was used to compare the total number of each parameter counted (acknowledging that a single animal could receive multiple instances/types of handling aid or handler noise). Comparisons are presented based on the original scale without transformation, and statistical significance was considered when P < 0.05. This showed a significant reduction in use of handling aids (P < 0.001) but no significant change in handler noise post-installation as compared with pre-installation (Figure 6).

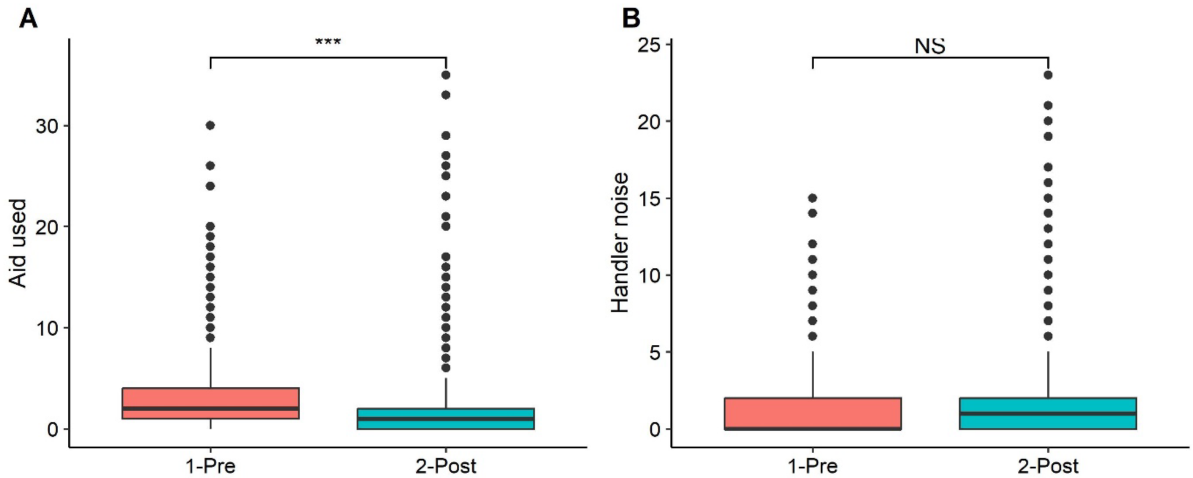


*Figure 6: Results of generalized linear model with Poisson distribution (Poisson regression) for A) Handling aid used and B) Handler noise for pre- and post-installation of the 'Stealth Box' (all processors combined). ***P < 0.001; NS – not significant.*

At one processor, on the data collection days post-installation, there was a significant increase in use of handling aids and handler noise, and a significant increase in animals backing up or baulking (processor 4 in Figure 7). Further detailed assessment of the recordings indicated that there was a noisy leaking pneumatic cylinder neck seal, associated with actuation of the gate between the pre knock box and race. This noise may have contributed to the backing up and concomitant frustration in the handlers, leading to increased use of handling aids. This issue has been addressed, and the team at the abattoir are monitoring the situation. It is possible that if these data were removed from the analysis, the positive benefits of the 'Stealth Box' on animal handling would be more obvious.

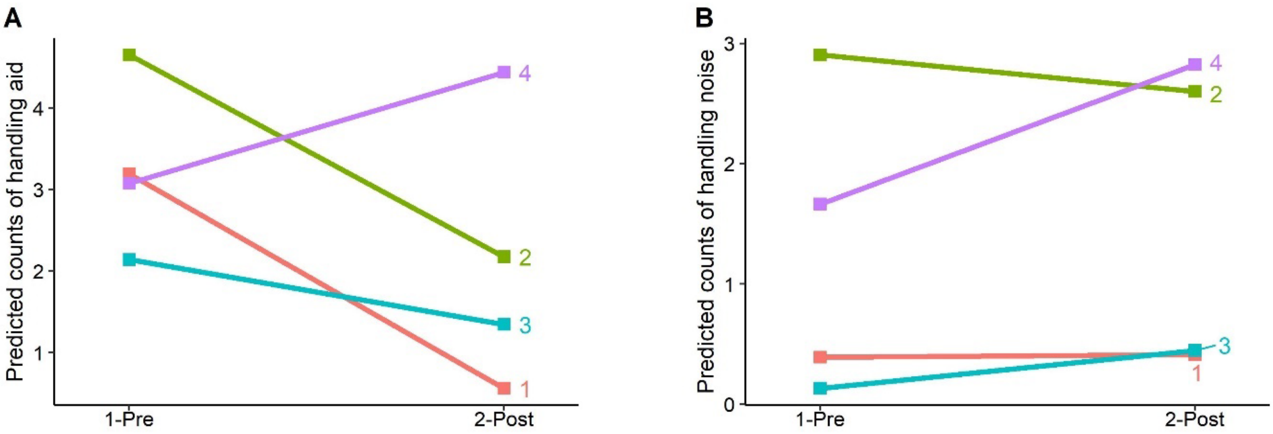


Figure 7: Comparison of individual processor results of generalized linear model with Poisson distribution (Poisson regression) for A) Handling aid used and B) Handler noise for pre- and post-installation of the 'Stealth Box'.

Processors with only post-install data: assessing the fit of those data with the post-install data from the other processors

The processor in which there was a significant increase in use of handling aids and handler noise, and a significant increase in animals backing up or baulking after 'Stealth Box' installation (likely due to noisy leaking pneumatic cylinder neck seal, associated with actuation of the gate between the pre knock box and race) was excluded from this analysis, because those data were considered to be not representative of the normal situation at the facility.

Figure 8 shows the comparison of (A) use of handling aid and (B) use of handler noise across seven processors in which the 'Stealth Box' had been installed. The analysis shows that handling interventions varied significantly between different processors and operational system had a significant effect ($P < 0.001$) on handling interventions.

Although not formally analysed, facilities with shorter straight sections into the single pen (i.e. the curve in the race ended close to the entrance to the single pen) generated lower counts of use of handling aid or handler noise (processors 1, 3, 4 and 6 in Figure 8). Furthermore, the staff member performing video annotation reported that in any of the facilities, animal handler had a visible impact on animal flow: when handlers stood back from the race and moved quietly and calmly, cattle moved freely; when handlers moved quickly, close to the race or 'interacted' more with the animals, cattle flow was impeded.

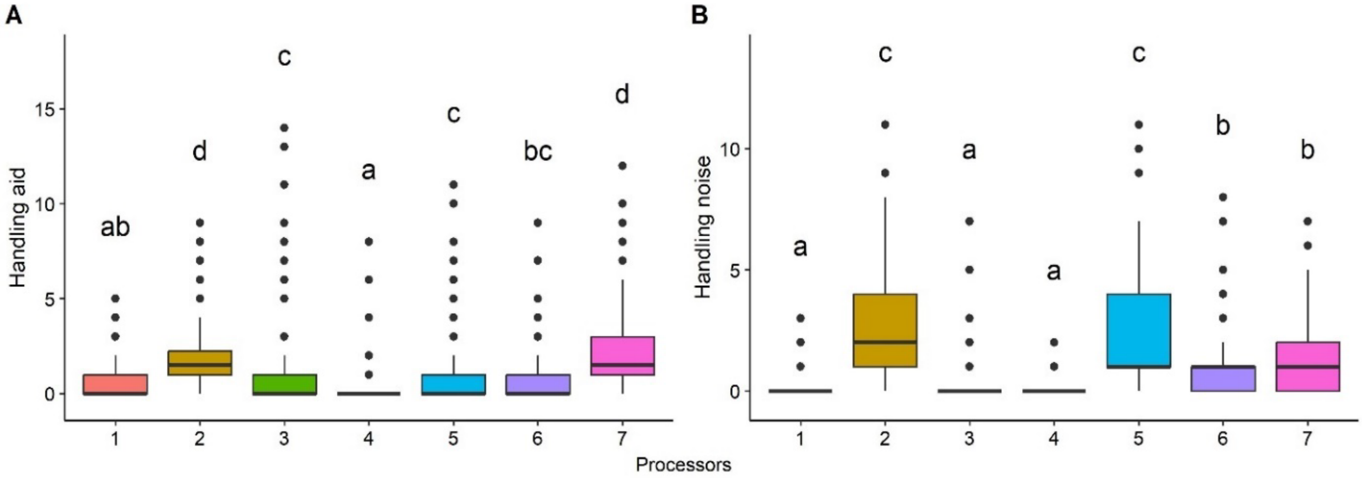


Figure 8: Results of post-installation analysis for A) Handling aid, and B) Handling noise across seven studied processors. Note: Processors sharing the same lowercase letters are not significantly different (P > 0.05).

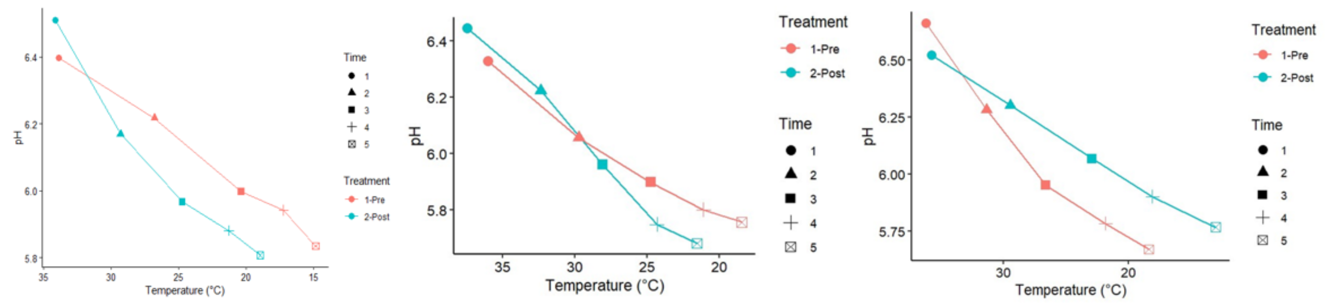
4.3 pH declines

Three processors supplied pH decline data. pH decline data were collected by CSIRO staff, from 20 carcases pre- and 20 carcases post-installation at 2 processors, while data for 60 carcases pre- and 60 carcases post-installation, based on their regular Meat Standards Australia (MSA) grading activities, were supplied by the third. CSIRO collected pH declines were measured using a TPS WP-80 pH meter with a polypropylene spear-type gel electrode (IJ 44) and temperature probe (TPS Pty Ltd, Brisbane, QLD, Australia). The probes were inserted into the *longissimus lumborum* between the 2nd and 5th lumbar vertebrae and the pH and temperature was recorded. The first reading was taken as the carcases entered the chiller and then subsequently at hourly intervals afterwards over a four-hour window. Calibration was performed using pH 4.00 and pH 7.00 buffers before each hourly reading.

