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ARTICLE

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Assessing animal welfare during a stranding of pygmy killer whales (*Feresa attenuata*)

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Abstract

Empirical assessment of cetacean welfare to inform stranding interventions is lacking. Here, potential welfare indicators are described for two stranded pygmy killer whales (*Feresa attenuata*), along with euthanasia procedures and pathology of potential relevance. The animals were filmed for 3.5 and 1.5 hr, respectively, allowing assessment of 19 indicators, including animal behaviors and human interventions. Eight interventions and 19 animal behaviors were identified; 17 and 11 behaviors were displayed by animal 1 and 2, respectively. Examination of ballistics euthanasia revealed atypical projectile placement and characterized animal behavioral responses, but welfare implications could not be assessed as insensibility was not verified in-field. Pulmonary edema and renal degeneration were documented in both animals; differential etiologies include ischemia–reperfusion, shock, and/or myopathy. Potential relationships among histopathology and welfare indicators are explored to infer affective experiences. For example, simultaneous head-lifting with respiration increased over time which, alongside pulmonary edema, suggests these animals experienced breathlessness. Other likely affective states include fatigue and discomfort; there are insufficient data to estimate the intensity or duration of these experiences or to provide an overall welfare grade/score. Further data are required to validate the proposed welfare indicators and to progress development of holistic approaches to welfare assessment at cetacean strandings.

KEYWORDS
animal welfare science, ballistics, behavior, cetacean, euthanasia, marine mammal, odontocete, pathology, stranded, welfare assessment

1 | INTRODUCTION

Live cetacean strandings are physiologically stressful situations where an animal is alive on the shore (Geraci & Lounsbury 2005; Moore et al., 2018). These animals can vary in their state from appearing outwardly healthy to those that are clinically ill or moribund (Arbelo et al., 2013; Câmara et al., 2020; Cowan & Curry 2008; Diaz-Delgado et al., 2018; Herráez et al., 2013). Regardless of their state, live strandings are life-threatening situations for cetaceans, which are poorly adapted to the terrestrial environment. Therefore, understanding the welfare state and survival likelihood of stranded cetaceans is crucial to inform appropriate decision-making for stranding response (Boys et al., 2022a; International Whaling Commission, 2016).

During live strandings, human intervention commonly occurs, generally with the aim of relocating and releasing or refloating as many animals as possible while improving animal welfare and increasing chances for postrelease survival through supportive care (Gales et al., 2008; Geraci & Lounsbury, 2005; Moore et al., 2018). First response interventions typically include minimizing pressure on internal organs by righting animals into a position on the sternum, and reducing the risk of hyperthermia and sunburn by covering animals with sheets and pouring water over the body, or reducing the risk of hypothermia in cold weather with dry blankets (Geraci & Lounsbury, 2005). Depending upon the animal's condition, it may then be relocated or refloated and released at sea; or undergo further intervention either through rehabilitation in captivity (where legal) or end-of-life procedures, including euthanasia or palliative care (Moore et al., 2007).

Although such response procedures have become common, there are limited data available to assess stranded cetacean welfare, survival likelihood or inform intervention decision-making (Gales 1992; Karns et al., 2019; Manire et al., 2018; Sampson et al., 2012; Sharp et al., 2014, 2016; Wells et al., 2013). Notably, in some regions, strandings response may not be coordinated under a management system. Even in regions where management policies and procedures are in place, protocols may lack the detail required to undertake decision-making (Boys et al., 2022d). This can lead to inappropriate intervention and unrealistic expectations from responders, particularly during discussions about emotive topics such as euthanasia (Boys et al., 2022d; Gales et al., 2008; Stockin et al., 2022). Delays to euthanasia and/or undertaking inappropriate interventions can prolong suffering of debilitated and/or moribund animals and cause further welfare compromise (Brownlow et al., 2015; Geraci & Lounsbury 2005; Perrin & Geraci 2008; Sharp et al., 2014).

However, the information required to inform end-of-life decision-making and guide euthanasia procedures is also lacking (Barco et al., 2016; Boys et al., 2021, 2022d). This may compound welfare concerns at stranding events where animals are deemed nonviable for refloatation. In cases where end-of-life procedures are necessary, evaluations should be undertaken by examining both animal-based (e.g., behavior) and resource–/management-based (e.g., equipment or personnel) indicators, to highlight potential welfare concerns and provide data to effectively address any welfare implications. In this way, intervention procedures can be further improved to ensure the best animal welfare outcomes possible.

Recently, major knowledge gaps for stranded cetacean welfare were identified by an international group of experts; these included interpreting behavioral/physiological parameters, diagnosing internal injuries, and euthanasia decision-making (Boys et al., 2022a). Notably, key barriers to assessing stranded cetacean welfare related to the limited data collection at strandings to inform decision-making (Boys et al., 2022a). These same experts suggested that assessments of stranded cetacean welfare may be undertaken in a structured, systematic manner by applying the Five Domains Model for assessing animal welfare (Mellor et al., 2020). In this framework, observable/measurable indicators are organized into four domains reflecting different aspects of the animal's welfare: three physical/functional domains relating to the animal's nutrition, physical environment, and health/functional status, and one situation-related domain relating to the animal's goal-directed behavioral interactions with its environment and other animals including humans. That information is used to *infer* the animal's associated mental/affective experiences in the fifth domain (Beausoleil & Mellor 2017; Beausoleil et al., 2018; Mellor & Beausoleil, 2015). These various mental/affective experiences, arising due to the animal's own perception of its health and physical state and its environment, are considered to most directly reflect its overall welfare state at any point in time (Mellor et al., 2020).

The international experts highlighted various animal and resource−/management-based indicators as valuable for assessing welfare (Boys et al., 2022b). A variety of these indicators, including animal body condition, respiration rate, body posture, behavior, and human intervention types, were found to be feasible to assess from video taken at live stranding events of long-finned pilot whales (*Globicephala melas edwardii*) (Boys et al., 2022c). However, additional assessment of similar indicators for other species in variable stranding contexts would provide further information on how they reflect animal welfare state. The collection of such data can then be used to scientifically validate observed indicators and develop a welfare grading system (Harvey et al., 2020).

The collection of pathological data and samples from individuals that do not survive a stranding but are monitored antemortem could be used to correlate features of internal physical state and externally observable indicators (e.g., behavior) to enhance understanding of welfare state antemortem (Câmara et al., 2020; Camps et al., 2019). Additionally, evaluation of behavioral and physiological indicators during euthanasia could provide information on the welfare implications of end-of-life procedures. Such welfare-relevant information would improve decision-making and effective application of euthanasia methods at future stranding events.

Many indicators that are likely relevant for assessing welfare have been evaluated in biology/health studies of small odontocetes. Most of these relate to the health or functional status of the animal, for example evaluation of body condition and wounds, cardiac and respiratory rates, and serum chemistry and/or hematological variables (Manire et al., 2018; Sampson et al., 2012; Sharp et al., 2014, 2016). Previous behavioral observations have focused on animal alertness or responsiveness and tended to note only those behaviors suggested to be indicative of significant physiological stress, such as arching and thrashing (Sampson et al., 2012; Sharp et al., 2016; Townsend 1999). Knowledge of the prognostic importance of these indicators, in particular hematological and serum chemistry parameters, has been enhanced by these studies through correlation with known deaths (Manire et al., 2018; Sharp et al., 2014) and postrelease survival data (Gales et al., 2012; Manire et al., 2018; Sampson et al., 2012; Sharp et al., 2014, 2016; Wells et al., 2009).

These studies suggest that the state of stranded animals can vary from those without significant abnormalities through to those that are clinically moribund (Sampson et al., 2012; Sharp et al., 2014). Such results highlight the importance of considering both preexisting and stranding-induced conditions, such as capture myopathy, when assessing the welfare state and likely survival of stranded cetaceans. Indeed, Sharp et al. (2014) noted that animals least likely to survive tended to exhibit acidosis, dehydration, and lower body condition when compared to surviving counterparts. However, in some cases health assessments were unable to distinguish between clinically healthy and questionable candidates for release, suggesting that further refinement of stranded cetacean assessments is required (Sharp et al., 2014, 2016). Until now, such studies have given little focus to cetacean behavior during stranding, and the indicators assessed have usually not been interpreted in terms of welfare state or integrated into a systematic, holistic structure for welfare assessment.

To the best of our knowledge, the only existing approach to assessing cetacean welfare in a structured and holistic way is the C-Well framework (Clegg et al., 2015). This is based on the European Union's Welfare Quality framework for assessing farm animal welfare and includes both animal- and resource-/management-based indicators that reflect similar dimensions of welfare to those included in the Five Domains: nutrition/hydration, health, physical environment, and social interaction (Clegg et al., 2015). However, many of the indicators cannot feasibly be measured or are irrelevant in the context of strandings because animals are in an abnormal environment where normal function, natural behaviors, and agency cannot be expressed.

Here we apply the recently gained knowledge on welfare assessments for live stranded cetaceans (Boys et al., 2022a,b) to a live stranding event of pygmy killer whales (*Feresa attenuata*). Specifically, we aim to describe the physical state, behavior, and conditions of live stranded pygmy killer whales to make preliminary inferences about their welfare state, using a holistic system developed for another odontocete species (Boys et al., 2022c). We further explore the relationship between externally observable indicators displayed by live animals and histopathological changes evident postmortem, to better interpret the live indicators displayed in the context of the stranding. Due to the context of this specific stranding event, we further describe the application of, and the animals' behavioral responses to, ballistics euthanasia, to make inferences about the welfare implications of this killing method. These types of preliminary data can be used to highlight potential experiences that may arise during stranding, focusing future research, and also provide important insights into welfare concerns associated with end-of-life procedures. These data are important to further develop a generally applicable welfare assessment framework for cetacean stranding events.

