



Animal welfare indicators for sheep during sea transport: Monitoring health and behaviour

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ABSTRACT

The welfare of sheep transported by sea is a pertinent issue for Australia's agriculture industry and a subject of concern amongst the general public. Evaluating the effect of sea transport on the welfare of livestock requires an accurate system of reporting. This study piloted an animal welfare monitoring protocol during two sea voyages from Australia to the Middle East. Sheep health and behaviour were analysed to determine if the protocol could detect animal responses to voyage conditions such as feeding management and changes to the pen environment. Data were recorded for target pens of five different commercial lines of Merino sheep. Principle Components (PC) analysis on behavioural data identified three principal components which were compared with voyage day, management and environmental conditions using general linear mixed modelling (GLMM). PC factor 1 scores (23.35 % of total variance) showed that resting and recumbency increased as the voyage progressed. PC factor 2 scores (17.09 % variance) demonstrated that behavioural responses to heat fluctuated by voyage day. PC factor 3 scores (9.48 % variance) showed reduced flight distances and increasingly competitive feeding behaviour as the voyage progressed. Environment and management conditions were associated with behavioural changes, with Wet Bulb Globe Temperature (WBGT) being the most influential measure affecting all three PC factors ($p < 0.001$). There were few diseases or symptoms of ill-health (including veterinary treatments) or mortalities recorded for sample pens; however, associations between ill-health and nutrition, environmental conditions and behaviour, were identified using GLMM. Increases in WBGT were linked to increased nasal discharge ($p < 0.001$), ocular discharge ($p < 0.05$), pink eye ($p < 0.05$) and Panting Score 4 ($p < 0.01$). With few recordings of ill-health, and a low mortality incidence, it was difficult to interpret the results of comparisons between health and behaviour. It is likely that associations between health indicators and behavioural variations will be more meaningful if a welfare monitoring protocol were applied to a larger sample size. The pen-side assessments presented in this study provided a practicable and comprehensive system for monitoring the behaviour and health of sheep during sea voyages. The application of such assessments on an industry basis could better inform all stakeholders about the welfare of sheep during sea transport.

1. Introduction

Animal welfare is a topic of increasing public concern, especially for animals during transport and under intensive management systems (Buddle et al., 2018; Sinclair et al., 2018). In 2019, Australia exported 1.1 million sheep (Meat and Livestock Australia Ltd, 2020) and the welfare of these animals during the live export process is of heightened interest (Hampton et al., 2020). There is much debate around the continuation of shipping livestock from Australia to overseas markets; however, it is recognised by both industry representatives and those who

oppose the trade that, while animals are transported by sea, their welfare should be closely monitored (Foster and Overall, 2014; McCarthy, 2018; Fleming et al., 2020a). Despite recent regulatory changes (Department of Agriculture Water and the Environment, 2020b), welfare reporting does not currently follow an evidence based method. An integrated welfare assessment protocol is required to record outcomes relating to livestock health, behaviour and mental state, as well as environmental conditions and resource provisions (Dunston-Clarke et al., 2020; Fleming et al., 2020b). The development of a standardised approach to the measuring and reporting of animal welfare could

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encourage ongoing improvements, address public concerns (McCarthy, 2018), increase regulatory capability and identify areas of unacceptable risk.

The majority of live sheep exported from Australia are transported from Fremantle, Western Australia to various destinations in the Middle East (Meat and Livestock Australia Ltd, 2020). Certified livestock vessels typically carry between 2000–90,000 sheep (Department of Agriculture Water and the Environment, 2020a) for an average of 21 days (Collins et al., 2018a). Shipments are commonly comprised of different types of livestock, divided into commercial lines by breed, sex, weight or age. Sheep are allocated pens according to pre-approved stocking densities calculated on body weight, fleece and horn length, and the time of year (Department of Agriculture Fisheries and Forestry, 2011). Stockpersons and veterinarians accompany voyages and monitor the health and welfare of animals throughout the journey, reporting to the industry regulator daily and at the end of each voyage. Introducing a standardised system of assessing health and behaviour from sample pens of animals could improve current evaluations of livestock welfare (Llonch et al., 2015; Messori et al., 2015).

Animal welfare is a multidimensional concept relating to both physical and mental state (Webster, 2005; Botreau et al., 2007); it is determined by an animal's ability to maintain biological homeostasis, fulfil innate behavioural preferences, and to adapt and cope with their environmental context (Mormede et al., 2018). Recently developed, land-based protocols for monitoring animal welfare consider a combination of resource- and environment-based measures, and animal-based indicators for assessing mental and physical states taken from a sample of animals (e.g., Blokhuis et al., 2013; Colditz et al., 2014; Ruiz and Dwyer, 2015). Animal health and behaviour indicators can be observed via repeated pen-side assessments and used to identify patterns of animal responses as sheep react to changing voyage conditions and on-board management practices (Dunston-Clarke et al., 2020; Willis et al., 2021). The development of a standardised pen-side welfare assessment protocol for implementation on livestock carrier vessels could be used as the basis for capturing animal responses during sea transport over multiple voyages and under varied industry conditions (Dunston-Clarke et al., 2020; Fleming et al., 2020b). These data can then enable the categorisation of indicators, by consensus agreement of welfare scientists, into thresholds of acceptable, marginal and non-acceptable welfare outcomes. Such thresholds could be used to better regulate industry and drive improvements in welfare outcomes.

For a protocol to be suitable in an industry setting, it must be non-invasive and comprehensive, yet efficient and practical to apply (Munoz et al., 2017). Fleming et al. (2020b) and Dunston-Clarke et al. (2020) proposed a welfare monitoring protocol based on the four welfare domains defined by Blokhuis et al. (2013) for the Welfare Quality® (WQ) project. Indicators under the criteria of good feeding, good housing, good health and appropriate behaviour were adapted to suit the Australian livestock export context by including pen-side observations used onboard livestock vessels (Jubb and Perkins, 2019). The aims of this study were to pilot the proposed assessment protocol on livestock carrier vessels during commercial consignments and to determine if the observed animal outcomes were influenced by voyage, resource and environmental factors. Pen-side assessments on target pens were conducted during two voyages travelling from Australia to the Middle East in contrasting seasons to capture environmental and management variations. Behaviour and health observations were compared between five commercial lines of livestock against environmental and resource variables.