Although the data indicated that carcases pass through pH6 during the optimal window (15-30°C) both pre- and post- "Stealth Box' installation, the three data sets revealed distinctly different pH decline outcomes.

In one data set, following 'Stealth Box' installation, carcase pH appeared to fall more rapidly relative to temperature than prior to 'Stealth Box' installation (Figure 9A), suggesting that the postmortem metabolic rate was faster in Post- 'Stealth Box' installation than under the previous system. It may be that use of the immobiliser to facilitate shackling has added to the electrical inputs post installation; or there may have been underlying animal or other processing parameter differences that have not been recorded and considered. Similarly, in the second data set, following 'Stealth Box' installation, there was a slight increase in the rate of decline in carcase pH relative to temperature compared to prior to 'Stealth Box' installation (Figure 9B).

In the third data set, following 'Stealth Box' installation, the rate of decline in carcase pH relative to temperature was slower than prior to 'Stealth Box' installation (Figure 9C), suggesting that, for this processor, the postmortem metabolic rate may be slower in Post- 'Stealth Box' installation than under the previous system. This could be a result of the animal being calmer and less agitated at the knocking box; or there may have been underlying animal or other processing parameter differences that have not been recorded and considered.



A: Data set 1　　B: Data set 2　　C: Data set 3

Figure 9: pH-temperature declines prior to (1-Pre) and after (2-Post) 'Stealth Box' installation. A: first data set, B: second data set; C: third data set. Hourly timepoint measurements are indicated using different symbols on each line.

4.4 Meat Quality

Samples of eye round (*m. semitendinosus*) and striploin (*m. longissimus lumborum*) were collected at a single processor prior to and after 'Stealth Box' installation. Samples were collected randomly from the normal intake at the abattoir. Ten carcases were sampled on each occasion (Dentition 0 – 4 tooth, HSCW 359.4 – 445.8 kg). Primals were harvested at boning, 24 h after slaughter, vacuum packed and supplied to the CSIRO laboratory, where they were stored for 2 weeks at -0.5°± 1°C prior to analysis (methods described below). Data were analysed by t-test comparison of means, with statistical significance assumed at P < 0.05.

4.4.1 Texture measurement and cook loss

Samples with dimensions 5 x 10 x 3 cm (l x w x d) were taken from the centre of each primal and cooked in a 75 °C water bath for 41 minutes to an internal temperature of 72 °C. After cooking, samples were immediately cooled by plunging into an ice bath for 20 mins. The cook loss was calculated as the difference in weight between raw and cooked samples, presented as a percentage of the initial weight. Samples were stored overnight at 4 °C prior to texture analysis.

Texture measurements were carried out using a Lloyd LS 2.5 with a 500 N load cell (Lloyd Instruments, West Sussex, United Kingdom) and a modified Warner-Bratzler shear device (Bouton and Harris, 1972). The samples were brought to room temperature prior to being cut into rectangular shapes (15 mm x 6.7 mm, giving a cross-sectional area of 1 cm2) and at least 25 mm long to enable secure clamping of the sample into the holder. A straight blade with a thickness of 0.64 mm was attached to an overhead clamp and pulled up through the muscle fibres, perpendicular to the fibre direction, at a speed of 100 mm/min. The maximum peak force (PF) and initial yield (IY) were determined using Nexygen Plus V3.0 software (Lloyd Instruments, West Sussex, United Kingdom). The difference between these measurements (PF-IY) was also reported. At least six measurements were made on each sample and the mean recorded.

There were no significant differences in cook loss before and after 'Stealth Box' installation in either eye rounds (Figure 10A) or striploins (Figure 10B). The values for cook loss obtained in the current study (25 – 33 %) align with previously reported data: for striploin, 33 – 35 % cook loss was reported by Warner et al. (2007), while Yancey et al. (2011) reported 26 – 32 % and Schönfeldt et al. (2011) reported 23 – 25 % cooking loss for striploin and 29 – 32 % for round.

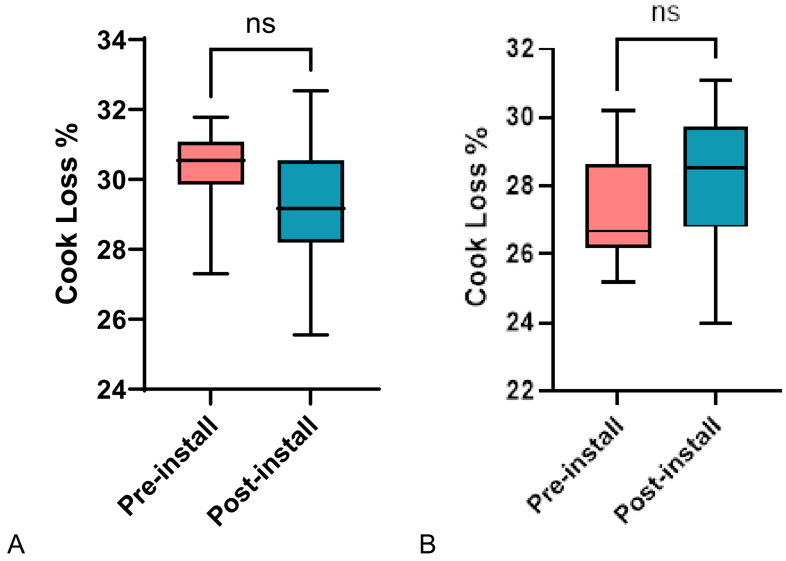


Figure 10: Cook loss prior to (Pre) and after (Post) 'Stealth Box' installation in (A) eye rounds (m. semitendinosus) and (B) striploin (m. longissimus lumborum).

For eye round (*m. semitendinosus*), there was a significant (P < 0.05) reduction in peak force after 'Stealth Box' installation (Figure 11A). The range of values around initial yield increased, while the mean decreased, but this was not statistically significant (Figure 11B). There was no significant difference in PF-IY before or after 'Stealth Box' installation (Figure 11C).

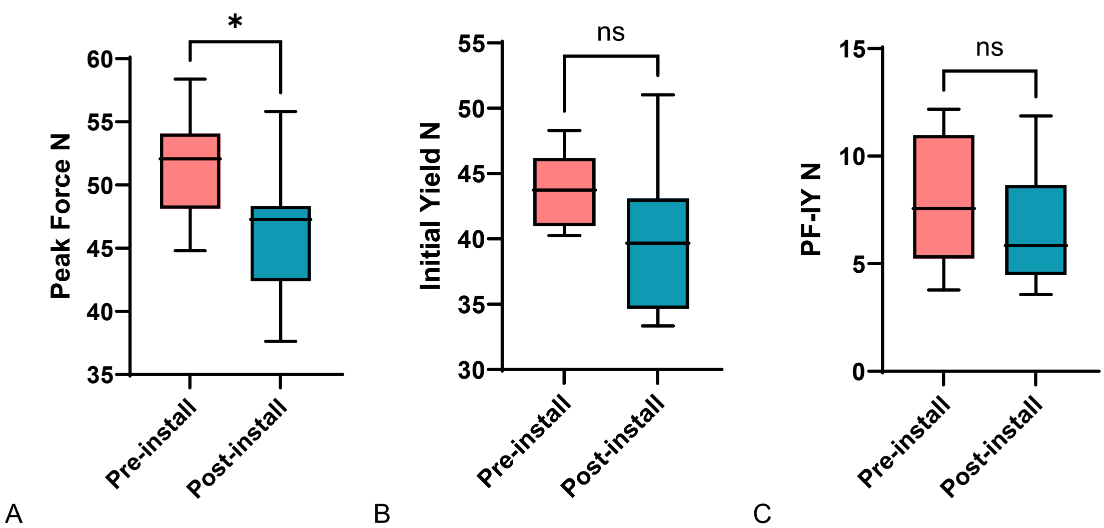


Figure 11: (A) Peak force, (B) Initial Yield and (C) PF-IY for eye round (m. semitendinosus) prior to (Pre) and after (Post) 'Stealth Box' installation.

For striploins (*m. longissimus lumborum*), Peak force, Initial Yield and PF-IY post installation was not significantly different from prior to installation (Figure 12).

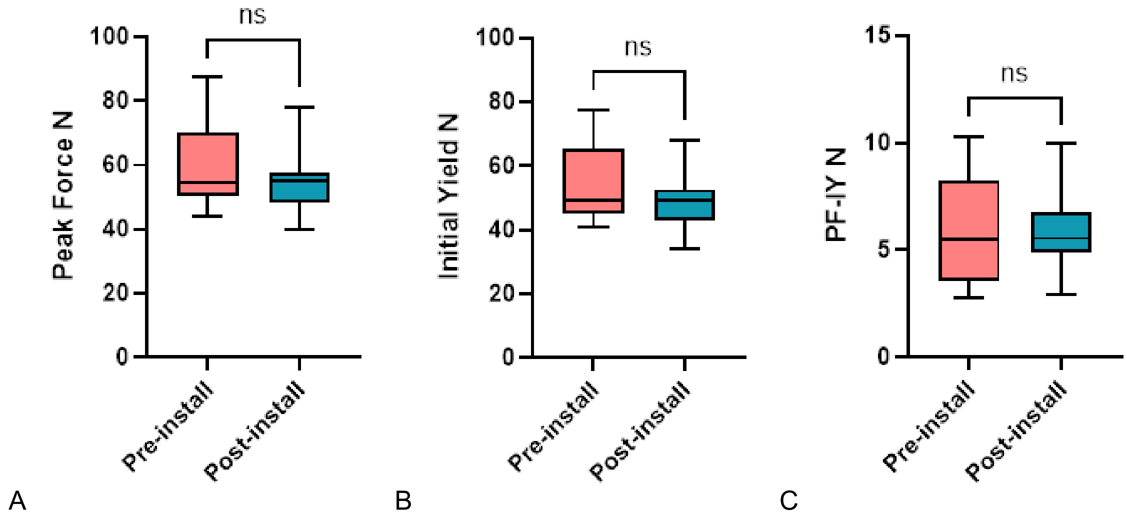


Figure 12: (A) Peak force, (B) Initial Yield and (C) PF-IY for striploin (m. longissimus lumborum) prior to (Pre) and after (Post) 'Stealth Box' installation.