2 | METHODS

2.1 | Stranding event

A mass stranding of pygmy killer whales occurred on the coast of the North Island, New Zealand in March 2020. To the best of our knowledge, there were no environmental or anthropogenic factors occurring at the time which may have contributed to the stranding. All animals ($n = 4$) were initially refloated, although one individual (adult male) restranded almost immediately and was subsequently euthanized by the agency responsible for managing stranding events, the Department of Conservation (DOC). The following day, beach patrols were conducted at first light to search for any other restranded animals. Two live restranded adult males (Animals 1 and 2) were located approximately 1 km north of the original stranding. Animal 1 was found alive in the shallows, buffeted by the incoming waves, while Animal 2 was discovered in the estuary floating on its side over rocks with the blowhole fully submerged. Animal 2 was unable to remain upright without assistance. The sea state hindered an immediate refloat attempt, and as the animals were judged by DOC to show signs of deterioration, they made the decision to euthanize in consultation with members of a subtribe (hapū) of local indigenous Māori (Patuharakeke). Euthanasia was undertaken by a warranted DOC officer via ballistics, following the Standard Operating Procedure (SOP) that guides strandings management in New Zealand (Boren, 2012; Boys et al., 2021, 2022d). Following euthanasia, Patuharakeke permitted opportunistic postmortem sampling of some tissues prior to burial. All tissue samples were fixed in the field in 10% buffered formalin and subsequently submitted for histopathology.

2.2 | Data collection: Welfare indicators

Prior to euthanasia, we collected data from the two restranded animals on a number of indicators reported to be valuable for understanding stranded cetacean welfare state and to be practically measurable from video footage (Boys et al., 2022b,c). As in the previous study, indicators were selected to represent multiple dimensions of animal welfare, as described by the Five Domains Model for animal welfare assessment (Mellor et al., 2020). Briefly, indicators were selected to represent each of three physical/functional domains (nutrition, physical environment, health) and the situation-related domain (behavioral interactions) (Table 1). According to the model, this kind of observable information is used to infer the mental/affective experiences of the animal in the fifth domain, which are most directly relevant to understanding its welfare state (Beausoleil & Mellor 2017; Beausoleil et al., 2018; Mellor & Beausoleil, 2015).

These selected indicators were split into two categories: animal-based indicators that directly reflect the animal's state (welfare status indicators), and animal- and resource-/management-based indicators that represent *risks* to

TABLE 1 Potential welfare indicators organized into the three physical/functional domains and one situation-related domain of the Five Domains Model for welfare assessment (Mellor et al., 2020) following Boys et al. (2022b, c). Within each domain, indicators are organized according to whether they provide direct information on the animal's welfare status or welfare risk (alerting) information.

	Indicators	
Domain	**Welfare status**	**Welfare alerting**
1: Nutrition	Body condition	Animal age class
2: Physical environment	Skin condition/blistering	Initial strand versus Restrand Dry-strand versus In-water strand Availability of equipment Substrate type Weather (including UV intensity), sea, and tidal conditions
3: Health	Signs of trauma, injuries Signs of skin illness and disease Respiration rate and character/effort Bleeding/fluids/mucus from orifices Eye condition Animal's level of response to stimuli/reflexes Body posture	
4: Behavioral interactions	Movements and behaviors[a] Vocalization	Presence and status of pod members Number of responders Type and duration of human interaction[a]

[a]Composite behavioral categories that include multiple indicators (described in Tables S1 and S2).

welfare (welfare alerting indicators). These can be used to provide context when interpreting welfare status indicators (Harvey et al., 2020). Indicators deemed feasible to assess in the stranding context (Table 1) were examined for each live focal individual ($n = 2$) via direct observations in-field during the stranding event and poststranding using video footage collected during the event.

Video footage was collected using two GoPro Hero 7 Black cameras per animal. Each camera was mounted 0.5 m high on a pole placed ~1.5 m in front and to each side of the head of the animal, aimed toward the animal at a 0°–45° angle (Figure 1). Filming occurred at 720 px and 60 fps in wide-angle view, which along with the camera placement, ensured that the entire body was filmed bilaterally. Filming commenced as soon as each individual had been safely (with regards to both human and animal welfare) placed in ventral recumbency and provided with necessary first aid (i.e., water cooling and covering in wet sheets). Animal 1 and Animal 2 were filmed continuously for 3.5 and 1.5 hr, respectively. Filming duration was dictated by ongoing assessment of the animals by DOC until euthanasia occurred. Permission was granted by DOC and Patuharakeke for video monitoring to continue throughout the cetacean euthanasia procedures.

2.3 | Welfare indicator assessment

Data were collected for welfare indicators following Boys et al. (2022c) as summarized below. Animal behaviors and human interventions were examined via video footage only, while eye condition and animal reflexes were examined by in-field observations only. All other welfare indicators were examined both in real-time in-field and retrospectively via video footage. Video footage was examined manually at 0.8× speed by one observer (R.M.B.) at least twice to identify all cetacean behaviors and human interventions. Additionally, a subset of videos was examined by two independent observers to confirm animal behavior and human intervention classification. Behaviors were not mutually exclusive, with multiple events occurring simultaneously.

FIGURE 1 Two GoPro cameras (highlighted in red circles) mounted cranio-laterally and angled caudally to enable continuous assessment of both sides of the body of the pygmy killer whale (*Feresa attenuata*) Animal 1 during stranding. Note the right lateral curvature of the peduncle. Photo credit: Rebecca M. Boys.

2.3.1 | Domain 1: Nutrition

Body condition score was assessed by the shape of the epaxial musculature and concavity of the nuchal crest (IJsseldijk et al., 2021; Joblon et al., 2014); animals were scored as emaciated, thin, normal, or robust (Boys et al., 2022c). Age class was based on body length and sexual maturity. Animals were classified as adult (≥236 cm and/or sexually mature following Clua et al., (2014), juvenile (over one third of the length of an adult), or calf (less than one third of the length of an adult and/or with visible fetal folds).

2.3.2 | Domain 2: Physical environment

Skin blistering was assessed qualitatively based on the presence of epidermal necrosis, cutaneous bullae formation, or epidermal sloughing and erosions/ulceration (Boys et al., 2022c; Groch et al., 2018). Data on the stranding circumstances were also recorded; animals were classified as either initial or restranded, and as either dry-stranded or in-water stranded. Equipment availability was noted based on the use of sheets over the animals' body and water being poured over the animal for cooling, as well as the availability of spades for digging, and refloatation mats. The substrate type, weather, sea swell, and tidal conditions were also observed (Boys et al., 2022c), with those most prevalent (>50% duration) throughout the filmed stranding period being recorded. UV intensity was based on the UV index calculated by National Institute of Water and Atmospheric Research (National Institute of Water and Atmospheric Research, 2020) for the specific date, time, and location of the stranding.

2.3.3 | Domain 3: Health

External injuries were qualitatively assessed based on presence of superficial or hypodermal wounds and the area of the body involved. Superficial wounds involved the epidermis only, while in the hypodermal wounds the blubber was visible. Skin disease was considered based on the presence or absence of cutaneous lesions following Van Bressem et al. (2007).

Respiration rate was measured based on visual observation of opening and closing of the blowhole in combination with audible exhalation (Kremers et al., 2016). Respiration rate was measured in-field for a period of 60 s every

30 min to monitor changes over the course of the stranding. Additionally, an average respiration rate across the total monitored period per individual was calculated from video footage. Respiratory abnormalities were qualitatively assessed by examining for immediate inhalation following exhalation (Martins et al., 2020; Mazzariol et al., 2015) and other unusual respiratory occurrences e.g., chuffing (Fire et al., 2020; Lusseau, 2006; Mazzariol et al., 2015).

Additionally, in-field observations were conducted every 30 min to examine the observable orifices for discharge, mucus, blood, feces, and/or vomiting; these were recorded as present or absent and based on the orifice of origin. Finally, the open eyes were examined for trauma and presence of any ocular abnormalities following Colitz et al. (2016) and Colitz (2019). Anomalies were recorded as either present or absent for each eye.

In the field, individual responsiveness was assessed every 30 min by testing the palpebral reflex (by gently tapping near the eye and looking for a blink response) and blowhole response (by gently pressing around the edges of the blowhole and examining for blowhole tightening). Additionally, the menace response was also monitored (Butterworth et al., 2004b), by rapidly moving the flattened palm of the hand toward the open eye and examining for a blink response or withdrawal of the eye into the socket. Each of these reflexes was assessed three times at every 30-min testing point. Reflexes were considered present if observed at least twice at the test point, reduced if only observed once, or absent if no response was observed. Such reflexes have been shown to be reliable indicators of sensibility in cetaceans and should not be influenced by learned behaviors or human presence (Butterworth et al., 2004b).

Body posture was assessed based on the recumbency position being ventral or lateral. Presence and type (lateral, dorsal, ventral) of body curvature was recorded (Boys et al., 2022c).

2.3.4 | Domain 4: Behavioral interactions

The frequency or duration of various behaviors, including vocalization, was quantified. Behaviors were identified and coded per second using BORIS v7.9.6 (Friard & Gamba, 2016) by applying the stranded odontocete ethogram (Boys et al., 2022c; Table S1). Respiration rate was also included in this ethogram (Boys et al., 2022c).