2. Materials and methods

2.1. Livestock and pen selection

Data were collected by the same single observer (RW) onboard two commercial livestock voyages transporting sheep from Fremantle,

Australia to Muscat, Oman in May 2018 (Voyage A, 13 days at sea) and November 2018 (Voyage B, 14 days at sea). The voyages involved two livestock carrier vessels of the same size and design. For each voyage, four or six target pens of 31–55 sheep were selected from different weight, sex and age categories. A total of 10 pens were observed on Voyage A (454 sheep), and 14 pens on voyage B (617 sheep). Pens were between 15.87–19.84 m² and selected from different decks, with two or four pens assessed per deck (Table 1). Target pens were chosen to include areas of the vessel expected to vary in environmental conditions during the voyage (e.g., pens affected by radiant heat from the engine room or sun deck). Pen selection involved consulting the ship's crew, the researcher's prior knowledge of the vessel and consideration of the stowage plan. Due to variations in stowage and stocking density, it was not practical to sample exactly the same target pens on each voyage. Animal types selected were representative for this supply chain. All wethers (castrated male sheep) were recently shorn Merino sheep, had a fleece length < 25 mm, and were of Body Condition Score (BCS) 3.5–4 (Russel et al., 1969; Department of Agriculture and Food Western Australia, 2018). The wethers assessed on Voyage A were of mixed ages (date of birth: 2015–2016) and averaged 49 kg. Voyage A ewes were of mixed ages (date of birth: 2013–2016) and breeds, and averaged 55 kg bodyweight with BCSs ranging from 2–4. Sheep assessed on Voyage B were divided into A Wethers (4 years old, date of birth: 2014 and averaging 64 kg); B Wethers (<1 year old, date of birth: 2017 and averaging 54 kg); or C Wethers (<1 year old, date of birth: 2017 and averaging 47 kg). Sheep were evenly drafted by bodyweight (Table 1) as per industry standard practice of visual examinations. Sheep were not individually weighed; therefore, ranges in body weight were not recorded. It is estimated that weights varied by less than 5 kg for wether lines and 8 kg for ewe lines.

2.2. Pen assessment technique

Pen-side indicators (Table 2) were collected on a group level using the mobile phone application *Kizeo Forms* (2017). Assessments were made daily at 14:00–16:00 h (14:00–17:00 on Day 2 on Voyage A), commencing on Voyage Day 2. Pen assessments were not made on Day 1 and on some voyage days due to the handling of livestock as stocking densities, trough accessibility and pens gates were adjusted, or due to ship factors such as a temporary power supply interruption to the cargo holds (Table 1). Observations were made by point sampling as the researcher stood stationary in the alleyway in front of each pen. Recordings firstly involved the collection of static information (time, date, location, voyage day and pen ID) while allowing sheep to acclimatise to human presence before collecting animal indicators outlined in Table 2a–b over a period of 5–8 min per pen. Behavioural indicators were used to record animal activities, and how animals were behaving (demeanor) during each assessment period (Willis et al., 2021). Health and mortality were then assessed (Table 2c), along with resources (Table 2d), and environmental data (Table 2e). During assessments the observer was able to view all sheep in the pen; however, it was not always possible to inspect the whole animal from a pen-side vantage point. Therefore, some health observations were prone to a degree of error.

When recording panting scores, a modal panting score for each pen of sheep was firstly recorded, and a subsequent record of any individual animal's panting score that differed from the modal score was then noted. This improved recording efficiency as the observer was not counting the number of sheep at each panting score, only those that presented outside the modal score. The percentage of sheep at panting scores 2–3 were combined and included in behavioural analyses (Table 2a) while sheep at panting score 4 were also included under health indicators (Table 4a).

Some indicators required retrospective information (e.g., clean water availability, fodder ration, feeding regimen, access to roughage and feeding behaviour); these observations were made by the researcher throughout each day and by stockpersons and crew who were

Table 1

Voyage and pen details. (a) Voyage A: May 2018 (Fremantle, Australia – Muscat, Oman) 49 kg B Wethers and 55 kg Ewes (b) Voyage B: Nov 2018 (Fremantle, Australia – Muscat, Oman) 64 kg A Wethers, 54 kg B Wethers and 47 kg C Wethers. The number of animals per pen varied during the voyage due to movements of livestock, the stocking rate listed here is for the majority of voyage days. Pen assessments were carried out daily on days 2-13 for Voyage A and days 2-14 for Voyage B, except where indicated (*Day(D) Number).

Pen Group	Class	Age (years)	Ave Weight (kg)	Deck	Pen No	No of Animals	Feed Trough Space (m)	Watering Points	Pen Size (m ²)	No of Pen Assessments
Voyage A										
1A	B Wether	1–3	49	4	34	48	4	2	18.91	115
2A	B Wether	1–3	49	4	33	44	3	2	15.87	12
3A	B Wether	1–3	49	3	16	41	4	2	18.91	11 (*D5)
4A	B Wether	1–3	49	3	15	44	4	1	16.10	11 (*D5)
5A	B Wether	1–3	49	1	16	41	3	2	16.00	11 (*D5)
6A	B Wether	1–3	49	1	29	47	4	2	18.81	11 (*D3 & 5)
7A	Ewe	2–5	55	7	90	45	3	2	19.69	12
8A	Ewe	2–5	55	7	67	48	3	1	17.56	12
9A	Ewe	2–5	55	8	20	31	4	3	16.22	12
10A	Ewe	2–5	55	8	9	41	4	2	16.41	12
Voyage B										
1B	A Wether	3–4	64	5	65	33	3	1	17.95	178
2B	A Wether	3–4	64	5	66	43	3	2	19.51	13
3B	A Wether	3–4	64	5	49	38	3	2	18.84	13
4B	A Wether	3–4	64	5	48	44	3	2	18.84	13
5B	B Wether	≤1	54	4	54	45	3	2	17.47	13
6B	B Wether	≤1	54	4	55	51	4	1	19.84	13
7B	B Wether	≤1	54	3	16	46	3	2	18.91	13
8B	B Wether	≤1	54	3	15	32	3	2	16.10	13
9B	B Wether	≤1	54	1	17	48	3	2	18.81	11 (*D8 & 12)
10B	B Wether	≤1	54	1	16	37	3	2	16.05	11 (*D8 & 12)
11B	C Wether	<1	47	8	3	45	3	2	16.45	13
12B	C Wether	<1	47	8	4	50	2	2	17.41	13
13B	C Wether	<1	47	8	5	50	2	2	16.81	13
14B	C Wether	<1	47	8	6	55	3	2	19.69	13

monitoring the deck environment. All pens were supplied by one or more automatic water troughs that were cleaned and maintained daily (Table 1). Water was provided *ad libitum* on all voyage days except days 2–4 of Voyage A. On these voyage days, automatic refill water systems were turned off for approximately 8 h from 21:00 h. It was, therefore, not possible to accurately predict when, or for how long water troughs were empty during this time period. On each voyage, sheep were fed pellets at 07:00 h and 15:30 h daily, and given additional pellets or roughage feeds at 10:30 h or 13:00 h on selected days. Feed was manually delivered by crew to troughs fitted outside each pen. The design of shipboard pens means that it is not possible for all animals to attend the feed troughs simultaneously, and, *ad libitum* feeding is not always feasible due to the finite reserves of fodder loaded for each voyage. Therefore, sheep behaviour at feeding was assessed using a five-point scoring system, hereinafter referred to as 'feeding behaviour score' (Table 2b). This score was designed to assess social competition at feeding and reflects how environment and management factors may impact appetite (Dunston-Clarke et al., 2020). Feeding behaviour was scored during the most recent feeding time prior to the pen-side observations listed in Table 2d. The amount of pellets and roughage provided each day was calculated from the exporter's voyage instructions document, the Chief Officer's daily records, and the Australian Standards for the Export of Livestock (ASEL) daily reports (Department of Agriculture Fisheries and Forestry, 2011).