The results obtained in the current study lie within normal ranges: for striploin, Warner et al. (2007) report peak force values of around 68 N at 6 days post slaughter, and 47 N at 21 days post slaughter; Gruber et al. (2010) report a range of 35 – 51 N measured over a range of ageing periods from 3 to 28 days; while Sazili et al. (2013) report 80 – 110 N at one week post slaughter and Holman et al (2019) report 26 – 29 N from 2 – 12 weeks aging under vacuum. For rounds, previously published ranges are, for example, 40 – 180 N (Odusanya and Okubanjo, 1983), 40 – 68 N (Otremba et al., 1999) and 46 – 95 N (Hwang et al., 2004).

4.4.2 pH

The pH of the samples was measured using a TPS WP-80 pH meter with a polypropylene spear-type gel electrode (IJ 44) and temperature probe (TPS Pty Ltd, Brisbane, QLD, Australia). The probes were inserted into 25 mm steak samples cut from each primal and the pH was recorded. Calibration was performed using pH 4.00 and pH 7.00 buffers equilibrated to the sample temperature (10 °C).

pH was not significantly different in eye rounds prior to and after 'Stealth Box' installation (Table 5), but for striploins, pH was significantly lower ($P < 0.001$) after 'Stealth Box' installation than before (Table 6). A limitation of the current study is that hot carcase glycogen levels were not assessed, so the findings could be confounded by differences in available initial glycogen levels in the animals processed. Differences between the animals processed could be accentuated due to the small sample size (n = 10 in each group)

Table 5: pH and Colour measurement data for eye round (m. semitendinosus) prior to (Pre) and after (Post) installation of the 'Stealth Box'.

Muscle	Timepoint	pH	L* (lightness)	a* (redness)	b* (yellowness)
Eye round	Pre-install	5.48 ± 0.03	47.88 ± 2.79	33.79 ± 1.30	29.72 ± 1.11
	Post install	5.46 ± 0.03	49.56 ± 0.92	33.30 ± 1.01	29.51 ± 0.77
	p-value	ns	ns	ns	ns

Table 6: pH and Colour measurement data for striploin (m. longissimus lubmorum) prior to (Pre) and after (Post) installation of the 'Stealth Box'.

Muscle	Timepoint	pH	L* (lightness)	a* (redness)	b* (yellowness)
Striploin	Pre-install	5.57 ± 0.05	40.09 ± 3.72	33.35 ± 0.90	26.19 ± 1.09
	Post install	5.48 ± 0.03	41.50 ± 2.29	33.55 ± 0.95	27.07 ± 1.03
	p-value	0.0003	ns	ns	ns

4.4.3 Colour

Objective colour measurements were made on the inside cut surface of 25 mm thick steaks taken from the middle of each primal sample after blooming for 60 ± 10 min at 10 °C. A Hunterlab Miniscan EZ 45/0 LAV (light source A, observer angle 10°, 25 mm viewed area) was used to measure L* (lightness), a* (redness) and b* (yellowness) attributes, in triplicate. The instrument was calibrated using white and black calibration tiles, as supplied with the instrument (Novasys group Pty Ltd, Ferntree Gully, VIC, Australia), at the same temperature as the samples (~10 °C).

There were no significant differences in colour measurements before and after 'Stealth Box' installation in either eye rounds (Table 5) or Striploins (Table 6). It is difficult to draw comparison with previously published data: the actual values generated in each study are influenced by many instrument and environmental variables. Nevertheless, the data generated in the current study are reasonably aligned with data from previously published data on Hunterlab colorimetry measurements of beef after 1 hr blooming (Table 7).

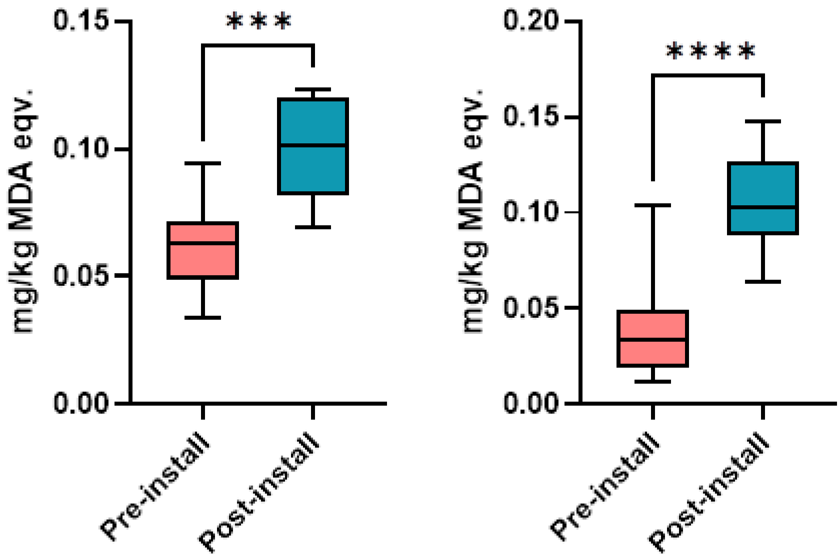
Table 7: Previously published data on Hunterlab colorimetry of striploin and eye round.

Parameter	Reported range	Reference
L*	31 – 47 striploin	English et al, (2016)
	33 – 39 cube roll	Hughes et al. (2023)
a*	24 – 28 cube roll	Hughes et al. (2023)
b*	15 – 20 cube roll	Hughes et al. (2023)

4.4.4 Lipid Oxidation

Lipid stability was determined by the thiobarbituric acid reactive substances (TBARs) assay as per Witte et al. (1970), with modifications. Samples of diced striploin and eye round were ground in an Oskar food processor and 2 ± 0.10 g were weighed into scintillation vials and capped. Samples were cooked in a 75°C water bath for 20 min and allowed to cool at 5°C for 30 min before extraction. Samples were homogenized on ice in 6 mL of chilled TCA solution (7.5% trichloroacetic acid, 0.1% propyl gallate and 0.1% EDTA) using an Ultra Turrax for 30 s at 13 000 rpm. The homogenate was filtered and rinsed with an additional 2 mL of TCA solution. Aliquots (2.5 mL) of the filtrate were transferred, in duplicate, to test tubes, diluted with 2.5 mL distilled water and reacted for 16 h with 5 mL of 0.02M thiobarbituric acid solution at room temperature, in a dark cupboard. The absorbance of the resulting solutions were read at 532 nm on a visible light spectrophotometer (UV5, Mettler Toledo) and the TBARs value as mg/kg malondialdeyde (MDA) equivalents was determined against a standard curve prepared form 1,1,3,3-tetraethoxypropane. Samples collected at each storage point were stored at -80°C before analysis.

TBARs values were very low (for example, values of 0.99 – 5 mg/kg MDA have been reported for vacuum-packed striploin after 14 days' aging (Bonny et al., 2017, Holman et al., 2019)), and the apparent increase in TBARs post installation of the 'Stealth Box' (Figure 13) should be interpreted with caution. Lipid oxidation is affected by the feedbase on which animals were raised (Mitsumoto et al., 1998, Faustman et al., 1989), age, distance transported (De la Fuente et al., 2010, Zhong et al., 2011), as well as other factors, and the origin, breed and sex of carcases sampled at each time point were not standardised. Differences between the animals processed could be accentuated due to the small sample size (n = 10 in each group).



A B

*Figure 13: Thiobarbituric acid reactive substances (TBARS mg/kg MDA eqv.) prior to (Pre) and after (Post) 'Stealth Box' installation in (A) eye rounds (m. semitendinosus) and (B) striploin (m. longissimus lumborum). *** P < 0.001; **** P < 0.0001*

4.4.5 Expressible moisture

Samples were prepared from each muscle sample slicing strips with the fibre direction into rectangular pieces (approx. 25x10x10 mm), each weighing 3.0 g (± 0.2 g), in triplicate. Strips of filter paper 175 mm long and approximately 15 mm wide were cut from Whatman No. 1 filter paper rounds (24 cm diameter). The sample pieces were then placed in the middle of a strip of filter paper, and the filter paper folded over so that the sample is resting in a sling formation. The sample and doubled-over filter paper were then placed into a 50 mL Falcon tube and the lid replaced.

Samples were centrifuged in the Sigma centrifuge at 5000 g for 15 min at 18°C. Immediately after centrifugation, the samples were removed from the tubes, weighed and the EM was calculated as follows where Wi is the initial sample weight and Wf is the final weight of the sample.

Equation 1. Expressible moisture calculation whereby Wi is the initial sample weight (g) and Wf is the final weight (g) after centrifugation.

$$\mathrm{EM}\ (\%) = \frac{(\mathrm{W}i - \mathrm{W}f)}{\mathrm{W}i} \mathrm{x}\ 100$$

For eye rounds, expressible moisture was significantly lower ($P < 0.001$) post installation than pre-installation (Figure 14A), whereas for striploins there were no significant differences (Figure 14B). Lower expressible moisture means increased water-holding capacity, so less purge would be expected during storage.

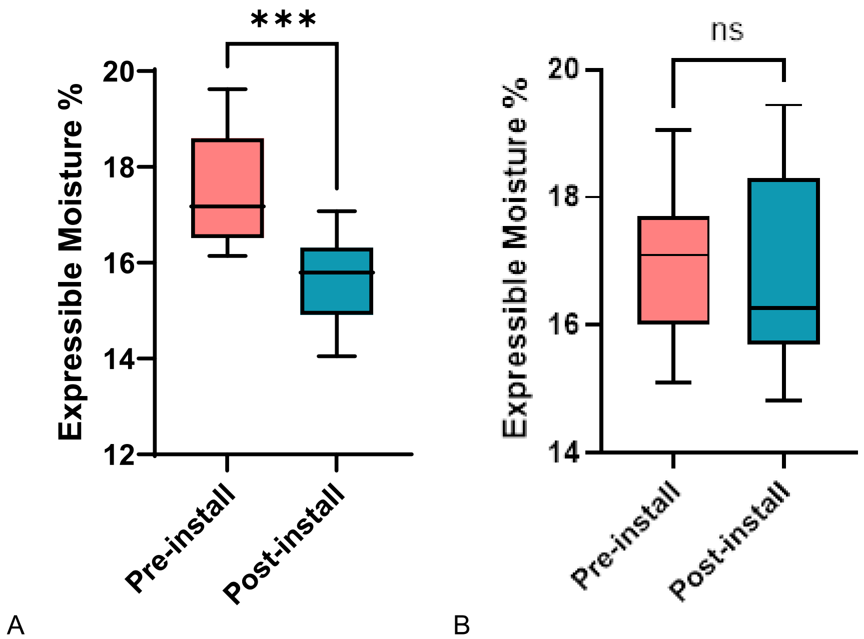


*Figure 14: Expressible moisture (%) prior to (Pre) and after (Post) 'Stealth Box' installation in (A) eye rounds (m. semitendinosus) and (B) striploin (m. longissimus lumborum). *** P < 0.001; ns – not significant.*

4.5 Forensic acoustic analysis

4.5.1 Aims

This analysis aims to assess the acoustic environment of meat processing sites before and after 'Stealth Box' installation. Using both acoustic energy and sound event-based metrics, the effect of 'Stealth Box' on overall sound activity is considered. Through the analysis of sound events that occur within a processing facility, the feasibility of an AI-based monitoring system is also explored. A real-time acoustic monitoring system would be a highly effective way to facilitate automated animal surveillance, allowing processors to receive rapid updates on important events occurring within the processing system. This would also ease the burden of auditing and legislative requirements by providing detailed reports on animal welfare indicators.