The presence and status of conspecifics were recorded as (1) stranded or floating and (2) alive or dead. Additionally, information on conspecific age class and sex were collected where possible. Finally, human intervention was characterized based on the number of people and following the ethogram constructed for intervention types (Boys et al., 2022c; Table S2). Duration of each intervention type was quantified based on its occurrence per animal and was coded in BORIS. Human intervention occurred when a human was within ~2 m of the focal cetacean.

2.4 | Data collection: Euthanasia

Video footage was reexamined, from 2 min prior to the initial shot, at 0.5× speed using BORIS to understand the ballistics euthanasia procedures applied. Data collected on the application of, and animal's response to, ballistics euthanasia were: orientation of firearm discharge (dorso-ventral or lateral; Hampton et al., 2014; International Whaling Commission, 2014), approximate projectile entry location based on animal anatomical features, number of shots, and fine-scale animal behavior during and post euthanasia. All animal behaviors were characterized and coded per second in BORIS and quantified based on their frequency or duration.

2.5 | Data collection: Postmortem sampling

The requirement for an immediate onsite burial prevented a systematic postmortem examination. However, external morphology and biometric measurements were recorded (see Tables S4 and S5) and basic, opportunistic in-field sampling was permitted during cultural flensing. Tissue samples collected for histopathological assessment included skeletal muscle (*longissimus dorsi*), taken at the epaxial section in-line with the dorsal fin, liver, kidney, lung, spleen, and bladder. The stomach was opened in situ, and chambers emptied and examined for contents and macro debris. Any gross obstructions and lesions were noted, and all prey remains were collected and subsequently identified where possible. The testes were extracted whole and subsequently sectioned and examined histologically to determine sexual maturity following Betty et al. (2019; see supplementary material).

All tissue samples were fixed in 10% buffered formalin and subsequently processed by standard methods into paraffin blocks, cut into 5-μm-thick sections, then stained with hematoxylin and eosin and submitted for histopathology. In addition, Perl's iron staining was used to differentiate lipofuscin from hemosiderin in sections of liver and kidney (Orchard, 2018).

2.6 | Data analysis: Welfare indicators

A quantitative assessment of behavioral and physiological indicators was conducted for each focal individual following Boys et al. (2022c). Briefly, the frequency and duration of animal behavioral and physiological parameters, and the duration of human intervention were calculated while accounting for video duration. All parameters were classified as either point events (nonvariable duration) or states (variable duration). To allow qualitative visualization of trends in behavior over time, the frequency of point events and duration of state parameters were mapped graphically and described over the monitored stranding period. In addition, the frequency of point events was calculated as the number of occurrences per minute over the total observation period and for each 30-min period for the focal animal to further explore changes over time. The duration of state parameters was calculated as the percentage of the total filming time and percentage of each 30-min period that the state occurred in each focal animal to allow for trends in behavior over time to be examined.

3 | RESULTS

3.1 | Welfare indicator assessment

Video footage was collected for 3.5 and 1.5 hr, respectively, for Animal 1 and Animal 2. Both sides of the body were observed for both focal animals throughout the filmed stranding period.

3.1.1 | Domain 1: Nutrition

Both individuals were evaluated to be in normal body condition, yet minimal stomach contents were present; there were only a few fisheye lenses. Both were classified as adults, based on size and sexual maturity (see Table S6, Figures S1 and S2).

3.1.2 | Domain 2: Physical environment

An external morphological assessment of both individuals detected no skin blistering of observable surfaces. A priori information through photo-identification revealed both to be restranded individuals, having been refloated the night prior. Both were dry stranded at the time of our assessment. Standard stranding response equipment available included sheets to cover the animals and buckets to pour water over the animals, spades, and one refloating mat. The substrate type was identified as sandy for both individuals. The stranding occurred at the end of austral summer

(March), with a maximum UV index considered to be high (6.4) recorded at the time of euthanasia. Prevailing weather and sea conditions were overcast, with medium to large swell on an outgoing tide.

3.1.3 | Domain 3: Health

Animal 1 was found to have no obvious injuries nor visible skin disease lesions. The respiration rate assessed in-field remained constant throughout the monitored period (range: 2.5–3.7 breath/min, $SD = 0.4$) and the average rate across the 3.5 hr monitoring was considered within the normal range for delphinid species (3.0 breath/min). However, double chuffing respiration, with two forceful exhalations prior to inhalation was noted in 16% of respiratory events ($n = 88$). No discharge from the blowhole or mouth was observed, and no vomiting or feces were produced by this animal throughout monitoring. Eye condition was also normal, with no trauma or abnormalities noted.

Animal 2 also had no significant injuries or trauma on gross examination, though superficial abrasions on the tail flukes, dorsal fin, and rostrum were evident (Figure 2). Skin condition was considered normal, and no skin disease lesions evident. The respiration rate assessed in-field was considered marginally elevated for delphinid species (6 breaths/min) at the start of observation, but at subsequent assessment (30 min into stranding) it had reduced and then remained constant (range: 2.0–3.0 breaths/min, $SD = 0.5$) for the remainder of the observation. The average respiration rate across the 1.5 hr monitoring period was within normal range (2.6 breath/min). No abnormal respiratory character was observed. No visible discharge from the blowhole or mouth was noted, and no vomiting or feces were produced throughout the monitoring period. No visible trauma or abnormalities were observed in either eye.

Animal 1 displayed palpebral reflex and menace response consistently (i.e., positive response three times at each test point) throughout the stranding, until the final hour of life. At this point, both reflex responses were reduced (i.e., present once) for the final two test points. However, the blowhole response was absent throughout the observation period. In contrast, although the blowhole and palpebral reflexes were present (i.e., positive response twice at each test point) in Animal 2 for the entire monitored period, the menace response was absent.

Animal 1 was in ventral recumbency throughout the observation period, with right lateral curvature of the peduncle beginning approximately 2 hr into monitoring (Figure 1). No postural changes (e.g., leaning) were observed at the time of curvature. Animal 2 was also in ventral recumbency throughout, though no body curvature was observed.

3.1.4 | Domain 4: Behavioral interactions

Of the 30 behaviors characterized in Boys et al. (2022c; Table S1), 19 were observed in this study (Figures 3 and 4). Fine-scale data on the behavioral and one physiological indicator (respiration) across the monitored period are provided in section 3.1.5. Audible vocalizations were not evident from either individual.

Both animals formed part of a mass stranding ($n = 4$). Aside from the focal animals being monitored, there was one dead conspecific present, an adult male. However, none of the individuals were in visual contact with each other. Eight types of human intervention were identified (Table S2) and all occurred with both animals. Further details and quantification of human intervention are provided in section 3.1.6.

3.1.5 | Quantitative assessment of behavioral indicators

Seventeen behavioral indicators were recorded for Animal 1 over the 3.5 hr observation period: three point events and 14 state behaviors (Figure 3). A total of 11 behavioral indicators were recorded for Animal 2 over the 1.5 hr observation period: two point events and nine state behaviors (Figure 4). The frequency and duration of behaviors

TABLE 2 Frequency (rate/minute) and relative duration (% of observation period) of behavioral events displayed by two live stranded pygmy killer whales (*Feresa attenuata*) during the total monitoring period (Animal 1: 3.5 hr observation period and Animal 2: 1.5 hr) and in the first and last 30 min of the monitoring period. See Table S1 for descriptions of behaviors.

	Overall frequency		Overall relative duration		Animal 1: Frequency		Animal 1: Relative duration		Animal 2: Frequency		Animal 2: Relative duration	
Behavior	Animal 1	Animal 2	Animal 1	Animal 2	0–30 min	180–210 min	0–30 min	180–210 min	0–30 min	60–90 min	0–30 min	60–90 min
State behavior												
Tail flutter			97.4	6.3			96.7	95.7			0.0	23.0
Dorsal fin flutter			46.6	15.8			36.3	14.6			25.0	4.2
Tail lift			46.6	0.3			45.6	57.5			0.6	0.0
Tail hover			43.2	1.6			40.3	31.4			0.1	0.0
Head side-to-side			35.5	26.2			13.1	65.2			20.2	33.3
Head lift			14.6	13.4			15.7	19.9			6.9	24.6
Body tremble			5	<0.01			13.6	0.8			0.0	0.0
Body rocking			1				0.0	5.9				
Pec fin flutter R			<0.01	0.3			0.0	0.0			0.0	1.0
Tail arch			7.2				11.3	8.2				
Pec fin flutter L			6.5				15.0	2.5				
Tail side-to-side			6.5				13.8	1.0				
Head arch			2				9.2	0.7				
Body tenses			0.1				0.0	0.4				
Mouth open				0.3							0.6	0.2
Point behaviors												
Blowhole twitch	0.5	0.2			0.8	0.1			0.2	0.3		
Movement in lower jaw	0.2	3.6			0.6	0.1			1.2	1.2		
Water from blowhole	0.1				0.0	0.0						

FIGURE 2 Superficial abrasion on the rostrum of pygmy killer whale (*Feresa attenuata*) Animal 2 during stranding. Photo credit: Rebecca M. Boys.