2.3. Statistical analysis

Multiple aspects of behaviour contribute to the overall welfare state of an animal, and these factors are not independent of each other (Webster, 2005; Mellor, 2016). Therefore, Principal Components (PC) analysis (Statistica, 2018) was used to simplify all mean standardised behavioural variables. PC analysis identified the behavioural

dimensions that captured the majority of the data variation. PC factors (eigenvalue >1.5) were described using the strongly loaded variables (variables with >75 % of the absolute value of the largest positive or negative correlation coefficient) on either end of the PC axis. Behavioural activities that were strongly loaded on the PC factors were examined separately against influential covariate measures for each line of livestock and on each voyage day (Fig. 1 a–f).

PC factors were graphed against covariates, voyage day, line and pen group. Generalised linear mixed modelling (GLMM) was used to test each PC factor (Statistica, 2018) (as a separate dependent variable) against independent factors: (1) voyage day, (2) voyage and (3) line, with pen group included as a random factor to account for repeated measures. Six covariates were also included: (4) WBGT, (5) pellet consumption, (6) roughage access, (7) sea swell score, (8) manure pad moisture score and (9) trough space per head. The factorial design allowed one degree of interaction between factors. Where a significant effect was detected on GLMM, a Tukey's post hoc test or graphic representation was used to identify where differences occurred. Resource- and environment-based measures were omitted from covariate comparisons if insufficient variation was seen within these voyages (Table 2); therefore, some potentially welfare relevant measures (such as water and feed trough contamination, or clean water availability) could not be included in the GLMM analyses.

Health-related indicators were tested as separate dependent variables against covariates, voyage day, line and pen group using the GLMM technique described above. Graphic representations and Tukey's post hoc tests were used to identify where significant differences occurred. As no sheep were recorded as 'unable to stand' during these two voyages, this variable was omitted from analyses.

Behavioural indicators were compared to each health-related indicator using a GLMM accounting for voyage day as a covariate factor (RStudio Team, 2019).

Table 2

a-e) Pen-side assessment indicators: animal-based (behaviour and health), resource- and environment-based measures. * Panting scores of 3 and 4 were infrequent; however, if observed, are important indicators of welfare (Collins et al., 2018; 2019). To achieve better comparisons for heat response, the percentages of the pen at panting scores 2, 3 and 4 were combined to produce the variable “Panting Score 2 or above (%)”.

Indicators	Method
(a) Quantitative behaviour	
1. Flight distance	Distance (m) the observer approached the pen before the animals moved away (Waiblinger et al., 2006; Llonch et al., 2015; Ruiz and Dwyer, 2015)
2. Recumbent	Percentage (%) of the pen lying down
3. Eating	Percentage (%) of the pen eating
4. Drinking	Percentage (%) of the pen drinking
5. Resting	Percentage (%) of the pen resting (in a state of sleep or minimal activity and engagement with their environment. Posture can be either standing or lying down)
6. Ruminating	Percentage (%) of the pen chewing their cud
7. Exploring the Environment	Percentage (%) of the pen performing seeking or searching behaviour, or physically interacting with or manipulating an object in their environment (licking, chewing, pushing about etc)
8. Antagonistic Behaviour	Percentage (%) of the pen performing antagonistic behaviour (riding, head butting, exerting pressure by pushing against another animal)
9. Panting Scores*	Percentage (%) of the pen at each panting score. 0: no panting; 1: slight panting; 2: fast panting, open grin; 3: open mouth panting; 4: open mouth panting, tongue out (Jubb and Perkins, 2019)
(b) Qualitative behaviour (demeanour)	
Scored on a continuous scale (0–100) where the observer indicated their perception to what degree the animals in the pen matched this description, where 0 = none of the animals, and 100 = all the animals, matched this description. Therefore, the score accounted for both the proportion of animals displaying the demeanour, and the extent that it was displayed. (Wemelsfelder et al., 2001; Wemelsfelder, 2007; Fleming et al., 2016)	
1. Anxious	Showing worry, nervousness or unease; increased vigilance behaviour
2. Settled	Quiet, calm, relaxed and not tense
3. Active	Energetic, lively, characterised by busy or lively activity (body movement and actions)
4. Uncomfortable	Showing signs of physical discomfort, unease or irritation
5. Alert	Wide awake, fully aware, attentive, (how engaged the animals are with their surrounding environment)
6. Lethargic	Lacking interest, dispirited, apathetic, slow moving, listless, dull
7. Inquisitive	Showing a positive interest or curiosity towards surroundings
(c) Health and Mortality	
1. Nasal Discharge	Percentage (%) of pen with serous or mucopurulent nasal discharge from one or both nostrils
2. Ocular Discharge	Percentage (%) of pen with serous or mucoid ocular discharge from one or both eyes
3. Hollow Sides	Percentage (%) of pen with a hollow behind the ribs indicating poor rumen fill (excluding illthrift animals)
4. Fleece Contamination*	Description of fleece contamination for the pen 1: all are clean and dry; 2: some with bellies, flanks and legs covered; 3: most with bellies flanks and legs covered; 4: muddy, dung contaminated or damp; 5: heavily soiled or wet; 6 filthy or very wet
5. Pink Eye	Number of sheep with conjunctival or ocular inflammation affecting one or both eyes
6. Scabby Mouth	Number of sheep with ulceration or scabbed lesions affecting the lips, mouth, nose or face.
7. Lameness or limb injury	Number of sheep able to stand but showing a gait irregularity of any severity or visible limb injury
8. Respiratory Disease	Number of sheep showing signs of primary respiratory disease e.g. dyspnoea, increased respiratory rate, or persistent mucopurulent nasal discharge.
9. Wounds / lesions	Number of sheep with a wound or lesion that penetrates the full thickness of the skin, not including minor grazes
10. Illthrift	Number of sheep failing to maintain body condition in the presence of adequate nutrition
11. Scouring	Number of sheep passing fluid faeces or diarrhoea
12. Unable to stand [†]	Number of sheep that are unable to stand without assistance
13. Mortalities	Number of mortalities since the previous pen assessment
14. Moved to hospital pen	Number of animals moved to a hospital pen since the previous pen assessment
15. Vocalisation*	Vocalisations per minute [‡]
16. Coughing*	Coughs per minute [‡] [‡] Where 0: nil; 1: 1 per minute; 2: >1 per minute – individual animal; 3: >1 per minute – multiple animals
17. Sneezing*	Sneezes per minute [‡]
(d) Resource-based measures	
1. Clean water availability [‡]	Hours of access to fresh clean water from at least one watering point in the last 24 h
2. Water contamination [‡]	A description of water trough contamination; 1: clean; 2: mild; 3: moderate; 4: marked contamination
3. Watering points contaminated [‡]	Percentage (%) of the watering points contaminated
4. Water Consumption	Water consumption for the decks/total sheep loaded (L/head/day)
5. Pellet consumption	Amount of feed as approximate % of body weight (BW) per head per day (total pellet consumption for the decks for 24 h /total heads/ average bodyweight x 100)
6. Feeding regimen [‡]	1: increased roughage/reduced pellets; 2: restricted fodder; 3: maintenance; 4: above maintenance; 5: <i>ad lib</i>
7. Roughage feeding [‡]	Grams of long fibre roughage fed per head per day
8. Roughage access	Number of feeds to include long fibre roughage in the previous 24h
9. Amount of feed in troughs [‡]	Amount of fodder in troughs – 0: empty troughs, 1: some crumbs left; 2: 1/4 full; 3: 1/2 full; 4: 3/4 full; 5: troughs full
10. Feed trough contamination [‡]	1: clean; 2: some fines; 3: majority fines; 4: some faeces/saliva/mould; 5: marked faeces/saliva/mould
11. Feeding behaviour score	Behaviour of sheep when fresh feed delivered at the most recent feeding time prior to the pen assessment – 1: disinterested (no animals attending troughs); 2: some interest (some animals eating, trough space available); 3: keen (no trough space available and animals waiting to attend troughs); 4: jostling (no trough space available, animals pushing to attend troughs); 5: smothering (no trough space available, some animals pushing, climbing or lunging to attend troughs)
(e) Environment-based measures	
1. Sea swell score	Scale based on the height of the swell; 1: no swell, 2: low swell (<2 m), 3: moderate swell (2–4 m), 4: heavy swell (>4 m), 5: phenomenal/confused swell