4.5.2 Acoustic Data

Audio recordings were obtained before and after 'Stealth Box' installation from four processors, while post-installation sound data was collected from a further four processors. At each site, a 2-channel recording unit was placed in the force pen, lead in race, knock box, and roll out.

Each recording unit contained 2 microphones which were directed at different areas of each processing location. Through both inspection and statistical analysis, it was found that each channel of audio data was significantly different enough to be treated as a separate data stream.

Analysis was performed on a per-location basis (e.g., only force pen data pre- and post- installation were compared), and a per-channel basis (i.e., the same channel was compared for each location).

4.5.3 Assessing Acoustic Activity

The question of how *loud* something is can be quite complex. Given that objective decibel sound pressure level (dB SPL) values are not available, true sound event pressure level cannot be known. Digital audio recordings only provide a relative measure of sound event energy but have the benefit of facilitating a deeper understanding of occurring acoustic events. For the purposes of preliminary assessment of acoustic activity pre- and post- 'Stealth Box' installation, *signal energy* can be described as a measure of loudness.

Root mean squared energy (RMSE) was used as a means of defining signal energy. A 500ms window, with 50% shift, was used to calculate RMSE for each audio recording and channel. RMSE provides insight into the overall level of acoustic activity contained within the audio file, without consideration to the nature of each sound event (e.g., what part of the frequency spectrum it inhabits, the origin of the sound, etc.).

To capture the power of the audio signal within the spectral domain, a power spectral density (PSD) representation was obtained using Welch's method. An interval of audio data was transformed into a set of frequency bands, with the resolution controlled by the PSD window length (*bands = (window length / 2) + 1*). By successively applying the PSD transform to sequential audio frames, the level and change of frequency bands over time can be determined. PSD features have the advantage of allowing audio data to be filtered based on a predefined frequency range. By only analysing a limited spectral range, an approximation of signal energy as it relates to sound events of interest (e.g., animal and staff vocalisations, handling-related sounds, etc.) is obtained. A narrow range of 750Hz – 3KHz was used based on aural and spectral observations of acoustic events: the automated selection and optimisation of this range could be an area of future research. To further reduce the dimensionality of the data, the mean of the filtered PSD (MFPSD) was calculated and used for analysis. MFPSD provides a coarse indicator of events of interest occurring, while simultaneously removing low and high frequency noise.

To facilitate pre- and post-installation comparison, numerous statistical measures were calculated across audio data files. Firstly, a statistical 5-number summary (min, max, median, Q1, Q3) was computed for both RSME and MFPSD. This was augmented by including the mean value to gain an understanding of the average signal energy. To further summarise the signal energy of each audio recording, the area under curve (AUC) was calculated, relative to file duration. For this application, AUC can be used as a coarse indicator of overall energy using RMSE and MFPSD, allowing for a 'one number' comparison of audio data files. It should be noted that this measure alone cannot be used to determine *loudness* but is still useful as an indicator of overall acoustic activity. To provide a succinct overview, the average mean and AUC of RMSE and MFPSD were calculated across pre- and post-installation audio recordings.

To observe the average overall changes in acoustic activity before and after 'Stealth Box' installation, the percentile changes in event rate, mean duration, and total event duration (relative to file length) were calculated for each processing location. Events were detected by calculating the MFPSD value for each frame, then applying energy-based segmentation, with the Q3 value for each file used as the threshold. The event rate is defined as the number of detected sound events occurring per minute. The mean event duration describes the average duration of detected sound events (ms). Total event duration is expressed as the percentage of events detected within a file compared to total file length (%). It is important to keep in mind that acoustic changes are relative to levels observed in pre-installation recordings, and the noise floor present in individual locations. Absolute values cannot be directly compared between processing facilities, and changes are nuanced in nature. An overview is provided to give some insight into how 'Stealth Box' may affect the quantity and duration of sound events. Although these metrics can be used as an indicator of changes to the acoustic environment's composition, they do not consider the nature of the sounds themselves.

4.5.4 Pre and Post Installation Comparison of Acoustic Data

Acoustic Energy Assessment

Acoustic energy metrics provide a coarse overview of the level of sound activity contained within an audio file captured during a processing session. RMSE assessment gives a raw, full spectrum view of energy levels. MFPSD looks at a narrower frequency range, filtering out some of the high noise levels experienced in a processing facility, while homing in on the area of sound inhabited by vocalisations and other sounds of interest. An increase or decrease in energy metrics should not be viewed as positive or negative, but rather as an indicator that a change in the acoustic environment has occurred. This could be due to changes in animal or staff behaviour, but may also be caused by many other factors, such as layout changes to incorporate 'Stealth Box' into the processing system. Given the highly variability inherent in both animals and staff, and the limited data available, it is difficult to make a definitive assessment as to the level of acoustic activity before and after 'Stealth Box' installation.

Sound Event Assessment

The energy of a recorded audio signal can only reveal limited information about the overall composition of the acoustic soundscape. To determine when sounds of interest are occurring, automated audio segmentation is required to identify when acoustic events are of a significant amplitude to warrant further investigation. Three metrics were used to express sound event activity: events per minute, mean event duration, and total event duration. By assessing the percentile change in these metrics after 'Stealth Box' installation, a more nuanced view of sound event occurrence can be obtained. The rate of change of each metric, and whether an increase or decrease was observed, can provide information about what type of change in acoustic environment has occurred. Table 8 provides an overview of how to interpret each combination of event metric changes.

Table 8: An overview of how to interpret each combination of sound event metric changes.

Events / Min	Mean Duration	Total Duration	Explanation
Decrease	Decrease	Increase	Less events with lower duration are highly unlikely to result in increased total duration: improbable scenario.
Decrease	Increase	Decrease	Less events, less activity overall, but longer events. A minimal to small increase in mean could still result in less activity.
Increase	Decrease	Decrease	Shorter but more frequent events, resulting in lower overall activity. The decrease in mean duration is enough to compensate for the increase in events.
Increase	Increase	Decrease	More sounds that are, on average, longer in duration. This scenario is highly unlikely to result in a decrease in overall event activity.

Increase	Decrease	Increase	The increase in events is large enough to compensate for the decrease in event duration, resulting in an increase in overall activity.
Decrease	Increase	Increase	A decrease in events, but with substantial enough increase in mean duration to result in an increase in overall event activity.

Sound event metrics were computed for all processors with acoustic data collected before and after 'Stealth Box' installation. Percentile changes to events per minute (Figure 15), mean event duration (Figure 16) and total event duration (Figure 17) are supplied. Table 9 provides an overview of the statistical changes to sound event metrics for each processing area, facilitating a comparison of results across sites. This is followed by a discussion of observed trends between processors and a breakdown of area-specific findings.

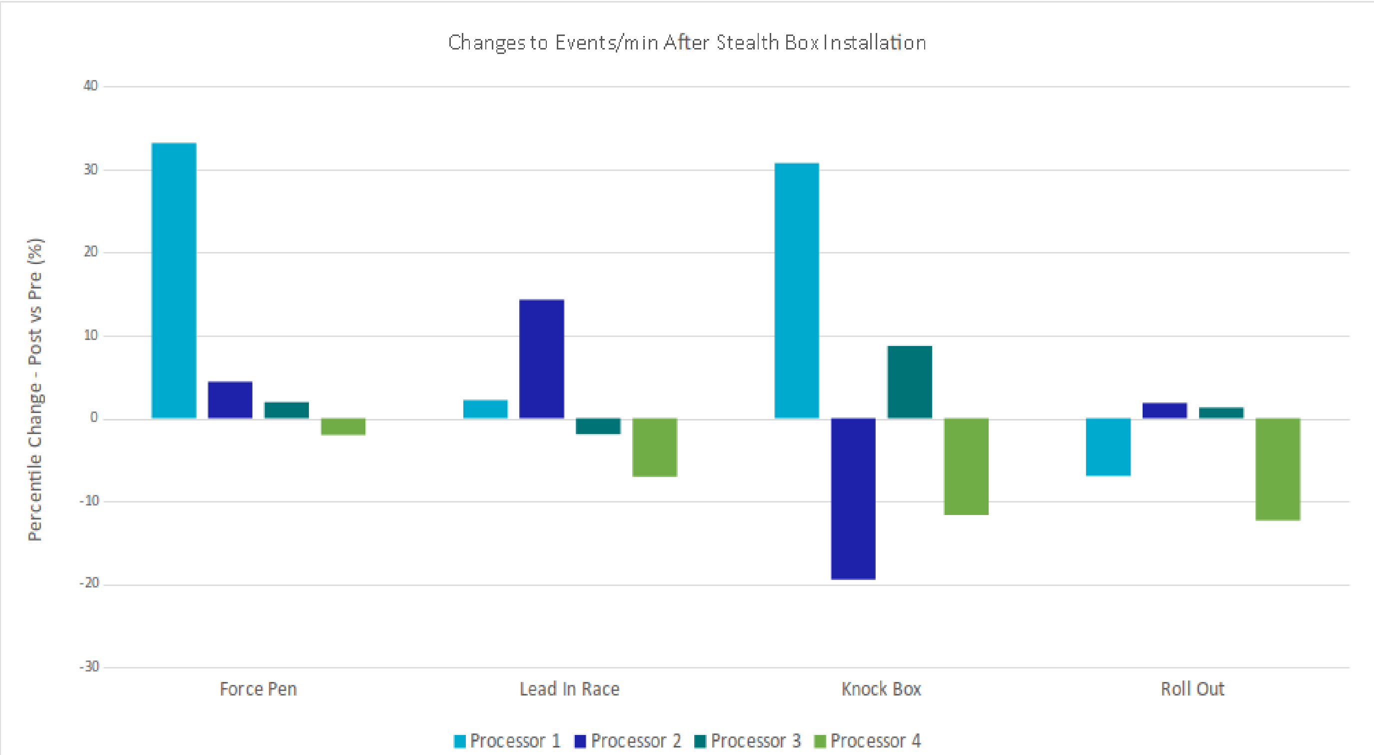


Figure 15: An overview of changes to events per minute after 'Stealth Box' installation for each of the 4 processors with pre and post data.