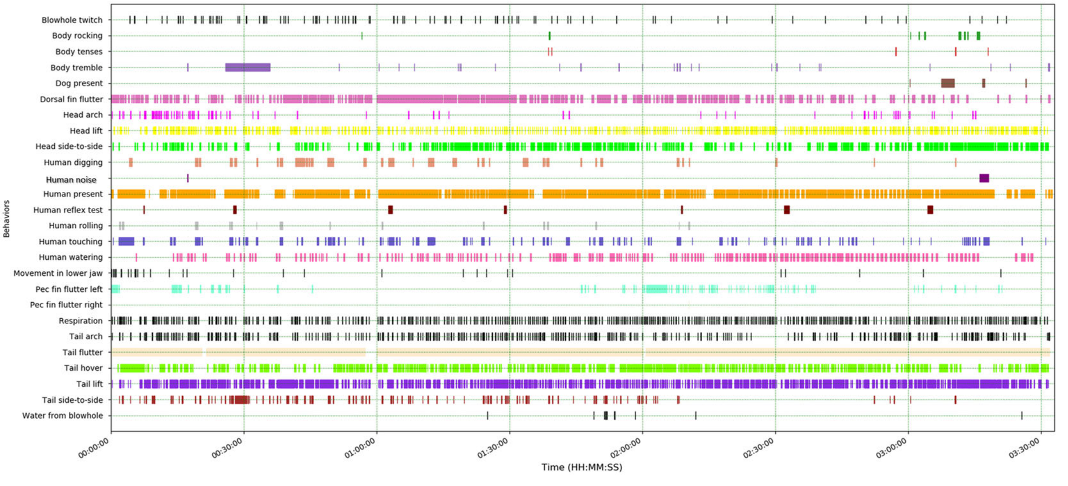

FIGURE 3 Frequency and duration of behavioral events displayed by Animal 1, a live stranded pygmy killer whale (*Feresa attenuata*), and the associated human interventions occurring with this animal. See Tables S1 and S2 for descriptions of behaviors and interventions. Only behaviors expressed by, or interventions occurring with, this animal at least once are displayed.

varied between individuals. In general, Animal 2 was less behaviorally active than Animal 1 over the observation period. For those behaviors observed in both individuals, Animal 1 generally displayed them at a higher rate of occurrence or for longer duration (Table 2).

For Animal 1, few clear trends were observed in the frequency or duration of behavior over the 3.5 hr observation period (Figure 3). Blowhole twitch and movement in the lower jaw appeared to decrease in frequency over time. In terms of state behaviors, more time was spent body rocking and tensing and moving the head side to side towards the end of the monitored period, while dorsal fin fluttering and moving the tail side to side decreased. The duration of head lift, tail arch, tail hover, tail lift, and tail flutter remained relatively consistent across the monitored stranding. Indeed, tail fluttering occurred almost constantly.

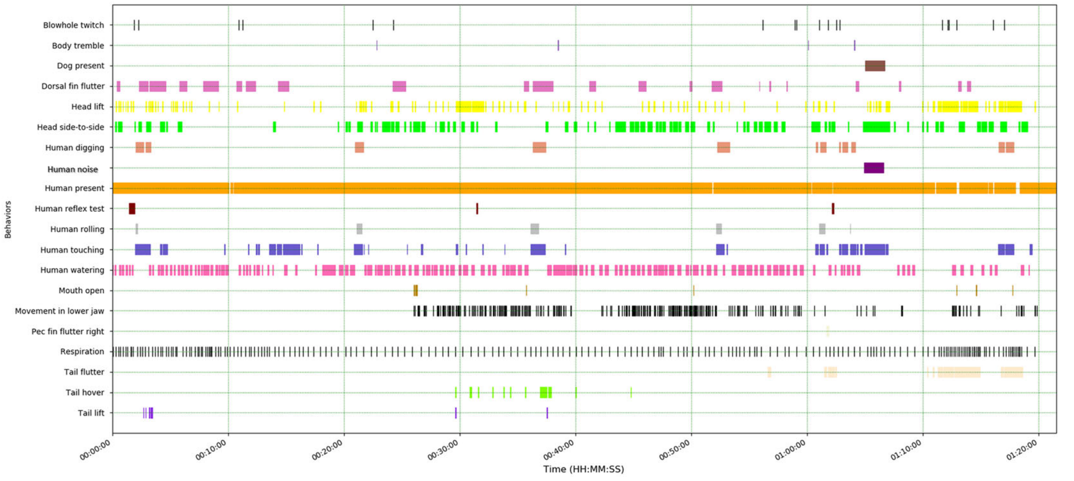

FIGURE 4 Frequency and duration of behavioral events displayed by Animal 2, a live stranded pygmy killer whale (*Feresa attenuata*), and the associated human interventions occurring with this animal. See Tables S1 and S2 for descriptions of behaviors and interventions. Only behaviors expressed by, or interventions occurring with, this animal at least once are displayed.

For Animal 2, movement in the lower jaw began approximately 25 min into the observation period and was frequently expressed for about half an hour, after which time it became less frequent (Figure 4). Dorsal fin fluttering occurred throughout the observation period, but the bouts appeared to decrease in duration over time. More time was apparently spent head lifting and moving the head from side to side over the observation period. Tail flutter only began to occur in the final 30 min of stranding and seemed to increase in duration over this time.

For Animal 1, 92% of the 76 head arching events occurred simultaneously with tail arching. Similarly, in Animal 1, 81% of 554 tail lifting events occurred simultaneously with head lifting. Interestingly, the relative duration of such simultaneous tail and head movements in Animal 1 increased over the observation period, occurring for just 25% of the first 30-min period of observation to 70% of the final 30 min. For both individuals, head lift occurred simultaneously with most respiration events. This synchrony occurred in 71% and 60% of respiration events for Animal 1 and Animal 2, respectively. Notably, this synchrony generally increased in frequency across the observation period for both animals, from approximately 20% of respiration events in the first 30 min of observation to >70% in the final 30 min.

3.1.6 | Quantifying human intervention

Seven personnel attended the stranding throughout the entire data collection period and a further six were present during the final monitored hour. These people included six indigenous Māori of the Patuharakeke subtribe who, together with the four DOC staff, undertook decision-making at the stranding, as well as two members of the public and one researcher (R.M.B.). For both cetaceans, eight types of human intervention occurred, with the duration of each intervention varying between focal animals (Figures 3 and 4). A total of 527 and 232 discrete human interventions occurred with Animal 1 and Animal 2, respectively. Humans were present with both focal animals (within ~2 m of an animal) for much of the observation period, approximately 2.6 hr of total 3.5 hr, and 1.3 hr of total 1.5 hr, respectively. In general, the relative duration of human intervention was lower for Animal 1 than Animal 2.

For Animal 1, human presence remained relatively constant. Time spent digging, rolling, and touching by humans generally decreased over time, whereas time spent watering increased (Figure 3, Table 3). Interestingly, in the final

TABLE 3 Relative duration (%) of human interventions occurring with two live stranded pygmy killer whales (*Feresa attenuata*) during the total monitoring period (Animal 1: 3.5 hr observation period and Animal 2: 1.5 hr) and in the first and last 30 min of the monitoring period. See Table S2 for descriptions of intervention types.

	Relative duration (% of observation)					
	Animal 1			Animal 2		
Human intervention	Overall	0–30 min	180–210 min	Overall	0–30 min	60–90 min
Present	74.4	71.4	70.0	97.9	99.3	93.8
Watering	23.6	9.9	21.5	36.4	40.2	16.0
Touching	12.2	20.1	7.6	15.7	17.5	25.1
Digging	8.1	7.9	0.3	8.2	6.1	12.4
Rolling	1.8	4.8	0.0	2.7	1.9	2.2
Dog present	1.6	0.0	10.6	2.1	0.0	7.9
Noise	1	0.7	6.0	2.1	0.0	7.9
Reflex test	1.3	1.8	1.8	0.5	1.1	0.5

30 min of observation, when the number of humans was highest, a dog (*Canis familiaris*) was also present and there was human noise, Animal 1's expression of body rocking, tensing, and head side to side behavior increased.

For Animal 2, human presence was almost continuous throughout the observation period. Generally, time spent digging increased over time, whereas watering decreased from 40% in the first 30 min to 16% in the final 30 mins of observation (Table 3). Rolling of the animal remained relatively constant, while touching decreased and then increased over time (Figure 4, Table 3). Notably, during the last 30 min of monitoring, there was increased human presence, associated noise, and presence of a dog. During this period Animal 2's side to side head movements increased and the longest continuous bout of this behavior was observed.

3.2 | Euthanasia assessment

Dorso-ventral orientation for firearm discharge was applied to euthanize both individuals using a Bergara .308 caliber rifle. It was determined a priori that a minimum of three shots would be employed per animal, each shot involved a Winchester soft-point 150 gr projectile. Firearm discharge occurred with the marksperson standing in front of the animal, with the firearm muzzle positioned ~50 cm above and in front of the animal's head, angled at approximately 70° ventro-caudally along the dorsal midline, aiming just caudal to the blowhole for all three shots (Figure 5). The first projectile for both focal animals entered anterior to the blowhole and likely into the melon based on the observation of clear fluid expelled from the projectile entry site in both individuals. For both animals, the second projectile entered the blowhole and the third was caudal to the blowhole. Notably, criteria for assessing insensibility were not examined in-field for either animal following application of the euthanasia method. A total of 10 animal behavioral responses were characterized during and post ballistics euthanasia from the video footage collected (Table S3).

For Animal 1, the time between the initial and secondary shot was 8 s, and between the first and final shot was 16.5 s. Stiffening of the peduncle, tail fluttering, tail arching, dorsal fin flutter, and body tremble occurred after every shot (Figure 6). Jaw open (slack lower jaw with mouth agape) occurred after the initial shot, while agonal convulsions (unprovoked violent, rapid thrashing movements), lasting 7.5 s, only commenced after the third shot. Relaxation of the epaxial musculature occurred 13.8 s after the final shot. Tail lifting was observed in this animal 35.3 s after the final shot and continued for 27 s.

