Received: 30 March 2022 | Revised: 31 July 2022 | Accepted: 2 September 2022

DOI: 10.1002/vetr.2238

Vet Record

ORIGINAL RESEARCH

Using a combination of midazolam and butorphanol is a safe and effective reversible field sedation protocol for Weddell seal (*Leptonychotes weddellii*) pups

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Funding information
National Science Foundation, Grant/Award Number: ANT-1543539

Abstract

Background: Weddell seals (*Leptonychotes weddellii*) are a well-studied species of phocid with an apparent sensitivity to immobilising agents. Mortality as high as 31% has been reported during field immobilisation. This study investigated the use of a benzodiazepine in combination with an opioid agonist/antagonist for sedation in Weddell seal pups as part of a physiological study.

Methods: During the 2017 and 2019 Antarctic pupping seasons, 18 Weddell seal pups were sedated by intramuscular administration of a combination of midazolam and butorphanol or intravenous midazolam alone. Individuals were sedated at 1, 3, 5 and 7 weeks of age. Naltrexone and flumazenil were used to reverse sedation. The combination was 100% effective in providing appropriate sedation for the intended procedures.

Results: Analyses were performed to investigate relationships between dose administered, age, individual reactions, adverse effects and changes in dive physiology. Transient apnoea (10–60 seconds) was the most frequently observed adverse effect. No sedation-associated morbidity or mortality occurred.

Limitations: The sample size is small and there is no pharmacokinetic information for either sedative or reversal in phocid species.

Conclusions: The combination of midazolam (0.2–0.3 mg/kg) and butorphanol (0.1–0.2 mg/kg) provided safe and effective sedation, with reversible effects, in Weddell seal pups.

BACKGROUND

Sedative and anaesthetic drugs are routinely used for chemical restraint of pinnipeds in field settings for purposes including health assessments, biological sample collection, application of telemetry instruments and disentanglement.[1–4] Benefits of chemical restraint as compared with physical restraint include improvements in sampling capability, instrument placement and handling safety, especially for large or aggressive species. Importantly, chemical restraint can reduce animal stress and can be designed to produce anxiolysis and analgesia. Despite numerous benefits, field sedation and anaesthesia can carry high risks. Risks are exacerbated in extreme and remote environments where access to monitoring and emergency equipment is limited. Additionally, the logistical constraints of these environments often dictate which agents and delivery methods are feasible. The development of chemical restraint protocols that are effective, safe and logistically feasible is a critical component of successful field research endeavours. Protocols must be tailored to the species, individual animal, environment, procedure and anticipated level of pain.[5]

Multiple field chemical sedation protocols for Weddell seals, with both injectable and inhalant agents, have been used with varying success.[6–11] Weddell seal adults have a large amount of peripharyngeal

tissue and a loose soft palate[11]; these features contribute to risk of airway obstruction and can make intubation difficult.[6,11,12] This, combined with the possible initiation of a dive response while sedated, has been suggested as a cause of the 10%–31% mortality observed with certain drug combinations in adult Weddell seals.[7,11,13] Over the past decade, the most extensively used protocol has been the combination of ketamine and midazolam for induction and maintenance of anaesthesia in adult Weddell seals,[9,14–16] and to a lesser extent in juveniles (1–2 years old) and weaned pups (more than 35 days old).[14] Ketamine is not reversible; however, it increases the anaesthetic risk should an emergency arise. Furthermore, few studies have required sedation of neonatal and dependent pups, and the combination of ketamine and midazolam has not been used in this vulnerable age class. In neonatal animals, reversible combinations of benzodiazepines and opioids are recommended, as they provide sedation and anxiolysis with minimal cardiovascular and respiratory depression.[17] Given that Weddell seals appear particularly sensitive to previously used anaesthetic agents,[7,10] care is needed to develop a safe and effective field immobilisation protocol that can be used in all age classes.