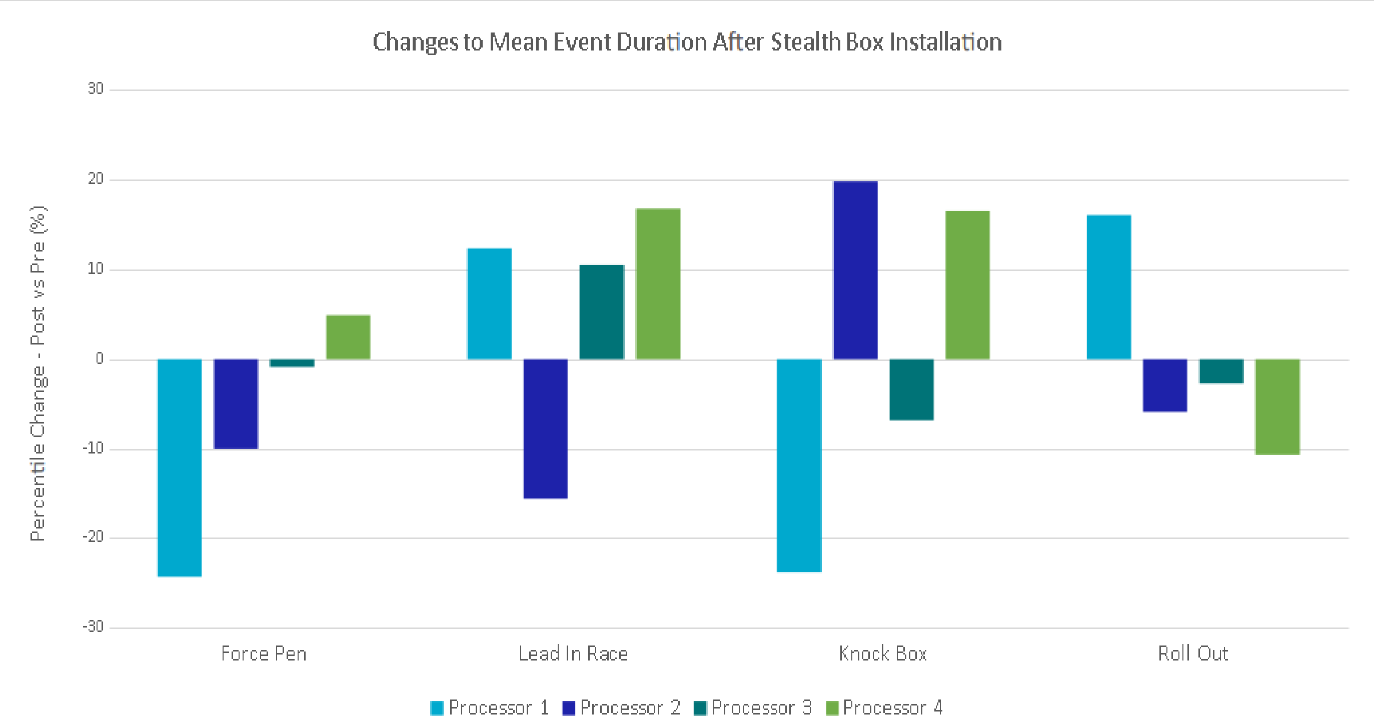


Figure 16: An overview of changes to mean event duration after 'Stealth Box' installation for each of the 4 processors with pre and post data.

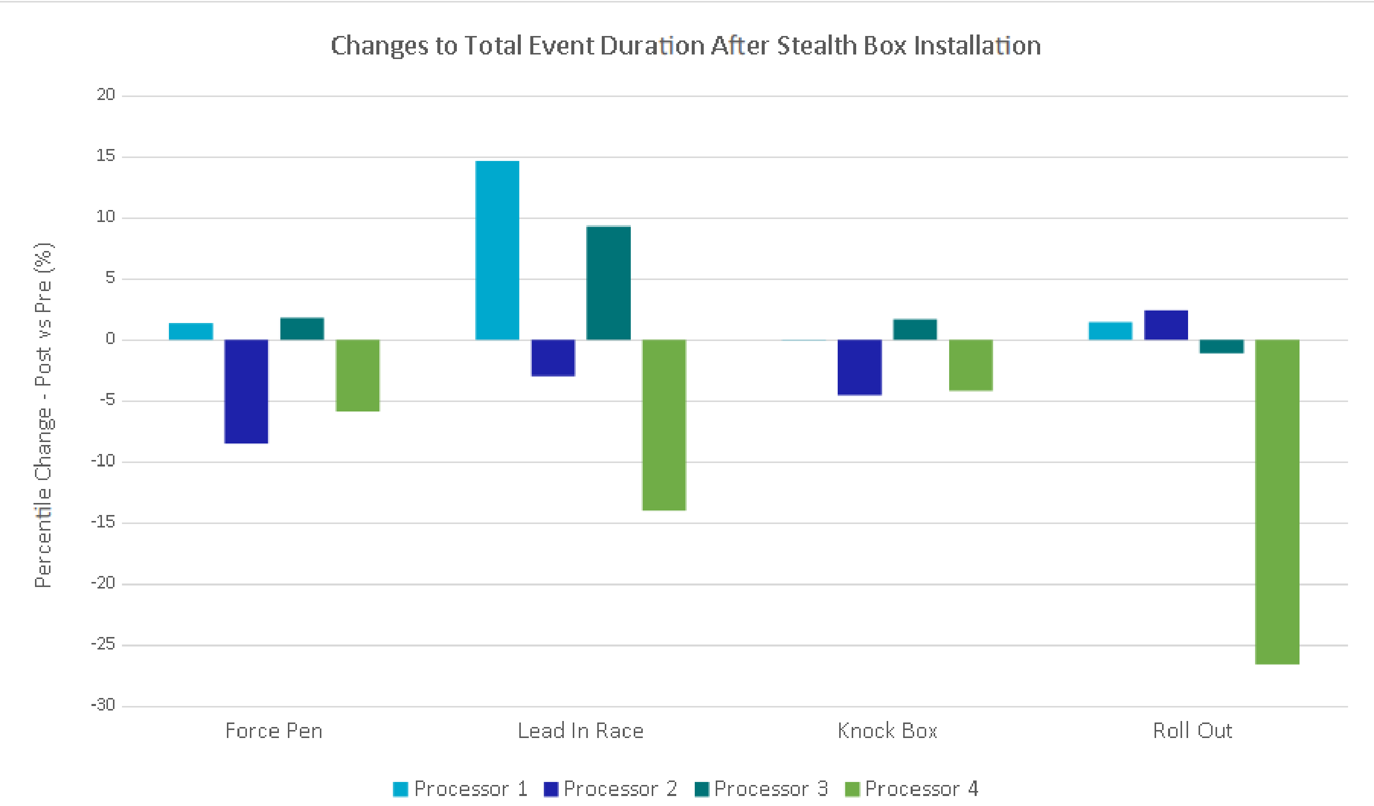


Figure 17: An overview of changes to total event duration after 'Stealth Box' installation for each of the 4 processors with pre and post data.

Table 9: A summary of the changes to sound event metrics across each of the 4 processing sites with acoustic data before and after 'Stealth Box' installation. Results have been colour-coded to denote their significance.

Force Pen			
Processor	Events / Min	Mean Duration	Total Duration
1	Increase	Decrease	Increase
2	Increase	Decrease	Decrease
3	Increase	Decrease	Increase
4	Decrease	Increase	Decrease

Lead In			
Processor	Events / Min	Mean Duration	Total Duration
1	Increase	Increase	Increase
2	Increase	Decrease	Decrease
3	Decrease	Increase	Increase
4	Decrease	Increase	Decrease

Knock Box			
Processor	Events / Min	Mean Duration	Total Duration
1	Increase	Decrease	Increase
2	Decrease	Increase	Decrease
3	Increase	Decrease	Increase
4	Decrease	Increase	Decrease

Roll Out			
Processor	Events / Min	Mean Duration	Total Duration
1	Decrease	Increase	Increase
2	Increase	Decrease	Increase
3	Increase	Decrease	Decrease
4	Decrease	Decrease	Decrease

Legend	
Minimal	0 - 9.9%
Notable	10 - 29.9%
Significant	>30%

Although there is no clear pattern to the changes to sound event metrics observed before and after 'Stealth Box' installation across sites, this is not a surprising finding given the vastly different layouts and management practices of each processor. Each site has dramatically different acoustic environments, making it difficult to compare results. The physical differences between sites are further complicated by the changes to floor layout and machinery position to accommodate the installation of 'Stealth Box'. For some processors, 'Stealth Box' could be incorporated with relatively minimal changes, while other processors had to substantially change their layout. This means that some processors had immensely disparate soundscapes before and after installation. Changes to layout also had an impact on data collection consistency, as recording units could not always be placed in the same location post-install.

The most common trend that can be observed between sites is that the types of sounds encountered in certain processing areas (i.e., the knock box and lead in race) appears to have changed. This is a significant finding, as it indicates that staff and/or animal behaviour may have been modified by the installation of 'Stealth Box'. An increase or decrease in sound event metrics does not signify a 'good' or 'bad' result, rather that the types of sounds has changed. Further analysis using machine learning classification and unsupervised clustering techniques is required to delve into exactly which types of sounds are occurring, how this has changed before and after installation, and how this relates to animal and staff behaviour. Across processing sites and areas, only 2 results show a definitive increase or decrease across all metrics. Even under these circumstances, it cannot be objectively stated that there is definitely more or less sound: there is too much variability to make this conclusion. More extensive data collection is required to gain a more complete picture of the acoustic environment at each processing site, particularly in relation to the variability inherent in changes to staff, animals, and other occurrences.

Force Pen

Results for the force pen followed a similar pattern for Processors 1-3: an increase in events per minute and a decrease in mean event duration. The effect on overall activity differed between Processors 1-3, with minimal increases for Processors 1 and 3, but a notable decrease for Processor 2. Of these results, those obtained from Processors 1 and 2 showed the most change, meaning it is likely that the types of sound events occurring differed after 'Stealth Box' installation, and may be an indicator of changed animal or staff behaviour. Changes to sound event metrics for Processors 3 and 4 were minimal, so it is unlikely that any significant changes have taken place.