FIGURE 5 Firearm muzzle for euthanasia was in front of the pygmy killer whales (*Feresa attenuata*) on the dorsal midline using the blowhole as an anatomical landmark. Photo credit: Rebecca M. Boys.

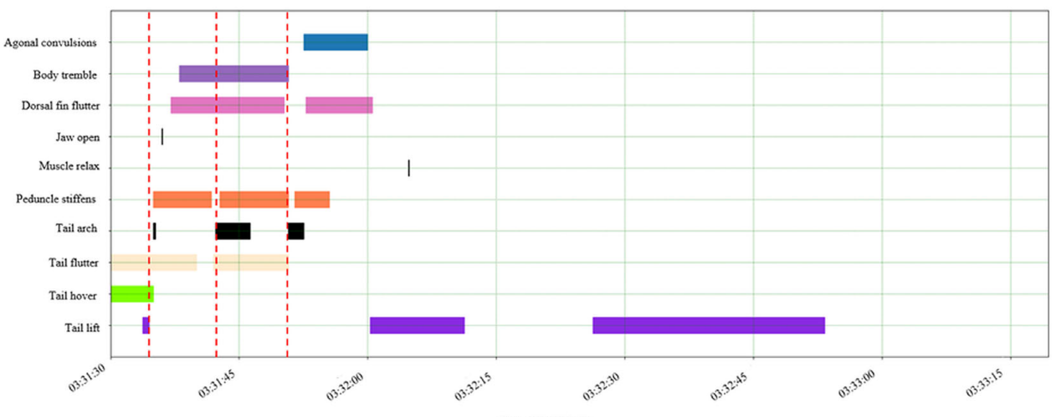

FIGURE 6 Behavioral events related to euthanasia of pygmy killer whale (*Feresa attenuata*) Animal 1. Each shot is indicated by a red dashed line.

For Animal 2, the time between the initial and secondary shot was 6.8 s, and between the initial and final shot was 14.8 s (Figure 7). Stiffening of the peduncle, tail fluttering, and tail lifting occurred after every shot. Additionally, body tremble occurred after the initial and second shots were discharged (Figure 7). Agonal convulsions occurred only after the third shot, lasting 3.3 s, followed by observation of the open jaw. Relaxation of the epaxial musculature only occurred 16.8 s after the final shot. Tail fluttering began with agonal convulsions after the third shot and continued for 52.5 s. Dorsal fin fluttering, which began after the initial shot, continued for an additional 90.7 s following the third shot.

3.3 | Histopathology

The lungs of both animals displayed marked diffuse congestion and the presence of proteinaceous fluid (edema) within multiple bronchioles and alveolar spaces. A mild increase in the number of alveolar macrophages and small

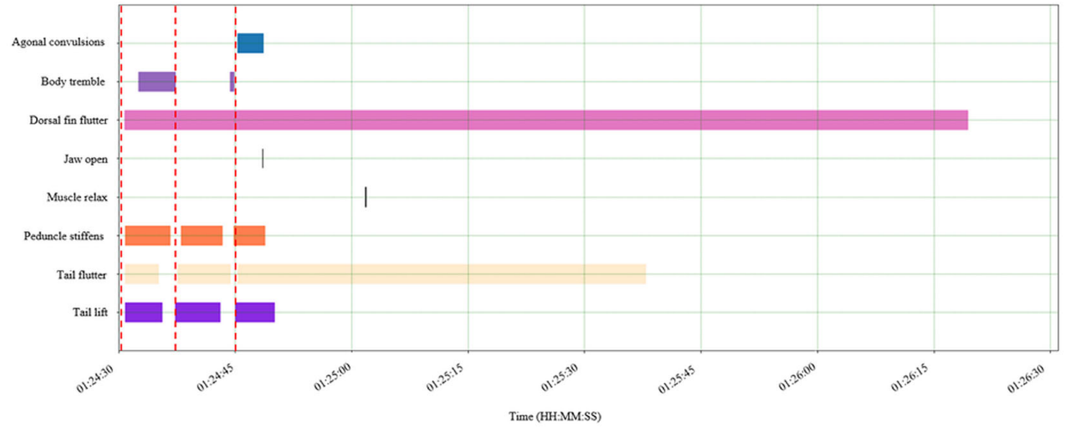

FIGURE 7 Behavioral events related to euthanasia of pygmy killer whale (*Feresa attenuata*) Animal 2. Each shot is indicated by a red dashed line.

numbers of erythrocytes were also noted. Additional patchy over-expansion of subpleural alveolar spaces was further evident. Within the hepatic tissues of both animals, large numbers of Kupffer cells contained iron in the form of hemosiderin, which was confirmed with a Perl's Iron stain. Similar pigment was not observed within hepatocytes.

Renal tissue sampled from both individuals revealed cytoplasmic swelling and vacuolation of the proximal renal tubular epithelial cells. No convincing evidence for intracytoplasmic myoglobin droplets or myoglobin casts within tubular lumina were evident, but many of these cells did contain iron in the form of hemosiderin which was confirmed with a Perl's Iron stain. Additionally, Animal 2 displayed rhabdomyolysis in the *longissimus dorsi* muscle; this was evidenced by multiple individual and small groups of myofibers exhibiting loss of cross striations, fragmentation, and hypereosinophilia of the sarcoplasm with pyknosis.

4 | DISCUSSION

Because stranding responses aim to optimize animal welfare, the scientific assessment of welfare indicators, euthanasia procedures, and stranding-associated pathology is crucial to inform decision-making. By applying the principles of welfare assessment articulated by Boys et al. (2022c) in the context of cetacean strandings, this study provides further evidence of the ability to undertake holistic assessments using a variety of potential noninvasive welfare indicators. Opportunistic collection of tissue samples postmortem enabled exploration of potential relationships among external welfare indicators and histopathology to support inferences about welfare state. Additionally, this study contributes a fine-scale evaluation of the application of, and animals' behavioral responses to, ballistics euthanasia procedures for stranded cetaceans. A systematic postmortem examination was not possible in this instance, limiting our ability to observe gross organ changes. Future work should undertake full examinations whenever possible, to understand any preexisting conditions that may influence the likelihood of surviving stranding interventions and to facilitate greater understanding of how externally observable parameters might reflect internal pathology.

4.1 | Preliminary welfare assessment: Welfare indicators

Potential indicators reflecting various animal welfare dimensions were assessed in an attempt to holistically understand the welfare state of these stranded pygmy killer whales. The Five Domains Model for assessing animal welfare was used to structure the collection of data, with indicators representing each of the three physical/functional domains (nutrition, physical environment, and health) and one situation-related domain (behavioral interactions) of the model (Mellor et al., 2020). Welfare status indicators provided direct information on the potential state of the animal at the time of assessment, while welfare alerting indicators were examined to provide further context for interpretation of those welfare status indicators and to highlight potential future welfare risks (Harvey et al., 2021). Importantly, the indicators assessed here were non- or minimally invasive, reducing the likelihood of inducing additional welfare compromise to physiologically stressed animals (Câmara et al., 2020; Cowan & Curry, 2008). While the application of hematology and serum biochemistry are informative for assessing the health status of animals (Barratclough et al., 2019; Schwacke et al., 2014; Sharp et al., 2016), noninvasive measures, where possible, are preferable in welfare assessments.

In terms of nutritional status (Domain 1), both animals were considered in normal body condition for adult males of this species, as has been reported in other mass stranding events (Bogomolni et al., 2010; Gales, 1992; Sampson et al., 2012). However, stomach contents revealed few prey remains. In contrast, stomach contents have commonly been reported at previous strandings involving this species and suggest a diet of cephalopods and fish (Elorriaga-Verplancken et al., 2016; Leatherwood & Reeves, 1989; Mignucci-Giannoni et al., 2000; O'Dwyer et al., 2015; Zerbini & de Oliveira Santos, 1997). Similarly, previous studies of other blackfish delphinids (long-finned pilot whales, *Globicephala melas edwardii*) in New Zealand have also commonly found recently ingested prey (Beatson & O'Shea 2009; Beatson et al., 2007, 2010; Hinton, 2023). Because the pygmy killer whales in this study were not observed to vomit or produce feces, and no dietary remains were found cast upon the shore, we suggest that the animals had not fed immediately prior to stranding (Sekiguchi & Best, 1997). With foraging impossible during the ~17-hr stranding event and considering the elevated energy needs and high caloric prey required by delphinids (Benoit-Bird, 2004; Hin et al., 2019), we suggest that the animals could have experienced progressively growing hunger and/or weakness during the assessment period.

Furthermore, because metabolized prey are necessary for cetaceans to remain hydrated (Nollens et al., 2018; Telfer et al., 1970), a prolonged stranding period could lead to dehydration and the associated mental experience of thirst (Denton et al., 2009). Future research should analyze blood samples to examine for clinical correlates of hunger (e.g., hypoglycemia) and dehydration/thirst (e.g., packed cell volume and total protein), and explore relationships between such test results and stomach contents data (e.g., Sharp et al., 2014).

In terms of physical state due to environmental conditions (Domain 2), no skin blistering was evident on either animal, which contrasts with the common occurrence at some stranding events (Boys et al., 2022c; Groch et al., 2018). Although both animals stranded at the end of austral summer on a day with high UV index, both were discovered and monitored on an overcast day and were rapidly protected with sheets and cooling water. This likely minimized the impacts of environmental exposure to both ultraviolet radiation (sun) and wind (Geraci & Lounsbury, 2005). However, other environmental factors may have impacted animal welfare. Both animals were known to have restranded and due to the large swell and outgoing tide, refloatation attempts were not possible immediately. The longer a cetacean is stranded and/or the more times an animal restrands, the more compounding damage and sustained physiological stress response it will endure (Fernandez et al., 2017), compromising both welfare and survival likelihood (Boys et al., 2022a).