The combination of the benzodiazepine, midazolam, and the opioid agonist/antagonist, butorphanol, has been used successfully in a variety of marine mammals. In phocids, the combination has been used in neonatal to adult harbour seals (*Phoca vitulina*),[18,19] adult leopard seals (*Hydrurga leptonyx*)[20] and weanling northern elephant seals (*Mirounga angustirostris*).[19] Midazolam and butorphanol together produce dose-dependent rousable sedation with anticipated anxiolysis and mild analgesia.[12,21] The combination has numerous clinical and logistical advantages. Both agents are readily administered by intramuscular (IM) or intravenous (IV) routes with rapid onset of effect and titratable dose ranges.[22,23] Depth and duration of sedation can be increased as needed by repeated administration of one or both agents. Each drug has a wide margin of safety demonstrated across taxa with minimal cardiovascular and respiratory impacts at low doses, and each has been safely and effectively used alone or in combination in a number of phocid species.[1,9,12,18,24–27] In addition, the effects of both midazolam and butorphanol can be counteracted with reversal agents. Flumazenil is a competitive antagonist at benzodiazepine receptors and can be used to reverse the effects of midazolam.[28] Naltrexone is a competitive agonist/antagonist at opioid receptors and has been used to reverse the effects of butorphanol.[29,30] A reversible protocol facilitates a rapid recovery and can reduce the risk of self-harm, predation and anaesthetic-related complications following immobilisation. Reversibility is a particularly valuable trait in remote and extreme field conditions where access to emergency medical equipment is limited and environmental conditions can change rapidly.

The aim of this study was to design a field sedation protocol for Weddell seal pups using midazolam and butorphanol in combination. Midazolam was also evaluated as a single-agent sedative for short-duration handling of neonates. The primary hypothesis of this study was that midazolam and butorphanol would provide safe and effective immobilisation of Weddell seal pups. The secondary hypothesis was that the effects of midazolam and butorphanol could be reversed with flumazenil and naltrexone. This study was part of a larger project that investigated the development of diving and thermoregulatory capability in Weddell seal pups during the dependency period, and correlations between changes in dive physiology and observed clinical effects of sedation were also investigated.

METHODS

Animal capture and handling

All sampling occurred on the sea ice of Erebus Bay, Antarctica (~77 44′44″ S, 166 46′26″ E), during the 2017 and 2019 pupping seasons (October–December). A total of 18 Weddell seal pups were sampled from a well-studied population[31,32] in one of three breeding colonies: Turtle Rock, Big Razorback Island and Hutton Cliffs.

Study objectives focused on the physiological development of pups aged 1–7 weeks. Eight pups were included in the study in 2017 and 10 pups were included in 2019. All pups were sampled at four ages relative to birth date: 1, 3, 5 and 7 weeks. Week 1 pups were considered neonates. Pups were selected for inclusion based on the reproductive history of the mother to mitigate risk; multiparous females have the highest likelihood of producing healthy pups[33–35] and were expected to experience the least disturbance associated with temporary separation and handling of the pup. Life history data on target mothers were obtained from the long-term monitoring study of this population.[36] Mother–pup pairs were gently separated with herding boards, and pups were moved approximately 100 m away for sampling during week 1 procedures. For weeks 3–7, procedures were targeted during times when the mothers were on foraging trips to reduce separation events. Mother–pup pairs were reunited after each procedure, unless the pup had been captured while alone or had been weaned.

The larger study objectives and methods are described in detail in other studies.[37,38] The number of animals included and the sampling timepoints in this study were determined based on the larger research objectives. Sample size was kept to a minimum in accordance with the United States Marine Mammal Protection Act. Briefly, pups in each study year were split into two cohorts (A and B) with different sampling protocols and handling durations. Each pup within these cohorts was sampled at weeks 1, 3, 5 and 7. Cohort A ($n = 9$) procedures were conducted entirely under sedation and included standard morphometrics, ultrasound measurement of blubber depth, venipuncture with Evans blue and tritiated water dilution, muscle and blubber biopsy, and instrument

attachment or retrieval (time depth recorder [TDR], accelerometer, very high frequency [VHF] tag). Pups of cohort B ($n = 9$) spent between 30 and 40 minutes in a custom-designed metabolic chamber prior to sedation. Cohort B procedures conducted under sedation included standard morphometrics, ultrasound measurement of blubber depth, venipuncture (2019 only), and instrument attachment or retrieval. Body mass (±0.25 kg) was determined prior to each sedation for accurate dosing. Mass was obtained by bundling each pup into a tarp and suspending it from a hanging electronic scale (Central Carolina Scale, Sanford, NC, USA) under a tripod. During the 2017 field season, pups in cohorts A and B were sedated to facilitate sampling at weeks 3, 5 and 7, and in 2019, the protocol was modified to include pups at week 1. Across the 2-year study period, there were a total of 64 planned sedation events. Time between sedation events in both cohorts was 14 ± 2 days.