Lead In

Of all the processing areas, the lead in race had the most varied results across processing sites. There was no discernible pattern between processors, with a different combination of changes to sound event metrics reported for each site. The lead in also had the most notable changes of any processing area, with 7 out of 12 metrics changing by a notable amount (+/-10 – 15%). Of all the processing areas, the layout and composition of the lead in has the most variation between sites. Each processor has substantially different lead in race configurations, which coupled with animal and staff variance, makes it very difficult to compare across processors. A lot of acoustic activity takes place in the lead in and being the area most susceptible to acoustic event variation, it is unsurprising that the results are so diverse.

Processor 1 reported the only result for any area where all metrics increased, with mean and total duration being notable increases. This indicates that there has been a significant change to the types and prevalence of sounds encountered in the lead in for Processor 1. This increase should not be viewed as a 'negative' result, and further analysis is required to understand how the acoustic landscape may have changed after 'Stealth Box' installation and whether this is a result of modification to animal or staff behaviour.

Knock Box

While a definitive pattern was not found between processing sites, substantial changes to the acoustic environment of the knock box were observed. All sites saw either an increase in events per minute with a corresponding decrease in mean duration, or its inverse. Given that changes to overall sound event duration were insignificant for all sites, it is highly likely that the types of sounds occurring in the knock box has changed after 'Stealth Box' installation. Indicators of a changed soundscape were the most prominent for Processors 1 and 4 but were still prevalent in the results for Processors 2 and 3.

The results for Processor 1 contained a statistically significant increase in events per minute: this is 1 of only 2 such findings for this study. The corresponding notable decrease in mean duration for Processor 1 is another strong indicator that the types of sound events occurring in the knock box has changed. Results for Processor 4 were the inverse of Processor 1, with a notable decrease in the event rate and an increase in mean duration. This result can be viewed in a similar way to that of Processor 1: the types of sound events regularly taking place during the operation of the knock box has changed. Without automated classification, the exact composition of the acoustic environment remains unknown, but the current findings lend weight to the hypothesis that the ‘Stealth Box’ has had an impact on staff and/or animal behaviour.

Roll Out

The roll out area saw the lowest overall changes to sound event metrics, but still had some notable findings. Minimal changes were observed for Processors 1-3, with the only notable increase being mean event duration for Processor 1. It appears that there has not been a substantial change to the operation or behaviours within the roll out area. Given the adjustments needed to incorporate the ‘Stealth Box’ into existing processing facilities, a lack of change in the roll out could be viewed as encouraging: the sounds and behaviours occurring are unlikely to have dramatically changed after installation.

Processor 4 reported a notable decrease in all sound event metrics for the roll out: this was the only area in the study where this occurred. These decreases may be attributed to changes in behaviour, facility layout, or microphone placement. More analysis is needed to ascertain the nature of the sound event reductions, and whether they can be ascribed to ‘Stealth Box’ installation.

4.5.5 Processors with only post-install data

Acoustic data from the period prior to ‘Stealth Box’ installation was unavailable for four processors: only post-installation data was collected. This makes it impossible to meaningfully assess any changes to acoustic activity resulting from ‘Stealth Box’ installation, as baseline measures cannot be established: there is no way to observe how the site operated before ‘Stealth Box’ installation. Acoustic signal energy and sound event metrics could be calculated on collected post-installation data, but there is no way to interpret the results without some form of comparison.

While it is possible to attempt a comparison of post-installation-only acoustic data to that of other processing sites, this would not result in any meaningful or legitimate findings. All sound energy and event metrics are relative to the acoustic environment present in the specific processing facility: a comparison of absolute measures is not possible. There is a very high amount of variability within each individual processing site, with many variables changing within and between processing sessions. The breed, temperament, and condition of animals is variable, leading to vastly different behaviours during processing. Staff members have different approaches to animal handling and management (e.g., some staff whistle more). Sites may process different quantities of animals during any processing session. The variability between processing sites is even more pronounced, given the vastly different layouts, operational directives, and management practices observed at each site. Without automated detection and classification of sound events, comparison of relative sound measures between sites is meaningless. If data collection was continued at post-installation-only sites, it would be possible to compare site operation over time. This could reveal pertinent information concerning animal, staff and processor behaviour, and could be used to monitor the progress and outcomes of different initiatives and programs.

4.5.6 Acoustic Data: Conclusion & Next Steps

Analysis of acoustic data collected from the knock box of the focal processor revealed a statistically significant change in sound event metrics after ‘Stealth Box’ installation. It appears to have been a change to the types of sounds regularly occurring within the knock box. This is an important finding, as it contributes to the hypothesis that ‘Stealth Box’ adoption can have a positive impact on animal welfare. It was found that the acoustic composition of the knock box changed after ‘Stealth Box’ installation for all processors with pre and post install data. Although a notable to significant change to events per minute and mean event duration was observed for all processors, overall event activity was only minimally affected. This is further evidence that installation of the ‘Stealth Box’ has an impact on animal and/or staff behaviour.

Although there were no significant changes to sound event metrics in the lead in (i.e., >30% change), there were the most notable changes (i.e., 10 - 29.9%) of any area. Three out of 4 processors reported a notable change in at least 2 sound event metrics. Given that the lead in race is the most variable location within a processing site, it is unsurprising that each processor had a different combination of sound event metric changes. A lot of animal movement and handling occurs within the lead in, so this area is arguably the most susceptible to variability in staff and animal behaviour. Further analysis is needed to understand the nature of the changes to the acoustic environment of the lead in for each processor.

Results for the force pen were mixed when comparing between processors, with some sites showing a notable to significant change (Processor 1), and others reporting very little change at all (Processors 3 and 4). The roll out area displayed the least changes to sound event composition of any processing area. Minimal changes were observed for most processors, with only 1 processor showing a notable decrease in all metrics, the only such finding. It remains unclear as to whether this decrease is due to changes in behaviour, or simple due to variation of plant layout and subsequent microphone position.

This project has provided the opportunity to refine sound data collection strategies and acoustic assessment methodology. Given the highly diverse nature of the data collected from different processors, subsequent data collection campaigns are required to gain an accurate representation of each plant's acoustic environment. All currently used acoustic measurements have been implemented using Python, with the developed codebase allowing rapid processing of entire data sets. This codebase could be extended as new acoustic measures are explored.

Data was collected before and after 'Stealth Box' installation, but only during 2 processing sessions each. Given the high level of variability observed between animals and staff, this is not enough data to be able to conduct a thorough analysis of each processing site's acoustic environment. There is a high level of variability between animals within processing groups, and between groups. Individual animals display different responses to stimuli experienced within the processing environment and may require differing levels of staff handling. Groups of animals arrive at the processing site from disparate locations, and their behaviour is largely based on their previous experiences, such as the level of human contact and handling they have been exposed to. These groups may require a higher level of staff intervention to successfully navigate the processing system. Staff members have individual preferences pertaining to animal handling and intervention. For example, some staff may whistle to keep animals moving, while others may use calls. These differences in staff behaviour will produce dissimilar acoustic environments as the types of sound events will change. Staff members can change over time, adding further variability to long-term data collection. There are numerous external factors that can also affect the acoustic environment, such as weather, seasonal conditions, and natural phenomena. Future studies should aim to collect more contiguous data over a longer period, to reduce the effect of variability on acoustic analysis. By gathering data from a wider range of processing sessions, a more accurate representation of the acoustic environment can be obtained.

The current approach focuses on the assessment of overall signal energy or level of activity but does not disclose any information concerning the nature of detected sound events. Discovering the *type* and rate of occurring sound events would reveal more useful insights than signal energy alone. A sound event's temporal proximity to other sounds could also uncover important information (e.g., do more vocalisations occur directly proceeding another sound event type?). The next stage of research should focus on the development of acoustic segmentation and machine learning classification algorithms to facilitate the automated detection and identification of sound events. Given the diverse nature of each abattoir's acoustic environment, it would be difficult to produce accurate and complete annotations of all sounds that occur during a processing session. Unsupervised learning and clustering methods should be explored to discover the discrete classes that comprise the soundscape for each processor. Once a system capable of accurate sound classification is developed, close input from livestock behavioural scientists will be needed to interpret how particular sounds relate to animal welfare, behaviour, and management practices. To further improve the accuracy and performance of the system, detections should be correlated with animal and staff behaviour observed from video footage using multi-modal techniques. This research could lead to the development of an automated acoustic monitoring system designed specifically for use in abattoirs, facilitating the real-time surveillance of animal behaviour and welfare, assistance in auditing and legislative requirements, and improvements in overall system efficiency. It is also important to liaise with industry partners to more accurately understand their use-cases, ensuring that the most valuable research directions are pursued. An AI-based system capable of detecting and interpreting sounds of interest that may occur in a processing site is most certainly feasible, but significant research is required.

Given the success of MFPSD features for assessing acoustic activity within a specified spectral range, their use in automated segmentation should be considered. By using an adaptive energy thresholding approach, only sound events that are likely to be of interest will can be extracted from collected audio data. This approach has the added advantage of reducing interference and unnecessary computational load caused by low and high frequency noise. MFPSD-based segmentation will not only reduce system throughput requirements but may also improve machine learning classification performance by reducing dimensionality. PSD calculations are fast to compute and would be well suited to a real-time environment. As an additional advantage, if a PSD-based automated segmentation approach was adopted, no further transform is required for feature input, further reducing the system's computational requirements.

5.0 Discussion

Improving animal welfare in abattoirs is crucial, particularly with increasing public concern about meat production. In many countries, people are increasingly demanding that farm animals be reared, handled, transported, and slaughtered using humane practices (Faucitano et al., 2022, Terlouw et al., 2008). Low-stress livestock handling techniques at slaughter plants not only enhance animal welfare but also improve meat quality (Grandin, 1980, Grandin, 2003, Grandin, 2010, Grandin, 2020, Hemsworth, 2003, Hemsworth et al., 2011). Modern commercial slaughter of cattle involves the use of restraint boxes to present the head of the animal for accurate placement of the stunning device. Restraint boxes constructed predominantly of concrete and steel can produce loud and unfamiliar noises during operation. Such noise is known to cause stress in animals, leading to baulking and requiring additional coercion to move them into the restraint. Distractions such as noise, darkness, and seeing people, can significantly impact their welfare (Bourguet et al., 2011a, Bourguet et al., 2011b). Exposure to such stressors can lead to an increase in vocalisation during handling or agitated behaviour (jumping or hitting fences) as a result of being in stress (Hemsworth et al., 2011). Losada-Espinosa et al. (2021, 2018) identified human-animal interactions, vocalizations, and falling as valid behavioural indicators for assessment of welfare at abattoirs. Other signs of stress during the pre-slaughter phase include increased instances of head lowering (Hemsworth et al., 2011), attempts to turn or refuse to re-enter the handling facility (Grandin and Shivley, 2015), baulk and back up (Grandin, 1996, Grandin, 2003).