(continued on next page)

Table 2 (continued)

(e) Environment-based measures	
2. Manure pad moisture score	Visual perception of the moisture content of the manure pad; 1: dry and dusty; 2: firm; 3: tacky; 4: high moisture; 5: sloppy; 6: flooded
3. Manure pad depth‡	Visual perception of the average depth (cm) of the manure pad; 1: 0–5 cm; 2: 6–10 cm; 3: 11–15 cm; 4: 16–20 cm; 5: >20 cm
4. Dry bulb temp (°C)	As measured by a hand-held Extech HT 30 Heat Stress WBGT Meter; readings taken in the pen from shoulder height at arm's length from the point of observation.
5. Wet bulb globe temp (°C)	
6. Relative Humidity (%)	

* Insufficient variation during these voyages to allow meaningful inclusion in statistical analyses.

† There were no sheep recorded as 'unable to stand' during these two voyages, hence this health variable was omitted from analyses.

‡ Insufficient variability in these measures across the two voyages; data have not been included in the GLMM analysis.

3. Results

3.1. Behavioural outcomes

Principle components analysis on behaviour identified three PC factors with an eigenvalue > 1.5. PC factor 1 (eigenvalue 3.97) accounted for 23.35 % of the total variance and captured sheep activity levels. The terms 'alert', 'inquisitive' and 'active', were loaded on one end of the axis, while 'resting' and 'recumbent' were identified on the opposing end (Table 3a). PC1 scores changed by voyage day on combined and separate voyage analysis. Percentages of resting and recumbency generally increased for all lines of livestock in the middle and later stages of each voyage (Fig. 1a–b). A Wethers had lower resting and recumbent percentages with a significant difference noted for PC1 scores compared to lines across all voyages, except ewes. After accounting for covariate influences, there was with no significant variation between voyages for scores on PC1 (Table 3b).

PC factor 2 (eigenvalue 2.9) accounted for 17.09 % of the total variance and suggest behaviour related to responses to heat. Heavily weighted terms described sheep as 'settled' on one end of the PC axis and 'uncomfortable', 'lethargic' and a 'Panting Score of 2 or above' on the opposing end (Table 3a). GLMM results showed that this heat response behaviour varied by voyage, and voyage day for each journey (Table 3b). Tukey's post hoc test explained that significant differences in scores occurred between individual voyage days with data varying day to day.

PC factor 3 (eigenvalue 1.61) accounted for 9.48 % of the total variance and captured animal habituation responses. Flight distance and the term 'anxious' loaded on one end of the axis, with feeding behaviour score on the opposite end (Table 3a). Significant differences in PC3 scores were found between the two voyages. Voyage A recorded a shift towards increasingly competitive feeding behaviour scores (Fig. 1c–d) and reduced flight distance as the voyage progressed. There was no significant relationship associated with voyage day for this PC on Voyage B (Table 3b). During both voyages, it was noted that flight distances decreased for all lines of livestock as voyage days progressed, with most sheep no longer showing an aversive reaction to the approaching observer in the final days of the voyage.

3.2. Health outcomes

Pink eye and scabby mouth were the two most prevalent diseases or syndromes observed, affecting between 4–12 or 0–18 sheep per line, respectively (Table 4a). Pink eye was more prevalent on Voyage A (statistical difference by voyage), but there was no variation with voyage day. Scabby mouth was more prevalent on Voyage B, and prevalence increased with voyage day (Table 4b). There was also a higher prevalence of nasal discharge on Voyage B. Percentages of sheep with nasal discharge peaked on Day 11 on Voyage A, and Day 8 on Voyage B. Observations for sheep at Panting Score 4 were recorded on Day 13 of Voyage A and no other days (Fig. 1e–f). One mortality was recorded on Day 4 of Voyage A, a post mortem examination revealed findings typical of clostridial myositis: dark red, emphysematous inflammation localised to muscles of the right hind quarter. Four

animals were moved to hospital pens for treatment on Voyage B. Cases included an animal showing signs of respiratory disease, another with an infected shearing wound and two illthrift sheep — one of which later died and has been included as a mortality. Necropsy findings for this animal included depleted fat reserves and a discrete inflammatory lesion affecting the duodenum.

Significant correlations were found for comparisons between nasal discharge and heat responses on PC2 ($p < 0.001$), and human-animal responses and feeding behaviour on PC3 ($p < 0.001$). There was a weak association between ocular discharge and PC3 ($p < 0.05$), but the percentage of the pen with hollow sides showed a stronger correlation with PC3 scores ($p < 0.001$). Scabby mouth prevalence was associated with all three PC factors ($p < 0.001$).