Both animals were located on a sandy beach, although Animal 2 had previously been floating over rocks in the estuary. This likely explains the superficial abrasions on the tail fluke, dorsal fin, and rostrum (health; Domain 3). While such wounds have minimal impact on survival (Sampson et al., 2012), they may cause some discomfort or pain (Boys et al., 2022b).

Respiratory rates for both animals were similar to those reported for other stranded delphinid species (Sampson et al., 2012). The character of Animal 1's breathing, with forceful double chuffing exhalations, may be suggestive of physiological response to respiratory irritation (Fire et al., 2020) or a behavioral response (Lusseau, 2006) to the stranding situation and/or human intervention. The cause of chuffing in this case remains uncertain as only a basic pathological assessment of the lungs was possible and no clear trend between chuffing and human intervention was observed. However, both individuals also frequently elevated the head during respiration, indicating potential breathing difficulties due to compression of the thoracic cavity (Mazzariol et al., 2015; Townsend et al., 2018). Notably, the frequency of head lifting simultaneous with respiration increased over the period of observation, suggesting increasing breathing difficulties over time during the stranding event. Such breathing difficulties were supported by histopathological findings (see section 4.2 for further discussion).

No skin lesions or eye abnormalities were noted in either individual, nor were discharges observed from any orifices throughout the monitored period. Other studies of live stranded delphinids have reported the voiding of normal feces by some individuals (e.g., Sampson et al., 2012). It is possible that the absence of feces production in the current study related to the lack of prey remains in the stomach.

Overall, there was no indication that preexisting health conditions significantly impacted the animal's welfare during the stranding. However, incomplete health and necropsy datasets mean that the role of preexisting conditions cannot be ruled out completely. Accordingly, we suggest that any welfare impacts observed likely occurred due to the stranding event itself.

In Animal 1, both the palpebral reflex and menace response diminished over the 3.5 hr monitored period. Specifically, being present during the first five tests to being reduced (i.e., only one positive response) at the last two reflex testing points in the final hour of life. In contrast, the palpebral reflex of Animal 2 was present throughout the 1.5 hr monitored period, though the menace response was never present. These indicators are considered reliable tests of consciousness for cetaceans and do not appear to be influenced by the animal interacting with its environment or to be context specific (Butterworth et al., 2004b). Diminished response or loss of these neurological indicators typically implies physiological compromise and may relate to capture myopathy (Butterworth et al., 2004b; Mazzariol et al., 2015; Nollens et al., 2018; Townsend, 1999; Townsend et al., 2018), indicating that animals may not be viable for release (Boys et al., 2022d). However, it is important to note that any response to a combination of reflex tests (even if reduced) suggests the animal retains some level of sensibility (Butterworth et al., 2004b). Therefore, if end-of-life procedures are undertaken while these reflexes and responses are present, as was the case here, it should be assumed that animals are conscious and aware of welfare-relevant experiences. This highlights the critical importance of verifying insensibility following application of euthanasia methods to ensure a humane death, and to further evaluate the potential duration of any suffering (Leary et al., 2020).

In Domain 4 (behavioral interactions), 10 of 18 behaviors were displayed by both animals. Animal 1 was relatively active displaying tail fluttering for most of the 3.5 hr observation period, as well as spending more than a third of the observation period displaying dorsal fin fluttering, tail lift, tail hover, and moving its head side to side. In contrast, Animal 2 was predominantly inactive throughout the monitored period. This animal spent approximately 25% of the 1.5 h observed period displaying head side to side movements and ca. 15% of time displaying head lifting (mostly occurring with respiration) and dorsal fin fluttering. Tail fluttering in Animal 2 only began in the final 30 min of the monitored period but increased in duration over that time. For both animals, the time spent moving the head side to side also increased throughout the monitored period.

The collection of ethological data has been limited at stranding events (Boys et al., 2022c), yet animal behaviors are relatively easy to measure, give us the most direct information on current welfare state (Dawkins, 2003; Yon et al., 2019) and provide information on animal prognosis (Cope et al., 2022; Greggor et al., 2019). To robustly interpret these behaviors in terms of welfare state, their expression must be correlated with specific stranding circumstances (e.g., duration of stranding, restranding, environmental and social conditions, human intervention), and with antemortem physiological (e.g., hematology and serum chemistry) and/or postmortem pathophysiological (e.g., from vitreous samples) data (Watters et al., 2021) reflecting the animal's physical and health status. Here we make a preliminary exploration of some of these associations.

The two pygmy killer whales observed here spent a large portion of monitored time displaying "fluttering" behaviors. Such fluttering behaviors may represent forms of muscle fasciculations, which are considered clinical signs of capture myopathy (Câmara et al., 2020; Fernandez et al., 2017), and so their expression might be expected to increase with duration of stranding or with restranding due to sustained physiological stress (Fernandez et al., 2017).

Tail and dorsal fin fluttering were also prevalent in stranded long-finned pilot whales (*Globicephala melas edwardii*; Boys et al., 2022c). Somewhat surprisingly, dorsal fin fluttering was more commonly observed in initially stranded animals within that population than in restranded animals (Boys et al., 2022c).

Capture myopathy can also lead to ischemia–reperfusion injuries and contribute to death (Câmara et al., 2020; Herráez et al., 2007) and is likely a factor in the restranding of animals that have been refloated (Herráez et al., 2013). In the two restranded animals observed here, tail fluttering was constant throughout 3.5 hr or only expressed towards the end of 1.5 hr observation period, as may be expected in animals experiencing a sustained physiological stress response. In contrast, dorsal fin fluttering in both animals appeared to decrease over the monitored stranding. This is somewhat surprising and suggests that these different fluttering patterns—which appear to be muscle fasciculations or tremors—may be related to distinct physiological processes that occur during stranding. However, further data collection and correlation with known hematological parameters, such as creatine kinase (Câmara et al., 2020; Herráez et al., 2007), are needed to understand such fluttering patterns. Alternatively, changes in fluttering behaviors, and the increased head side to side movements observed in both animals towards the end of the observation period, may relate to two clinical signs of capture myopathy: ataxia and dystonic torticollis, respectively (Bonsembiante et al., 2017; Herráez et al., 2007).

It is also plausible that some muscle fasciculations represent a behavioral response to the specific stranding situation. Indeed, in our previous study, dorsal fin fluttering appeared to be context-specific, evidently displayed by a significantly greater proportion of animals stranded in-water than dry-stranded (Boys et al., 2022c). Notably, in the present study the animals were stranded on an outgoing tide, i.e., becoming increasingly dry-stranded; therefore, it is possible that the decrease in dorsal fin fluttering over the stranding observation was contextually related. Further work should specifically explore associations among the expression of fluttering behaviors and stranding scenarios, as well as with physiological indicators of capture myopathy (e.g., via hematology and serum biochemistry) (Bonsembiante et al., 2017; Câmara et al., 2019; Herráez et al., 2013; Seguel et al., 2014).

In the current study, Animal 1 also displayed lateral curvature of the peduncle, without any postural changes, after prolonged stranding, potentially due to muscular contractions. Such body curvature has been reported previously in stranded and rehabilitated animals that were compromised due to rhabdomyolysis, secondary to lack of swimming (Câmara et al., 2020; Gulland et al., 2018; Wells et al., 2013). Where rehabilitation and appropriate treatment can be rapidly provided such curvature, (which can lead to scoliosis) may resolve. However, in cases where curvature is permanent, animals are not considered viable for release (Wells et al., 2013). In this case, right lateral curvature of the peduncle began as dorsal fin fluttering decreased and left pectoral fin fluttering increased. Further data collection is recommended to investigate whether associations exist between fluttering behaviors and such muscular contractions. Notably, temporary body curvature during stranding may be substrate related, for example if a rock is present or the body becomes embedded in soft, muddy substrate. However, this is unlikely to be the case at this stranding as both animals were on compact sandy substrate and no other postural change occurred.

Tail and head arch behaviors were only displayed by Animal 1 and occurred for short durations. However, 92% of head arches occurred simultaneously with tail arching. Such body arching has been noted previously in stranded odontocetes (Sampson et al., 2012) and is indicative of severe physiological stress (Townsend 1999; Townsend et al., 2018). Furthermore, Animal 1 spent more time displaying simultaneous head and tail arching and/or head and tail lifting over the course of the 3.5 hr observation, which supports the idea of increasing physiological stress over time. Therefore, observations of these behaviors provide valuable information as part of a welfare assessment.

All event behaviors were expressed by these two animals at a low rate of occurrence. Therefore, these behaviors may not be useful as direct indicators of welfare status in welfare assessment frameworks (Watters et al., 2021). However, future research should note their occurrence to better evaluate their sensitivity, feasibility and reliability. Overall, the state behaviors displayed by these two restranded pygmy killer whales were similar to those previously reported in pilot whales (Boys et al., 2022c), emphasizing their wider applicability in a welfare assessment framework for stranded odontocetes. Their prevalence across these studies and prolonged duration also indicate their suitability to examine the effects of differing stranding circumstances and/or human interventions on behavior (Yon et al., 2019).