The movement of livestock and the subsequent reduction of stress at abattoirs can be enhanced through the implementation of innovative and quieter operational systems. Jarvis Australia has developed a conceptual design for a restraint unit, known as the 'Stealth Box,' which incorporates a variety of noise-reduction strategies. These strategies include silencer manifolds on actuators and valve exhausts, nylon lining inside the box and on the roll out cradle, and rubber matting on the floor. This study evaluated the performance of the 'Stealth Box' in terms of its potential for stress reduction. Specifically, the study aimed to investigate: 1) the ability of the 'Stealth Box' unit to facilitate animal handling and movement, and 2) the variation in processing outcomes after the installation of the 'Stealth Box', to identify operational-related issues that often impact animal welfare. This study demonstrates that the new 'Stealth Box' has improved animal movement into the race by reducing the use of handling aids across all processors (approximately 80% pre-installation vs. 60% post-installation). Statistical analysis confirmed a significant reduction in handling aid usage post-installation. However, there was no reduction in handling noise. Despite this, it can be expressed that the 'Stealth Box' has shifted animal handling from physical methods, which can be more stressful and impact meat quality, to a more efficient and potentially less stressful process, marking as a positive improvement.

An increase in handling noise may be attributed to the higher use of whistles following the post-installation period. This can be explained by 1) easier movement of the animals through the race post-installation, where handlers preferred to employ low-stress methods such as whistles instead of tools to encourage movement, 2) the potential challenges in accurately measuring this parameter, and 3) it may be a common habit for the staff to whistle unnecessarily. In this study, back up behaviour increased post-installation. This may be explained by an increased in handling noise, as there was a positive correlation between backup behaviour and handling noise, specifically whistle sounds. This finding is consistent with the fact that cattle have better hearing than vision, causing them to back up when they hear a noise. Additionally, as reported by Grandin, (1996, 2003), baulk and back up behaviours may result from various distractions, such as moving people, sparkling reflections, shiny metal that jiggles, or air blowing into the faces of approaching cattle, all of which cannot be mitigated by the ‘Stealth Box’.

Operational system had a significant impact on the handling interventions, suggesting that variations in system design (from distractions such as shiny mental or lack of lighting to operation shape- for instance concrete-sided S-shape or straight line from force pen to knock box) or implementation play a crucial role in influencing how animals are handled. Therefore, any differences, particularly regarding operational improvements to increase animal welfare, such as non-slip flooring or using light to attract animals into the race, could contribute to this variation. The differences in frequency of handling interventions between processors, suggest that additional factors may be influencing animal movement within the race (Grandin, 2014). Different breeds, origins of the animals, or handling personnel employed at the processing facilities may have resulted in this variation. All these operational, physical and environmental factors can influence the assessment of animal welfare at the slaughterhouse level (Losada-Espinosa et al., 2021) and were not recorded or controlled for in this study. During installation, some processors made some modifications to the lead in race and roll-out areas to accommodate the ‘Stealth Box’, which was larger than their existing box. In some processors, this modification was reasonably minor, e.g. removal of a wall, or relocating a door or gate in the lead in race; in other processors, the entire lead in race was replaced and reconfigured. In those which undertook major reconfiguration of the lead in area, it is difficult to assign any changes in behaviour or handling to the ‘Stealth Box’ alone.

In terms of meat quality, there is a well-established relationship between animal welfare at abattoirs and meat quality. Animals might be exposed to a variety of stressors, including noise, unfamiliar smells, social breakdown, close confinement, and often overcrowding in the abattoirs. Behavioural and physiological responses to these stressors can have negative effects, if extreme, on carcase and lean meat quality (Warriss, 1990). Meat quality, particularly aspects of pH, tenderness, water holding capacity and colour can be influenced by physiological effects of pre-slaughter stress (Ferguson and Warner, 2008, Lowe et al., 2004, Birhanu, 2020, Gregory, 2009). During the normal postmortem process, muscle glycogen is converted into lactic acid, which contributes to the lowering of meat pH. However, when animals experience excitation before slaughter, glycogen is depleted more rapidly due to the release of stress hormones. This rapid depletion of glycogen results in a faster decline in pH, which can lead to heat toughening, if the meat passes through pH 6 while carcase temperature is above 35°C. Heat toughening can negatively impact meat colour, water-holding capacity, and texture.

In the current study, three processors provided pH decline data prior to and post installation of the ‘Stealth Box’. In one processor, pH declines were more rapid post installation, but this could be due to the addition of an immobiliser in the roll-out cradle adding to the electrical inputs during processing. In a second processor, there was no real difference in pH decline pre- and post-installation. However, this processor actively manages pH declines and electrical inputs as a routine. In a third processor who had made no changes to electrical inputs, the pH decline was slower post installation of the ‘Stealth Box’, suggesting that there was a reduction in excitation of animals immediately prior to slaughter. These outcomes should be interpreted with caution, as the sample sizes were small and results could be confounded by animal and operational factors. The processors who provided pH decline data were using percussive stunning – if electrical stunning were implemented, this would increase the electrical inputs to the carcase and increase the rate of pH decline.

For objective meat quality, there was a reduction in Peak Force and expressible moisture (i.e. samples were more tender hand had better water holding capacity) in eye round samples after ‘Stealth Box’ installation, but no significant differences in striploin samples. Significant differences in colour or cook loss were not observed, and although there appeared to be an increase in lipid oxidation, the TBARs levels were extremely low, and thus the observed results are more likely to be related to animal factors than to the ‘Stealth Box’ itself. Lipid oxidation is affected by the feedbase on which animals were raised (Mitsumoto et al., 1998, Faustman et al., 1989), age, distance transported (De la Fuente et al., 2010, Zhong et al., 2011), as well as other factors, and the origin, breed and sex of carcases sampled at each time point were not standardised. Differences between the animals processed could be accentuated due to the small sample size (n = 10 in each group).

Although there is no clear pattern to the changes to sound event metrics observed before and after ‘Stealth Box’ installation across sites, this is not a surprising finding given the vastly different layouts and management practices of each processor. Each site has dramatically different acoustic environments, making it difficult to compare results. The physical differences between sites are further complicated by the changes to floor layout and machinery position to accommodate the installation of ‘Stealth Box’. For some processors, ‘Stealth Box’ could be incorporated with relatively minimal changes, while other processors had to substantially change their layout. This means that some processors had immensely disparate soundscapes before and after installation. Changes to layout also had an impact on data collection consistency, as recording units could not always be placed in the same location post-install. The most common trend that can be observed between sites is that the types of sounds encountered in certain processing areas (i.e., the knock box and lead in race) appears to have changed. This is a significant finding, as it indicates that staff and/or animal behaviour may have been modified by the installation of ‘Stealth Box’. An increase or decrease in sound event metrics does not signify a ‘good’ or ‘bad’ result, rather that the types of sounds has changed. Further analysis using machine learning classification and unsupervised clustering techniques is required to delve into exactly which types of sounds are occurring, how this has changed before and after installation, and how this relates to animal and staff behaviour. Across processing sites and areas, only 2 results show a definitive increase or decrease across all metrics. Even under these circumstances, it cannot be objectively stated that there is definitely more or less sound: there is too much variability to make this conclusion. More extensive data collection is required to gain a more complete picture of the acoustic environment at each processing site, particularly in relation to the variability inherent in changes to staff, animals, and other occurrences.

Analysis of acoustic data collected from the knock box of the focal processor revealed a statistically significant change in sound event metrics after ‘Stealth Box’ installation. It appears to have been a change to the types of sounds regularly occurring within the knock box. This is an important finding, as it lends weight to the hypothesis that ‘Stealth Box’ adoption can have a positive impact on animal welfare. It was found that the acoustic composition of the knock box changed after ‘Stealth Box’ installation for all processors with pre and post install data. Although a notable to significant change to events per minute and mean event duration was observed for all processors, overall event activity was only minimally affected. This is further evidence that installation of ‘Stealth Box’ has an impact on animal and/or staff behaviour.

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6.0 Limitations of the study

Data

- Data was only collected over 2 days pre and post install.
- Animal source and breed were not standardised.
- Personnel handling animals differed on different days at each processor.
- This is not enough acoustic data to be able to understand the highly variable environment of each location
    - High variability between animals, groups of animals, staff, etc. Thus, more data area required to ‘smooth out’ acoustic data and account for other operational and animal effects.

Integration between acoustic and behavioural annotations

- Although behavioural annotation included listening for specific sounds listed on the ethogram, we did not correlate detected sound events in the acoustic analysis with observed behavioural annotations.
    - This would allow for a greater understanding of occurring sound events.
    - Could be used as ground truth for Machine Learning (ML).

Machine Learning

- While feasibility of ML has been shown, ML techniques have not been used to classify sound events.
    - This would provide a greater understanding of acoustic environment.
    - Would lead to develop of automated real-time system.

7.0 Conclusions / Recommendations

The findings of behavioural parameters indicated that using the ‘Stealth Box’ in abattoirs improved animal handling by reducing the need for physical interventions, as evidenced by the 1.74-fold decrease in the use of handling aids post-installation. Despite no reduction in handling noise, this may indicate a shift toward less stressful handling methods that require less physical interaction. The difficulty of measuring these parameters- handling noise- makes objective measurement an alternative methodology to accurately measure them. Further, due to the significant differences between studied processors, other parts of the process may need to be replaced or reviewed to better facilitate animal handling. Meat quality results suggest that a benefit may be achieved through use of the ‘Stealth Box’, but outcomes are heavily influenced by other operational and animal factors. Overall, humane and efficient handling appears to be enabled by the ‘Stealth Box’.

This project has provided the opportunity to refine sound data collection strategies and acoustic assessment methodology. Given the highly diverse nature of the data collected from different processors, subsequent data collection campaigns are required to gain an accurate representation of each plant’s acoustic environment. All currently used acoustic measurements have been implemented using Python® software, with the developed codebase allowing rapid processing of entire data sets. This codebase could be extended as new acoustic measures are explored.

Given the success of MFPSD features for assessing acoustic activity within a specified spectral range, their use in automated segmentation should be considered. By using an adaptive energy thresholding approach, only sound events that are likely to be of interest will can be extracted from collected audio data. This approach has the added advantage of reducing interference and unnecessary computational load caused by low and high frequency noise.