3.3. Feeding

Analysis of behavioural changes against nutritional covariates showed that all three PC factors were associated with changes in pellet consumption for combined voyage data. Changes to activity and resting behaviour (PC1) and heat responses (PC2) were associated with access to roughage. Heat responses (PC2), and the flight distances and competitive feeding behaviour scores described by PC3, were both correlated to trough space per head (Table 3b).

Average pellet consumption on Voyage A ranged from 3.08 to 3.94% body weight per head per day for B Wethers and 2.75–3.51 % for Ewes (mostly mature animals) (Table 4a). Voyage A results showed a correlation between activity scores (PC1) and roughage access, while the heat response behaviour (PC2) was correlated with both pellet consumption and roughage access. PC3 scores were not correlated with nutritional covariates on this voyage (Table 3b). There was less feed available on Voyage B compared with Voyage A. During Voyage B, average pellet consumption was recorded at 2.03–3.25 % body weight per head per day for A Wethers (mature animals), and slightly more for the younger wethers (2.41–3.85 % for B Wethers, and 3.09–4.43 % for C Wethers). Roughage feed was provided on most days on Voyage B. (Table 4a). During this voyage, activity and rest (PC1) was associated with pellet consumption but not roughage access. Changes on PC2 (heat response) were not associated with any nutritional covariates, but flight distances and feeding behaviour (PC3) were significantly correlated with pellet consumption, roughage access, and trough space per head (Table 3b). Feeding behaviour scores increased in the first two days of the voyage for all lines of sheep. Feeding behaviour was more competitive on Voyage B with most pens described as Score 3 (Keen) and Score 4 (Jostling) on most voyage days (Fig. 1c–d). Younger sheep (B and C Wethers) recorded higher feeding behaviour scores than older sheep (A Wethers and Ewes).

Recordings for hollow sides, scabby mouth and mortality incidence were linked to pellet consumption. Provision of ration type varied according to animal and climatic factors. Pellet consumption was increased on days with higher percentages for hollow sides, scabby mouth or mortality. Roughage access was increased on days with higher nasal discharge and scabby mouth. No health variables were significantly correlated to trough space per head (Table 4b).

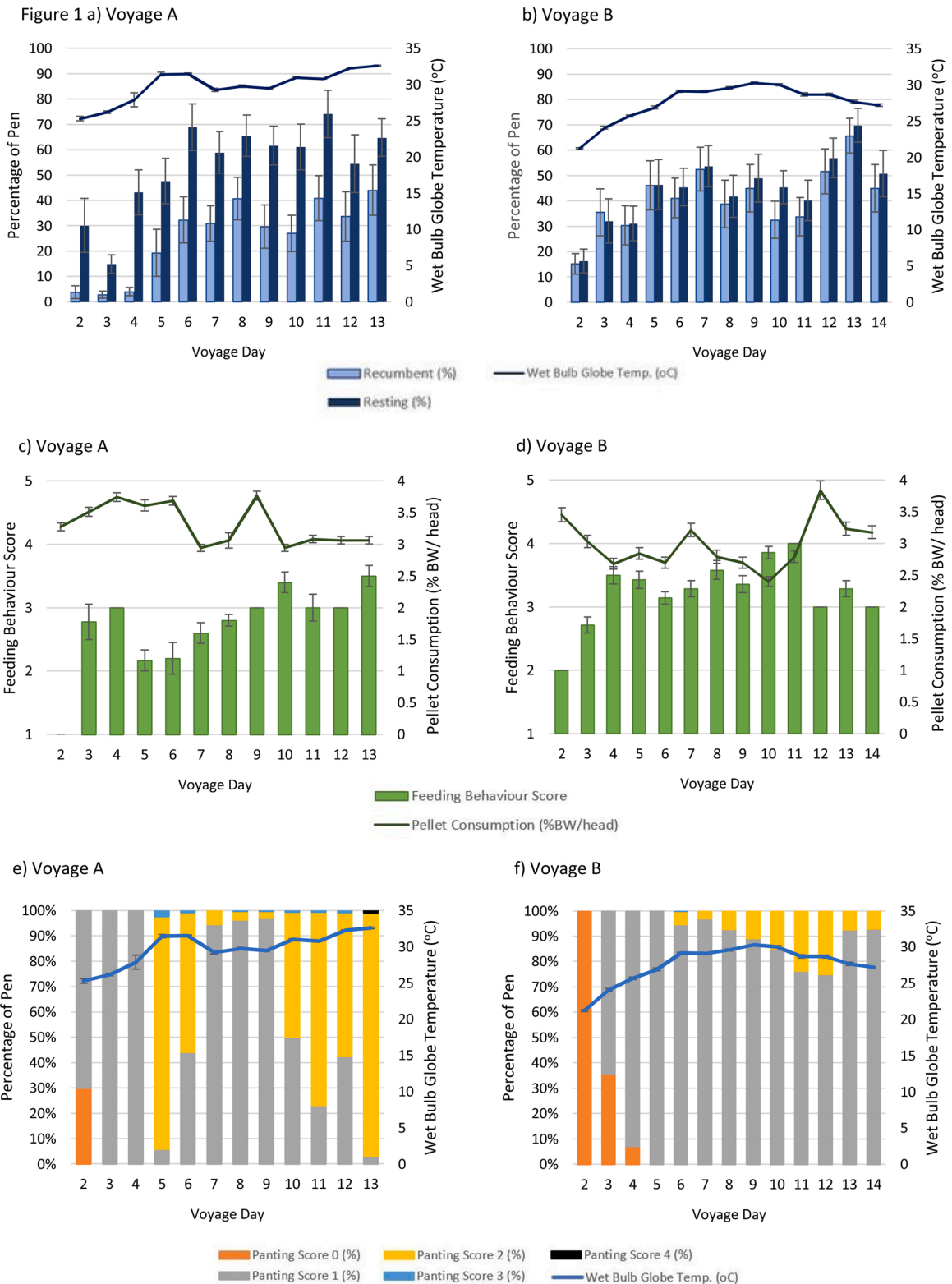


Fig. 1. a–b) Average percentage of animals resting and recumbent compared to wet bulb globe temperature (°C) by voyage day for Voyage A and Voyage B. c–d) Average feeding behaviour score compared to average pellet consumption (% body weight/head/day) by voyage day for Voyage A and Voyage B. Where feeding behaviour scores: 1 = disinterested, 2 = some interest, 3 = keen, 4 = jostling, 5 = smothering. e–f) Percentage of animals at each panting score (PS 0–4) compared to wet bulb globe temperature (°C) for each voyage day for Voyage A and Voyage B. Where panting scores: 0 = no panting, 1 = slight panting, 2 = fast panting, open grin, 3 = open mouth panting, 4 = open mouth panting, tongue out.