Lastly, human intervention occurred with both animals throughout the observation period and included several procedures, some of which are part of standard stranding response (e.g., watering; Geraci & Lounsbury, 2005). All intervention types were similar to those observed previously for pilot whales (Boys et al., 2022c), though they occurred for shorter relative durations, suggesting these pygmy killer whales were subject to less human interaction during the monitored period. This was likely due to the limited public attendance at the current stranding event, with most interventions involving only DOC, hapū, and a researcher (R.M.B.).

Because stranded cetaceans are unaccustomed to humans, it is likely that interactions cause additional stress and may be perceived as threatening (Boys et al., 2022a; Mellor et al., 2020). It is therefore somewhat notable that Animal 1, with less human intervention, was more behaviorally active than Animal 2. We propose this was likely due to the less responsive and fatigued state of Animal 2 because it was unable to remain upright unassisted when located, suggesting loss of the vestibulo-ocular reflex (Butterworth et al., 2004b), debility, and/or metabolic exhaustion. Notably, both animals displayed more head side to side movements during the period of observation with the most people present, which was also associated with increased noise and the presence of a dog. Further data collection should explore associations between specific patterns of animal behavior and human interventions (Lesimple 2020; Palmer et al., 2021; Watters et al., 2021; Yon et al., 2019). To minimize any undue stress, only minimal, necessary, and appropriate interventions should be undertaken as part of strandings response (Geraci & Lounsbury, 2005).

Some interventions undertaken at this stranding event likely reduced potential welfare compromise. For example, righting animals onto their ventrum should minimize pulmonary compression, though both animals still demonstrated breathing difficulties. These may have occurred since the animals were restranded for an extended period, which likely limited lung function due to prolonged compression of the thoracic cavity (Fahlman et al., 2021), and potentially led to the pulmonary damage observed on histopathology (see section 4.2). Covering and watering of both animals likely reduced hyperthermia and sunburn risk (Geraci & Lounsbury, 2005). Indeed, neither animal exhibited skin blistering despite the high UV exposure.

4.2 | Preliminary welfare assessment: Welfare relevant histopathology

Histological findings were consistent with stranding-associated injury, including pulmonary congestion and edemas, and renal tubular degeneration and vacuolation in both animals. Additionally, acute degenerative lesions were evident in the skeletal muscle (rhabdomyolysis) in Animal 2. Such findings are suggestive of a compartmental-type syndrome caused by prolonged stranding including compression of the skeletal muscle, reduced perfusion, metabolic acidosis, and ischemia potentially associated with neurogenic shock and capture myopathy (Fernandez et al., 2017; Herráez et al., 2007, 2013). Such damage is often untreatable and commonly contributes to death (Câmara et al., 2020; Herráez et al., 2007; Roe & Spraker, 2012), therefore such animals would typically not be considered viable for refloatation.

Compartmental-type syndrome, including crush muscular injury, is the likely cause of muscular lysis observed in this study, and has previously been reported in stranding events (Herráez et al., 2007, 2013). Such pathology has been noted in animals observed arching and with lateral curvature of the peduncle (Câmara et al., 2020; Sharp et al., 2014), as was observed in the present study. Such injuries are notable welfare concerns due to the potential pain and discomfort caused (Pongratz et al., 2002), with prolonged exertion leading to significant fatigue, potentially impacting the likelihood of survival (Fernandez et al., 2017). It is suggested, that where such behaviors are persistent and veterinary care (e.g., rehabilitation) is not available, animals should not be released (Wells et al., 2013; Zagzebski et al., 2006).

Notably, rhabdomyolysis was evident only in Animal 2, which did not display arching behaviors or peduncle curvature. This animal displayed fewer behaviors and those occurring were observed for shorter durations than in Animal 1. This may be explicable due to weakness and/or exhaustion since this individual was first observed floating with the blowhole submerged in the estuary and was unable to remain upright without assistance (See section 4.1 for further discussion). We also hypothesize that the lack of degenerative lesions reported in the skeletal muscle of Animal 1, may reflect the limited in-field tissue sampling undertaken. Similarly, the absence of myoglobinuric nephrosis in either animal may further be an artefact of sampling limitations. An alternative explanation is that such damage was not evident, as advanced capture myopathy may simply not have yet occurred in these animals (Roe & Spraker, 2012). Future investigations should aim to collect hematological and serum chemistry or vitreous samples, as well as tissue samples across all regions of each organ and musculature to improve accuracy of pathological assessment (Câmara et al., 2019; Sharp et al., 2014).

The histopathological findings further support the interpretation of behavior as reflecting breathing difficulties; both individuals exhibited significant pulmonary congestion and edema which would have limited oxygen exchange due to fluid filled alveolar spaces. Such pulmonary congestion/edema are commonly observed postmortem in stranded cetaceans and may be related to shock (Diaz-Delgado et al., 2018; Domiciano et al., 2016). We suggest that observation of head lifting in synchrony with respiration may be used to infer some sort of unpleasant breathlessness (Beausoleil & Mellor, 2015b) in stranded cetaceans due to compression of the thoracic cavity limiting lung function (Fahlman et al., 2021). Respiratory compromise is likely to increase with prolonged stranding durations (Fahlman et al., 2021), this was supported in our study as head lifting simultaneous with respiration increased in frequency over the observed stranding period for both individuals. Such respiratory impairment will impact significantly on welfare during stranding, as well as being life-threatening (Beausoleil & Mellor, 2015b; Boys et al., 2022a,b). Indeed, respiratory dysfunction may play a role in restranding following refloatation.

Finally, hepatic histology from both animals indicated the presence of iron within Kupffer cells, but not hepatocytes (Kupffer cell hemosiderosis). Previous studies of cetaceans have associated this pathology with iron overload disease (Venn-Watson et al., 2012). While the etiology and pathogenesis remain poorly understood, excessive dietary iron consumption, chronic inflammation, hemolysis/anemia, emaciation, and peracute sepsis with iron sequestration have been suggested (e.g., Raverty et al., 2006). In other cases, Kupffer cell hemosiderosis has been associated with hemorrhagic diathesis (Ewing et al., 2020) and chronic hepatitis and lipidosis (Hiemstra et al., 2015; Venn-Watson et al., 2012). Iron was also present in the proximal tubular epithelial cells of the kidney, in the absence of a concurrent hemoglobinuria or myoglobinuric nephropathy. Concurrent hepatic and renal hemosiderosis could suggest previous bouts of intravascular hemolysis (Venn-Watson et al., 2012). Given that these individuals were in normal body condition and exhibiting no evidence of chronic inflammatory disease within the organs examined, the significance of the hemosiderosis and its association with this stranding remains unclear.

Previous studies have elucidated prognostically useful hematological and serum chemistry parameters for disposition decisions of stranded delphinids but have provided limited information on how these data relate to any physical and/or behavioral examination undertaken (Gales, 1992; Manire et al., 2018; Sampson et al., 2012; Sharp et al., 2014). Future studies should seek, where possible, to correlate hematological and/or serum chemistry analysis with the noninvasive welfare indicators presented here, as well as with pathological and/or known survival data. In this way, indicators that can be practically applied at strandings (in the absence of veterinary and/or specialized equipment) can be scientifically validated to determine those most useful for decision-making based on immediate welfare impact and survival prognosis.

4.3 | Preliminary welfare assessment of euthanasia procedures

Criteria to verify cetacean insensibility/death were not assessed in-field immediately following application of the euthanasia method, despite the mandate to do so within the New Zealand SOP (Boys et al., 2022d). Behavioral responses specific to the euthanasia procedure in these animals included agonal convulsions, body trembling, jaw open, muscle relaxation, and peduncle stiffening. Onset and cessation of agonal convulsions and muscle relaxation were only observed following the third shot in both animals, while body trembling and peduncle stiffening occurred after each shot. In this context, the peduncle stiffening was likely a form of tonic spasm, while the agonal convulsions likely represent clonic spasms (Close et al., 1996; Leary et al., 2020).

Notably, jaw open occurred at different points for the two individuals, despite seemingly identical euthanasia procedures. For Animal 1, jaw open occurred following the first shot, whereas for Animal 2, it occurred only after the third shot. In other species, mouth gaping is suggested to indicate loss of cerebral cortex control (Erasmus et al., 2010), while cessation of musculoskeletal movements may be indicative of spinal cord dysfunction (Dawson et al., 2007). However, there is a lack of data to support correlation of loss of consciousness and behavioral and/or physiological responses in cetaceans (Brakes et al., 2006; Butterworth et al., 2004a).

Although both pygmy killer whales had reduced reflexes/responses (see section 4.1 for further discussion) prior to the application of the euthanasia method, the animals still reacted to the combination of reflexes tested, suggesting some level of sensibility (Butterworth et al., 2004b). This indicates that both cetaceans were capable of welfare-relevant experiences at the time of euthanasia and reinforces the importance of assessing insensibility following the application of ballistics.

Following the final shot and observation of slack lower jaw and agonal convulsions, both animals displayed movements; for Animal 1 this involved tail lifting, while for Animal 2, both tail and dorsal fin fluttering were observed. Although such movements may be involuntary clonic spasms (Leary et al., 2020; Woods et al., 2010), without verification of insensibility in-field, unconsciousness cannot be confirmed. Verification of insensibility in cetaceans should include the assessment of a combination of criteria: lack of jaw tone, absence of menace, palpebral and corneal reflexes, fixed dilated pupils, lack of response to painful stimuli, no capillary refill time, and ocular/skin temperature differential whereby the eye cools more rapidly after blood circulation ceases (Brakes et al., 2006; Butterworth et al., 2004a,b). Unfortunately, such indicators have not been validated to understand how they reflect different stages of insensibility. Future research should seek to examine for correlations between these behaviors and the loss of various reflexes, absence of heartbeat and/or electrical brain activity incompatible with conscious experience (Verhoeven et al., 2015, 2016) to further elucidate how they reflect insensibility/death in cetaceans.