MFPSD-based segmentation will not only reduce system throughput requirements but may also improve machine learning classification performance by reducing dimensionality. PSD calculations are fast to compute and would be well suited to a real-time environment. As an additional advantage, if a PSD-based automated segmentation approach was adopted, no further transform is required for feature input, further reducing the system's computational requirements.

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9.0 Bibliography

BIRHANU, A. F. 2020. Pre-slaughter Stress, Management of stress and its effect on Meat and Carcass quality. *International Journal of the Science of Food and Agriculture,* 4**,** 30-37.

BONNY, D. S. S., LI, X., LI, Z., LI, M., DU, M. T., GAO, L. L. & ZHANG, D. Q. 2017. Colour Stability and Lipid Oxidation of Beef Longissimus Lumborum under Different Packaging Conditions. *Polish Journal of Food and Nutrition Sciences,* 67**,** 275-281.

BOURGUET, C., DEISS, V., BOISSY, A. & TERLOUW, C. 2011a. Reducing stress at slaughter: examples taken from studies in cattle. *Bulletin des G.T.V.***,** 107-112.

BOURGUET, C., DEISS, V., TANNUGI, C. C. & TERLOUW, E. M. C. 2011b. Behavioural and physiological reactions of cattle in a commercial abattoir: Relationships with organisational aspects of the abattoir and animal characteristics. *Meat Science,* 88**,** 158-168.

BOUTON, P. E. & HARRIS, P. V. 1972. The effects of cooking temperature and time on some mechanical properties of meat. *Journal of Food Science,* 37**,** 140-144.

COCKRAM, M. S. & CORLEY, K. T. T. 1991. Effect of preslaughter handling on the behaviour and blood composition of beef cattle. *British Veterinary Journal,* 147**,** 444-454.

DE LA FUENTE, J., SANCHEZ, M., PEREZ, C., LAUZURICA, S., VIEIRA, C., DE CHAVARRI, E. G. & DIAZ, M. T. 2010. Physiological response and carcass and meat quality of suckling lambs in relation to transport time and stocking density during transport by road. *Animal,* 4**,** 250-258.

ENGLISH, A. R., WILLS, K. M., HARSH, B. N., MAFI, G. G., VANOVERBEKE, D. L. & RAMANATHAN, R. 2016. Effects of aging on the fundamental color chemistry of dark-cutting beef. *Journal of Animal Science,* 94**,** 4040-4048.

FAUCITANO, L., MARTELLI, G., NANNONI, E. & MANTECA, X. 2022. Chapter 24 - Fundamentals of animal welfare in meat animals and consumer attitudes to animal welfare. *In:* PURSLOW, P. (ed.) *New Aspects of Meat Quality (Second Edition).* Woodhead Publishing.

FAUSTMAN, C., CASSENS, R. G., SCHAEFER, D. M., BEUGE, D. R., WILLIAMS, S. N. & SCHELLER, K. K. 1989. Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E. *Journal of Food Science,* 54**,** 858-862.

FERGUSON, D. M. & WARNER, R. D. 2008. Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? *Meat Science,* 80**,** 12-19.

GALLO, C., LIZONDO, G. & KNOWLES, T. G. 2003. Effects of journey and lairage time on steers transported to slaughter in Chile. *Veterinary Record,* 152**,** 361-364.

GRANDIN, T. 1980. The effect of stress on livestock and meat quality prior to and during slaughter. *Int J Stud Anim Prob,* 1**,** 313-337.

GRANDIN, T. 1996. Factors that impede animal movement at slaughter plants. *Journal of the American Veterinary Medical Association,* 209**,** 757-759.

GRANDIN, T. 2003. Solving livestock handling problems in slaughter plants. *Animal Welfare and Meat Science.* Oxon, UK: CABI Publishing.

GRANDIN, T. 2010. Auditing animal welfare at slaughter plants. *Meat Science,* 86**,** 56-65.

GRANDIN, T. 2014. *Improving Welfare and Reducing Stress on Animals at Slaughter Plants*.

GRANDIN, T. 2020. Livestock Handling at the Abattoir: Effects on Welfare and Meat Quality. *Meat and Muscle Biology,* 4.
GRANDIN, T. & SHIVLEY, C. 2015. How Farm Animals React and Perceive Stressful Situations Such As Handling, Restraint, and Transport. *Animals,* 5**,** 1233-1251.
GREGORY, N. G. 2009. Pre and Post Harvest Factors of Beef Quality - Cattle Welfare and Beef Quality. *Reciprocal Meat Conference.* Rogers, Arkansas: American meat Science Association.
GRUBER, S. L., TATUM, J. D., ENGLE, T. E., CHAPMAN, P. L., BELK, K. E. & SMITH, G. C. 2010. Relationships of behavioral and physiological symptoms of preslaughter stress to beef longissimus muscle tenderness. *Journal of Animal Science,* 88**,** 1148-1159.
HEMSWORTH, P. H. 2003. Human–animal interactions in livestock production. *Applied Animal Behaviour Science,* 81**,** 185-198.
HEMSWORTH, P. H., RICE, M., KARLEN, M. G., CALLEJA, L., BARNETT, J. L., NASH, J. & COLEMAN, G. J. 2011. Human-animal interactions at abattoirs: Relationships between handling and animal stress in sheep and cattle. *Applied Animal Behaviour Science,* 135**,** 24-33.
HOLMAN, B. W. B., BAILES, K. L., KERR, M. J. & HOPKINS, D. L. 2019. Point of purchase fatty acid profile, oxidative status and quality of vacuum-packaged grass fed Australian beef held chilled for up to 12 weeks. *Meat Science,* 158.
HUGHES, J., MCPHAIL, N., WATKINS, P., STARK, J. & WARNER, R. D. 2023. Increased light scattering in electrically stimulated beef longissimus muscle fibres contributes to the observed meat colour at grading. *Animal Production Science,* 63**,** 673-680.
HWANG, I. H., PARK, B. Y., CHO, S. H. & LEE, J. M. 2004. Effects of muscle shortening and proteolysis on Warner-Bratzler shear force in beef longissimus and semitendinosus. *Meat Science,* 68**,** 497-505.
LEE, K. H., PEDROZA, C., AVRITSCHER, E. B. C., MOSQUERA, R. A. & TYSON, J. E. 2023. Evaluation of negative binomial and zero-inflated negative binomial models for the analysis of zero-inflated count data: application to the telemedicine for children with medical complexity trial. *Trials,* 24**,** 613.
LOSADA-ESPINOSA, N., ESTÉVEZ-MORENO, L. X., BAUTISTA-FERNÁNDEZ, M., GALINDO, F., SALEM, A. Z. M. & MIRANDA-DE LA LAMA, G. C. 2021. Cattle welfare assessment at the slaughterhouse level: Integrated risk profiles based on the animal's origin, pre-slaughter logistics, and iceberg indicators. *Preventive Veterinary Medicine,* 197**,** 105513.
LOSADA-ESPINOSA, N., VILLARROEL, M., MARÍA, G. A. & MIRANDA-DE LA LAMA, G. C. 2018. Pre-slaughter cattle welfare indicators for use in commercial abattoirs with voluntary monitoring systems: A systematic review. *Meat Science,* 138**,** 34-48.
LOWE, T. E., DEVINE, C. E., WELLS, R. W. & LYNCH, L. L. 2004. The relationship between postmortem urinary catecholamines, meat ultimate pH, and shear force in bulls and cows. *Meat Science,* 67**,** 251-260.
MITSUMOTO, M., OZAWA, S., MITSUHASHI, T. & KOIDE, K. 1998. Effect of dietary vitamin E supplementation for one week before slaughter on drip, colour and lipid stability during display in Japanese Black Steer Beef. *Meat Science,* 49**,** 165-174.
NATIONAL HEALTH AND MEDICAL RESEARCH COUNCIL 2013. *Australian code for the care and use of animals for scientific purposes,* Canberra, National Health and Medical Research Council.
ODUSANYA, S. O. & OKUBANJO, A. O. 1983. Shear Force Values for Steaks from the Semitendinosus Muscle of Pre-Rigor Leg-Twisted Beef Carcasses. *Journal of Food Science,* 48**,** 1577-1578.
OTREMBA, M. M., DIKEMAN, M. E., MILLIKEN, G. A., STRODA, S. L., UNRUH, J. A. & CHAMBERS, E. 1999. Interrelationships among evaluations of beef longissimus and semitendinosus muscle tenderness by Warner-Bratzler shear force, a descriptive-texture profile sensory panel, and a descriptive attribute sensory panel. *Journal of Animal Science,* 77**,** 865-873.
R CORE TEAM 2021. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.
SAZILI, A. Q., NORBAIYAH, B., ZULKIFLI, I., GOH, Y. M., LOTFI, M. & SMALL, A. H. 2013. Quality Assessment of Longissimus and Semitendinosus Muscles from Beef Cattle Subjected to Non-penetrative and Penetrative Percussive Stunning Methods. *Asian-Australasian Journal of Animal Sciences,* 26**,** 723-731.
SCHÖNFELDT, H. C. & STRYDOM, P. E. 2011. Effect of age and cut on cooking loss, juiciness and flavour of South African beef. *Meat Science,* 87**,** 180-190.
TERLOUW, E. M. C., ARNOULD, C., AUPERIN, B., BERRI, C., LE BIHAN-DUVAL, E., DEISS, V., LEFEVRE, F., LENSINK, B. J. & MOUNIER, L. 2008. Pre-slaughter conditions, animal stress and welfare: current status and possible future research. *Animal,* 2**,** 1501-1517.

WARNER, R. D., FERGUSON, D. M., COTTRELL, J. J. & KNEE, B. W. 2007. Acute stress induced by the preslaughter use of electric prodders causes tougher beef meat. *Australian Journal of Experimental Agriculture,* 47**,** 782-788.
WARRISS, P. D. 1990. The handling of cattle pre-slaughter and its effects on carcass and meat quality. *Applied Animal Behaviour Science,* 28**,** 171-186.
WITTE, V. C., KRAUSE, G. F. & BAILEY, M. E. 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *Journal of Food Science,* 35**,** 582-585.
YANCEY, J. W. S., WHARTON, M. D. & APPLE, J. K. 2011. Cookery method and end-point temperature can affect the Warner-Bratzler shear force, cooking loss, and internal cooked color of beef longissimus steaks. *Meat Science,* 88**,** 1-7.
ZHONG, R. Z., LIU, H. W., ZHOU, D. W., SUN, H. X. & ZHAO, C. S. 2011. The effects of road transportation on physiological responses and meat quality in sheep differing in age. *Journal of Animal Science,* 89**,** 3742-3751.