Table 3

a) Principal Components (PC) analysis. Variables that were >75 % of the highest absolute correlation coefficient were highlighted on each end of the PC factor axes b) F Values listed for generalised linear mixed modelling (GLMM) comparing the effect of several environmental or management measures on each PC factor. F Values are also listed for GLMM results comparing voyage, voyage day, line and pen group effect. Significant variations are highlighted in bold (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

a) PCA Variables		PC 1	PC 2	PC 3
Flight Distance (m)		-0.372	0.412	-0.549
Feeding Behaviour Score		0.196	-0.501	0.447
Panting Score 2 or Above (%)		0.326	0.690	0.357
Recumbent (%)		0.761	-0.309	-0.257
Eating (%)		-0.113	-0.036	0.269
Drinking (%)		0.078	0.249	0.295
Ruminating (%)		0.104	-0.400	0.223
Resting (%)		0.862	-0.009	-0.183
Exploring Environment (%)		-0.187	-0.147	0.294
Antagonistic Behaviour (%)		-0.272	-0.007	0.278
Anxious		-0.471	0.229	-0.462
Settled		0.506	-0.604	-0.166
Active		-0.667	-0.277	0.199
Uncomfortable		0.308	0.787	0.277
Alert		-0.789	0.245	-0.104
Lethargic		0.365	0.631	0.241
Inquisitive		-0.672	-0.310	0.292
Eigenvalue		3.97	2.90	1.61
Total Variance (%)		23.35	17.09	9.48
b) GLMM comparisons – F values				
Voy	WBGT (°C)	57.20***	52.20***	58.94***
A&B	Pellet Consumption (% BW/head/day)	4.13*	5.84*	9.55**
	Roughage Access	11.99***	67.15***	0.79
	Sea Swell Score	0.21	0.25	0.11
	Manure Pad Moisture Score	7.17**	46.37***	2.93
	Trough Space per Head	0.03	11.53**	7.39*
	Voyage Day	4.80***	17.18***	2.76**
	Voyage	0.05	23.14***	9.60**
	Line	3.68*	8.18***	7.54***
	Pen Group	1.32	4.65***	0.99
Voy A	WBGT (°C)	84.74***	30.53***	81.57***
	Pellet Consumption (% BW/head/day)	0.53	7.00*	1.07
	Roughage Access	11.49**	9.45**	3.27
	Sea Swell Score	1.92	0.85	2.13
	Manure Pad Moisture Score	3.74	0.01	1.51
	Trough Space per Head	2.18	0.08	1.13
	Voyage Day	3.14**	7.01***	2.13*
	Line	1.56	0.56	0.14
	Pen Group	3.00**	6.80***	1.43
Voy B	WBGT (°C)	13.74***	42.41***	24.58***
	Pellet Consumption (% BW/head/day)	12.25**	3.37	4.77*
	Roughage Access	1.95	3.76	9.90**
	Sea Swell Score	0.38	0.28	2.09
	Manure Pad Moisture Score	0.04	0.00	0.28
	Trough Space per Head	0.17	7.31*	5.14*
	Voyage Day	3.69***	7.72***	1.81
	Line	2.22	3.59*	2.06
	Pen Group	0.70	2.26*	1.46

3.4. Housing and environment

3.4.1. Wet bulb globe temperature

Wet bulb globe temperatures were marginally higher on Voyage A (average 29.76 °C ± 0.23, range 20.9–33.3 °C) than Voyage B (average 27.53 °C ± 0.20, range 20.6–31.0 °C). All three behavioural dimensions were correlated with variations in WBGT. Hotter temperatures were associated with less activity, more heat response behaviour, and a shift from aversive behaviour to an increase in competitive feeding behaviour. (Table 3b). During Voyage A, panting scores elevated sharply on Days 5 and 6, with some decrease in panting scores then observed before increasing again from Days 10–13 (Fig. 1 e). Panting scores during Voyage B were predominantly below Panting Score 2 on all voyage days,

one animal was recorded at Panting Score 3 on Day 6, and no animals were recorded at Panting Score 4 (Fig. 1 f). GLMMs found an association between increasing WBGTs and prevalence of nasal discharge, ocular discharge, pink eye, and Panting Score 4. In the early stages of Voyage B, lower WBGTs were associated with prevalence of scouring (Table 4b).

3.4.2. Manure pad moisture

Scores for PC1 and PC2 were correlated with manure pad moisture scores for the combined voyage analysis. As the manure pad scores increased, sheep were less active (PC1) and were more likely to be showing a heat response (PC2). Increased prevalence of nasal discharge, scabby mouth and Panting Score 4 were correlated to higher manure pad moisture scores. There was an increased prevalence of lameness in the

Table 4

a) Prevalence of physiological signs, ill-health, mortality rates and numbers of animals moved to a hospital pen. b) Health factors compared to covariate and voyage factors. F Values for generalised linear mixed modelling (GLMM) comparing the effect of environmental or management measures on each sign of ill-health. F Values are also listed for GLMM comparing voyage, voyage day, line and pen group effect. Significant variations are highlighted in bold (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