Based on video analysis, the first shot likely entered the melon of both animals. This suggests inappropriate shot placement and the potential for the animals to suffer pain and distress from initial application of the method until insensibility occurred. Notably, the initial shot placement observed appears misaligned with the euthanasia guidance in the New Zealand SOP which states that shots should be a "handspan behind the blowhole" (Boren, 2012; Boys et al., 2022d). Skull morphology among cetacean species is highly variable (Galatius et al., 2020; Gol'din, 2014) and thus species-specific reference points are required for accurate projectile placement (Boys et al., 2021). However, the cranial morphology of pygmy killer whales, including the anatomical location of the blowhole, is similar to that of pilot whales detailed in the generic New Zealand SOP, meaning that species differences are unlikely to explain melon penetration on this occasion. Experience levels of warranted officers who perform euthanasia are highly variable, therefore we recommend adequate, ongoing training be undertaken to ensure that potential welfare impacts are minimized during euthanasia.

Species-specific differences can also affect which equipment is most appropriate for technically enacting euthanasia. In this study, soft point projectiles were used. While these are recommended in the New Zealand SOP (Boren, 2012; Boys et al., 2022d), soft point projectiles are understood to have reduced penetration ability and lower killing efficiency (Hampton et al., 2014; Knox et al., 2018; Øen & Knudsen, 2007). Indeed, international recommendations suggest the use of only solid projectiles for cetaceans (Boys et al., 2021; Duignan & Anthony, 2000; Hampton et al., 2014; Øen & Knudsen, 2007). The implications of using soft projectiles at this event are unknown since postmortem examination of the cranium was not possible. However, future studies should apply dissection and/or imaging techniques such as computerized tomography and magnetic resonance imaging (Gascho et al., 2020; Schwenk et al., 2016; Thali et al., 2003) of the cranium to provide detailed evaluations of the welfare impacts of ballistics euthanasia.

4.4 | Next steps towards the development of a welfare assessment framework for cetacean strandings

For both theoretical and practical reasons, thus far, there have been limited attempts at specifically assessing the welfare of cetaceans (Boys et al., 2022c; Clegg et al., 2015; King et al., 2021; Nicol et al., 2020). The most commonly applied, multicriteria welfare assessment framework is the C-Well model that was developed to assess and monitor the welfare of cetaceans, specifically common bottlenose dolphins (*Tursiops truncatus*), living in captivity (Clegg et al., 2015). While appropriate for captive cetaceans, many of the indicators used in this model, such as "appropriate behavior," are not measurable or relevant in a stranding context as the animals are in an alien environment where normal function, natural behaviors and agency cannot be expressed. Therefore, C-Well as a holistic framework is not directly applicable for assessing the effects of physical and environmental conditions and human interventions on the welfare of stranded animals. Instead, we applied the more generic Five Domains model to guide systematic collection of context-specific data and interpretation of those data in terms of the animals' affective states relevant to overall welfare. The Five Domains has also been found useful to theoretically assess various welfare impacts of human activities on free-living (nonstranded) cetaceans (King et al., 2021; Nicol et al., 2020; Rae et al., 2023). Reassuringly, both the Five Domains and C-Well models aim for holistic assessment and cover similar dimensions relevant to overall welfare state. However, to ensure their application and component parameters are valid and justified, the same steps are required.

The next step in the development of a robust welfare assessment framework is the scientific validation of the proposed welfare indicators, to ensure that they reflect what we understand them to be measuring (Beausoleil & Mellor, 2017). In the C-Well model, this was undertaken via literature reviews and comparison of parameters among captive animals known to be healthy and unhealthy, though the authors acknowledge further scientific validation is required (Clegg & Delfour, 2018). The actual assessment of welfare in facilities is then largely based on how the indicators reflect normal/abnormal function or natural behavior (Clegg et al., 2015; Clegg & Delfour, 2018).

Here, we approached validation of specific indicators included in the Five Domains assessment by beginning to explore relationships among behavioral patterns, other observed indicators (such as external indicators of physical and health status) and limited histopathology that could be examined (Beausoleil & Mellor, 2017). This is the beginning of a robust validation process and ongoing research with this kind of fine detail is needed at strandings. Specifically, correlation with multiple other measures, including known stranding outcomes, behavioral, physiological, and pathological findings is required to further validate the observed indicators.

Once indicators to be included in multicriteria assessment models are considered reasonably valid in general terms, further steps are to consider the methods of grading the intensity/severity of any impacts and of aggregating various individual parameters or dimensions into a reflection of overall welfare state. Grading systems that are qualitative in nature (e.g., ordinal categories) are often recommended over quantitative/numerical systems to avoid implying precision that is not possible in frameworks based on inferring animals' affective experiences to understand their welfare state (Beausoleil & Mellor, 2015a). In the C-Well model, grading is based on the extent to which various indicators reflect normal/abnormal function or natural behavior and these are overlaid with ethical judgements of "good," "suboptimal/acceptable," or "poor" to reflect overall welfare state (Clegg et al., 2015; Clegg & Delfour, 2018). In the context of free-living cetaceans generally, and stranded animals in particular, there are currently insufficient data available to characterize the relative intensity/severity of impacts that various welfare indicators may reflect. Accordingly, focused collection of further data at strandings is necessary to understand the relative intensity and duration of welfare impacts required to inform such grading.

While multicriteria approaches to welfare assessment are recommended, a reflection of overall welfare state or impacts is often desired. Specifically, this can facilitate comparison across animals, groups or facilities, among procedures or management options or to chart changes in welfare over time (Botreau et al., 2007; de Graaf et al., 2017; de Vries et al., 2013). To achieve this overall understanding, ways of integrating individual indicators and types of parameters (e.g., welfare status and welfare alerting or resource/management-based and animal-based) within a domain, as well as ways of integrating impacts across various domains or dimensions of welfare are needed. The methods chosen for aggregation have significant ethical implications and should be carefully considered and transparently represented (Sandøe et al., 2019).

To provide an overall welfare score for an individual cetacean using the C-Well framework, Clegg et al. (2015) aggregated both animal- and resource-based indicators in an additive way. This means that indicators of different types and representing different aspects of welfare were given equal weighting in the overall score, which may be problematic for various reasons. For example, resource-based indicators may or may not reflect an individual's current welfare state (Harvey et al., 2020). Additionally, simple nonweighted aggregation can allow some "good" scoring indicators to cover up or compensate for "poor" scoring indicators, leading to an overall welfare assessment that does not necessarily accurately reflect the animal's welfare state (Sandøe et al., 2019).

As noted for grading welfare impacts, we are currently only beginning the process of validating putative indicators of welfare state in stranded cetaceans. Accordingly, it is too premature to confidently develop an approach to aggregation. For example, there are insufficient data to apply weightings to individual parameters for the purposes of calculating an overall stranded cetacean welfare score. Further, it would be remiss of us to attempt to do so without a clear understanding of the implications of our chosen approach. The systematic collection of additional data will ensure that the relative importance/influence of different aspects of welfare are well understood when aggregating indicators and grading welfare state (Sandøe et al., 2019). By undertaking development of a welfare assessment framework for stranded cetaceans in this stepwise manner, we are providing a robust, transparent method that should highlight true welfare compromise and provide evidence-based data to support strandings management.

4.5 | Conclusion

We provide a holistic approach to welfare assessment during restranding of pygmy killer whales and highlight potential welfare implications associated with ballistics euthanasia as applied. Our findings suggest that both individuals were experiencing compromised welfare due to stranding. Based on the findings presented, the decision of euthanasia was warranted; these animals showed evidence of compartmental-type stranding syndrome likely associated with ischemia–reperfusion injury, neurogenic or vasogenic shock and capture myopathy. Accordingly, these animals likely experienced negative affective states of breathlessness, discomfort, fatigue, and malaise which would be expected to impact both their long-term welfare and survivorship. Although our findings suggest potential links between externally observable welfare indicators (primarily behavior) and aspects of opportunistically sampled pathology, both dedicated live monitoring and veterinary pathology via systematic necropsies are required to conclusively elucidate links between welfare indicators, survival probability and observed pathology. Such data will better inform decision-making around refloatation versus euthanasia. Both animals appeared conscious and likely capable of welfare-relevant experiences at the time of ballistics application. Following each shot, behavioral responses were displayed by both animals and additional animal movements were observed after application of ballistics. Yet, verification of insensibility was not undertaken in-field, hindering confirmation of unconsciousness, and understanding of any associated welfare implications. It is vital that such verification and recording of time to insensibility always be undertaken following application of euthanasia to enable an evaluation of welfare implications and to allow for improvements in end-of-life procedures. By applying the knowledge acquired in this study, strandings response can be more scientifically informed and provide the best welfare outcomes for future stranded cetaceans.

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AUTHOR CONTRIBUTIONS

Rebecca M Boys: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; validation; visualization; writing – original draft. **Ngaio J Beausoleil:** Supervision; validation; writing – review and editing. **Stuart Hunter:** Investigation; methodology; writing – review and editing. **Emma Betty:** Investigation; supervision; writing – review and editing. **Bethany Hinton:** Investigation; writing – review and editing. **Karen Stockin:** Investigation, Methodology, Resources, Supervision, Validation, Writing – review & editing

INSTITUTIONAL REVIEW BOARD STATEMENT

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None.

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