a) Covariate factors and health prevalence		Wet Bulb Globe Temp. (°C)	Pellet Consumption (% BW/head/day)	Roughage Access	Nasal Discharge (%)	Ocular Discharge (%)	Hollow Sides (%)	Pink Eye	Scabby Mouth	Lameness	Respiratory Disease	Wounds	Illthrift	Scouring	Panting Score 4	Moved to Hospital Pen	Mortality
Line	Number of sheep (n)	Average (range)	Average (range)	Total feeds	Average Percentage (\pm SE)			Number of cases (% incidence)									
Ewes (Voy A)	165	29.9 (20.9-33.3)	3.10 (2.75-3.51)	4	21 (2.82)	4 (0.46)	5 (0.82)	6 (3.6)	0 (0)	1 (0.6)	0 (0)	0 (0)	1 (0.6)	0 (0)	1 (0.6)	0 (0)	0 (0)
B Wethers (Voy A)	265	29.6 (22.9-32.7)	3.45 (3.08-3.94)	7	21 (2.32)	5 (0.52)	2 (0.39)	12 (4.5)	6 (2.3)	0 (0)	0 (0)	0 (0)	2 (0.8)	0 (0)	3 (1.1)	0 (0)	1 (0.4)
A Wethers (Voy B)	158	27.5 (21.1-30.2)	2.51 (2.03-3.25)	9	31 (3.88)	4 (0.41)	8 (1.20)	4 (2.5)	6 (3.8)	0 (0)	0 (0)	1 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
B Wethers (Voy B)	259	27.1 (20.6-30.7)	2.98 (2.41-3.85)	10	28 (2.87)	4 (0.36)	2 (0.33)	9 (3.5)	18 (6.9)	0 (0)	1 (0.4)	2 (0.8)	1 (0.4)	2 (0.8)	0 (0)	2 (0.8)	0 (0)
C Wethers (Voy B)	200	28.1 (20.8-31.0)	3.44 (2.77-4.43)	11	40 (3.60)	4 (1.19)	2 (0.34)	6 (3)	10 (5)	0 (0)	0 (0)	1 (0.5)	2 (1)	0 (0)	0 (0)	2 (1)	1 (0.5)
Totals	1047	28.4 (20.6-33.3)	3.11 (2.03-4.43)		28 (1.43)	5 (0.28)	4 (0.32)	37 (3.5)	40 (3.8)	1 (0.1)	1 (0.1)	4 (0.4)	6 (0.6)	2 (0.2)	4 (0.4)	4 (0.4)	2 (0.2)
b) GLMM Comparisons -F values																	
			WBGT		41.99***	6.69*	1.53	5.01*	2.29	0.31	2.15	0.17	2.51	6.61*	10.67**	0	0.3
			Pellet Consumption		3.17	1.85	11.06**	4.08	4.58*	0.12	0.06	0.17	0.44	0.03	0.07	0.23	8.70**
			Roughage Access		9.78**	0.02	0.05	0.45	11.32***	1.9	1.53	0.33	0.81	1.58	1.62	0.13	1.02
			Sea Swell		16.13***	0.22	0.02	0.31	6.31*	0.93	0.63	0.14	0.95	0.07	0.6	0.16	2.08
			Manure Pad Moisture		9.26**	0.01	1.18	2.19	5.03*	4.01*	0.36	0	0.08	0.77	26.35***	0.04	0.02
			Trough Space Per Head		3.36	1.73	0.02	3.31	4	0.29	0.62	1.51	0.38	0.1	0	2.37	0.13
			Voyage Day		3.59***	1.28	1.37	1.73	4.52***	1.04	0.71	0.83	1.79	0.34	3.95***	0.85	0.95
			Voyage		14.85***	0	0.04	9.06**	32.93***	3.22	0.45	1.54	0.73	0.57	0.01	1.24	0
			Line		0.22	1.3	3.95*	0.86	10.98***	2.07	0.67	0.27	0.03	1.18	0.94	0.35	0.49
			Pen Group		2.01**	1.58	5.84***	2.20**	1.79**	0.95	1.15	0.84	1.3	0.96	1.1	0.89	1.12

early stages of Voyage B, and this was correlated with dry and dusty manure pad scores (Table 4b).

3.4.3. Sea swell

None of the three behavioural dimensions were influenced by sea swell. For both voyages, sea swell scores ranged between Score 1 (no swell) and 2 (low swell <2 m). As sea swell scores reduced, the prevalence of nasal discharge and scabby mouth increased.

4. Discussion

Repeated pen-side assessments reflected sheep health and behavioural variations during their transport. Findings indicate that the proposed monitoring protocol was informative and gave a meaningful representation of the animal experience during the two sea voyages studied. Animal-based indicators showed that predominant behavioural patterns were associated with activity and rest, and reactivity to humans. Sheep had a low prevalence of ill-health; however, heat responses and competitive feeding behaviour were observed.

4.1. Behaviour, mental state and health outcomes

During both voyages, primary behavioural responses were described by variables associated with resting and activity, and no observed negative demeanour. These results demonstrated that sheep showed periods of calm engagement with their environment while having the opportunity and inclination to rest at other times. When transported by sea, sheep are confined to pens for many days, and their ability to perform innate behaviours is restricted during this period (OIE, 2018). Periods of positive engagement with their surroundings and subsequent periods of rest, suggests that sheep were displaying the ability to adapt and cope with their transport conditions (Mellor, 2016). Although the onboard environment can be challenging and freedom of movement is restricted, sheep demonstrated behaviour indicative of habituation.

The significant effect of voyage day on activity and flight distances indicated that sheep became less reactive to their surrounding stimuli and habituated to human presence at the pen-side as the voyages progressed. Aversion to stock handlers can be a significant cause of stress (Hemsworth and Coleman, 2011); this is particularly relevant during sea transport, as animals are frequently exposed to humans in their proximity. Flight distances can illustrate either aversion or habituation to stock handlers and are influenced by age, breed, pen size (Cramer et al., 2020), and prior stock handling experiences (Grandin and Shivley, 2015; Cramer et al., 2020). Our study involved sheep sourced from extensive grazing enterprises; therefore, they not been handled intensively before entering the live export supply chain. The reduction in flight distances indicated that sampled pens of sheep became less reactive to observer presence as they habituated to their surroundings. Monitoring the ability of sheep to adapt to intensive management is an integral component of understanding the welfare implications of sea transport.

Sheep responses to heat challenge likely contributed to their reduced activity and reactivity to humans during each voyage. This assertion was supported by changes on PC2 (heat responses), where sheep showed increased heat response associated with environmental temperatures in the mid to later stages of each journey. Heat stress is recognised as one of the most significant welfare issues for Australian sheep transported from winter in southern Australia across the equator and into the northern hemisphere summer (Phillips, 2016; Collins et al., 2018b). Wet bulb globe temperatures (WBGT) had the most substantial impact on the behavioural domains identified, and the voyage day effect on heat response behaviour (PC2) is likely to indicate cumulative heat loading on voyage days with consecutively high WBGTs (Willis et al., 2021). Traditionally, panting scores have been used as the primary indicator of heat stress; however, panting is a physiological response for shedding body heat and does not necessarily indicate poor welfare outcomes

(Webster, 2005). Understanding the welfare implications for sheep during heat challenge requires a more holistic approach. For example, reduced appetite can be an associated sign of heat stress (Barnes et al., 2004); notably, competition to attend the trough at feed times increased on both voyages suggesting that environmental conditions did not cause decreased appetite during this study. Quantifying the impact of high WBGTs on physiological responses (panting scores) and behaviour, mental state and health, will enable better identification of sheep that are no longer coping with heat and beginning to show signs of distress and poor welfare outcomes (Mellor, 2016; Broom et al., 2019).

Increases in WBGT were linked to increased nasal and ocular discharge, and incidence of Panting Score 4. Both voyages encountered high WBGTs; however, temperatures were hotter on Voyage A, and the prevalence of elevated panting scores was markedly higher amongst these sheep. High panting scores also reflect the selection of target pens from areas of the ship known to be affected by radiant heat from the engine room or below the sun deck (HSRA Technical Reference Panel, 2019). Detecting animal responses to heat and humidity, specifically during periods of cumulative heat loading or times of respite and body heat dissipation, will inform industry about the suitability of transporting sheep under different environmental conditions and seasons (McCarthy, 2018; HSRA Technical Reference Panel, 2019).

Generally, the incidence of disease was low and the causes for mortality, or moving animals to hospital pens, were varied and not likely to be interrelated. Contagious diseases observed in this trial included scabby mouth and pink eye, although, without the availability of diagnostic pathology, these observations were made based on clinical signs only. The prevalence of diseases, such as scabby mouth or pink eye, is primarily influenced by factors determined prior to sea transport, including livestock immunocompetence and disease exposure. Still, contagious disease prevalence can also be exacerbated by stress or immunocompromise during sea transport (Jubb and Perkins, 2019).

Pink eye is a lay term for a common ocular syndrome characterised by corneal and conjunctival inflammation. Pink eye can result in temporary or permanent blindness in one or both eyes; it has been described as 'an ongoing challenge' for the live export industry (Murdoch and Laurence, 2014). Welfare implications include localised pain and inflammation, reduced ability for affected sheep to access feed and water, and difficulties associated with handling sight impaired animals (Jubb and Perkins, 2019). The prevalence of pink eye in this study varied between voyages and pen groups, but not lines of livestock, which perhaps indicates that localised disease spread occurred in some areas onboard the vessel. Understanding pink eye prevalence under different voyage conditions can improve management practices and reduce the welfare impact of pink eye during transit.

Scabby mouth is a viral infection endemic to sheep populations worldwide; it causes scabbed lesion and pustules to form, usually around the mouth and nose (Scott, 2014). Although infections tend to result in low morbidity, scabby mouth is of particular importance to the Australian live export industry as quarantine implications have previously been related to the rejection of livestock when inspected on entry to destination markets (Keniry et al., 2003; Stinson, 2008). The voyage day effect noted for scabby mouth was indicative of cumulative disease incidence and the time periods associated with possible exposure to the virus when sheep are in the pre-export facility. Sheep showing clinical signs of scabby mouth are removed during pre-export inspections (Department of Agriculture Water and the Environment, 2020b); however, animals incubating viral infections may begin showing clinical signs and start shedding the virus while at sea. Environmental contamination then results in subsequent disease exposure of susceptible animals and a further increase in disease prevalence in the later stages of a voyage (McCarthy, 2012). Although the scabby mouth disease process has minor welfare implications, the impact of livestock rejection in foreign ports can be severe, therefore, it is crucial to document the prevalence of scabby mouth disease on voyages.

4.2. Feeding and water access

Nutritional management of sheep during sea transport is a significant welfare component (Hodge et al., 1991; Barnes et al., 2008). Feeding during sea transport is aimed to provide nutrients to maintain or increase body weight while also managing competitive feeding behaviour to reduce the risk of injury and smothering at feeding time (Fleming et al., 2020b). Ships have a finite fodder reserve onboard, and feed must be managed to ration available fodder according to the expected voyage length. Feed management is also an essential part of reducing the risk of heat stress, where management can include restricting or changing feed type in an attempt to minimise heat production from rumen fermentation (Jubb and Perkins, 2019). Feed allocation on Voyage B complied with minimum requirements for the provision of shipboard rations (Department of Agriculture Fisheries and Forestry, 2011) and had less overall fodder availability than Voyage A. This deficit was reflected by animals showing more jostling behaviour and pushing to attend troughs at feeding times. Our results demonstrate that recording pen-side behavioural observations can show the impacts of daily feeding management and have the potential to drive improvements in nutritional allocation management during sea transport. Although water access didn't vary sufficiently within the data set, the availability of clean water was recorded, and is recommended for inclusion in this welfare monitoring protocol.

4.3. Manure pad moisture

Manure pad integrity is an important aspect of good housing: providing animals comfort in resting and hygienic pen conditions (Collins et al., 2018a). Observations of manure pad moisture did not change markedly across either voyage. Dry and dusty manure pad scores in the early stages of the voyage were correlated with an increased prevalence of lameness. However, this finding may be confounded with loading injuries or associated with abrasion of hooves on the ship's flooring before the manure pad had formed (McCarthy and Banhazi, 2016). Manure pad moisture is affected by WBGT and will influence air quality (i.e., ammonia), which is likely to explain the correlations with Panting Score 4 and nasal discharge observed in this study. With very few recordings of high manure pad moisture scores, the detection of associated health and behavioural outcomes requires further testing over multiple voyages and larger sample sizes.

4.4. Sea swell

Sea swell is an animal welfare factor unique to the live export industry. There is little regulation around voyage planning to mitigate travel during heavy seas. Stress responses, such as increased stepping to correct balance, have been demonstrated by land-based studies on a small sample of sheep exposed to brief periods of simulated sea motion (Santurtun et al., 2014, 2015; Navarro et al., 2020). However, studies have not previously assessed the effect of sea swell on sheep behaviour and welfare during sea transport. In the present study, low sea swell scores were recorded on both voyages, and there was minimal correlation with behaviour. The application of a shipboard monitoring protocol over more varied sea conditions could provide valuable information about the effect of sea swell on sheep behaviour and welfare.

4.5. Summation and limitations of the study

As only two voyages were studied, it is not possible to determine the extent to which behavioural outcomes and disease prevalence were influenced by voyage factors or by livestock selection and management prior to loading. The health and behaviour indicators presented here can be compared to other covariate factors (such as water availability or air quality, i.e., ammonia) on future voyages. Animal behavioural changes are a dynamic response to environmental challenge and can signal

adverse conditions before health issues arise (Blokhuis et al., 2003). The data from this study (collected under standard commercial conditions) are limited in that extreme behavioural responses were not recorded. Data collection under a broad range of conditions is required to capture diverse behavioural responses before the meaning of such responses is apparent and their application in risk management can be achieved. A more extensive data set can help define contributing factors, compare disease prevalence across industry and domestic flocks, and help set thresholds for industry regulation and enable ongoing improvement in welfare outcomes.

5. Conclusion

The welfare of sheep transported from Australia by sea is a significant social concern. Where the transport of animals by sea occurs, there have been calls for improved monitoring and reporting of conditions. The health and behavioural measures piloted in this study reflected animal outcomes as sheep responded to changing conditions during sea transport. These findings show that a monitoring protocol using multi-criteria pen-side evaluation of animal outcomes can be used to gain insight into the impacts of sea transport on sheep welfare. Taking data on an industry-wide basis will allow comparisons of animal outputs between voyages and under different environmental conditions or resource access. More comprehensive welfare monitoring can also improve industry transparency and the understanding of the welfare implications of sea voyages.

Declaration of Competing Interest

We wish to draw the attention of the Editors to the following facts which may be considered as potential conflicts of interest and to significant financial contributions to this work. The presenting author was contracted to provide veterinary services as an Australian Government Accredited Veterinarian during the sea transport of the animals assessed in this study. Co-authors Teresa Collins and Anne Barnes undertake occasional advisory roles for the Australian Government Department of Agriculture, Water and the Environment, and all authors have received funding from Meat and Livestock Australia /Livecorp.

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