Several sampling procedures were expected to cause transient discomfort and mild pain. Venipuncture was performed through the extradural vein via 38–90 mm 18 Ga spinal needle for blood collection and drug administration. For cohort A, blubber and longissimus dorsi muscle biopsies were collected at weeks 1, 3, 5 and 7 from alternative sides of the epaxial musculature at the level of the lumbar vertebrae using a standard 6 mm sterile disposable biopsy punch or a sterile biopsy cannula. Lidocaine HCl 2% (1 ml, Hospira Worldwide, USA) was administered intradermally and subcutaneously at the site prior to each biopsy for local analgesia associated with biopsy procedures. Flipper tags and TDRs were applied to the hind flipper webbing manually by tag punch after intradermal injection of lidocaine HCl 2% (1 ml).

Sedation

A combination of midazolam (West-Ward Pharmaceuticals, USA) and butorphanol (Merck, USA) was used to immobilise pups for procedures. Prior to sedative administration, a veterinarian with marine mammal experience performed a physical examination, including cardiopulmonary auscultation, to evaluate the general health of the pup and assess anaesthetic risk. For the IM combination, initial midazolam dose ranged between 0.1 and 0.2 mg/kg, and initial butorphanol dose ranged between 0.1 and 0.2 mg/kg. In 2019, a subset of week 1 pups from cohort B ($n = 4$) was administered with midazolam (0.2–0.3 mg/kg) IV alone to determine if this could provide sufficient sedation for short duration, minimally invasive procedures in neonates. If midazolam alone provided insufficient sedation, butorphanol (0.1 mg/kg) was subsequently administered IV. In any procedure, if initial sedation doses were insufficient in achieving the desired level of sedation or if additional sedatives were needed to maintain an appropriate level of sedation at any point during the procedure, then half of the initial dose of each drug was administered IV or IM, and this was repeated as necessary until the appropriate level of sedation was achieved. Emergency medications, including doxapram (West-Ward Pharmaceuticals), epinephrine (Vet One, USA) and atropine (Vet One), and equipment for endotracheal intubation were available in the event of an emergency. At the end of all procedures, midazolam and butorphanol were reversed using flumazenil (0.7–8 µg/kg IM, West-Ward Pharmaceuticals) and naltrexone (0.1 mg/kg IM, ZooPharm, USA), respectively. Where possible, reversals were administered just prior to moving pups back to their original capture location to minimise the stress of transport and shorten handling time.

Monitoring

Respiratory rate (RR), heart rate (HR), rectal temperature, capillary refill time and mucus membrane colour were monitored throughout procedures by a veterinarian or veterinary technician. The animals were monitored for 10–15 minutes post-induction to ensure that sufficient sedation was achieved before beginning sampling procedures. Time to first effect of sedation (eye closure and/or relaxation of muscle tone in the jaw) and time to immobilisation (no body movement, no head lifting, no vocalisation and/or snoring) were noted. Vital signs were recorded every 5 minutes at minimum. Breath-hold events under sedation, defined as transient apnoea (10–60 seconds), were recorded during each procedure. If apnoea events occurred, interventions including tactile stimuli and body repositioning were initiated to stimulate spontaneous respiration. Pups were placed on an insulating foam mat during immobilisation on the sea ice or inside a wind shelter to help maintain body temperature. All pups were monitored for a minimum of 30 minutes following the administration of reversal agents, and time to full recovery (alert and fully ambulatory) was noted.

Statistics

All analyses were performed in R (version 3.6.3).[39] For each sedation procedure, mean RR and range, mean HR and range, and the number of apnoea events were determined from the data. RR, HR and number of apnoea events were compared between cohorts for a given age to determine if differences in sampling protocols, such as sedation duration and level of anticipated discomfort (biopsy or venipuncture), had any significant effect. The first recorded RR and HR were excluded from analyses because they were often taken within minutes of capture, before sedatives had been administered, resulting in elevated levels neither representative of sedation nor baseline pre-capture conditions. Correlations between number of apnoea events and length of sedation were investigated. Data were tested for suitability for linear modelling, response variables were examined for normality using QQ Plots and Shapiro–Wilk normality

test, and data were examined for outliers using the box-plot method (function identify_outliers in R). All data are presented as mean ± 1 standard deviation.

Changes in vital signs under sedation and apnoea events with age

Mean RR and mean HR for each individual were compared within and between each age group using repeated measures ANOVA with post hoc pairwise *t*-test and Bonferroni adjustment. Apnoea events were compared among ages using a general linear mixed model (LME), and estimated marginal means were compared pairwise among age groups using the emmeans package in R.[39,40] Only the initial drug doses administered were included in the analysis. Apnoea events at week 1 were compared between the routes of initial drug administration (IM vs. IV). An LME was used to investigate if apnoea events were related to the initial dose of midazolam or butorphanol administered via IM injection; midazolam or butorphanol dose (mg/kg) was the predictor variable, and number of apnoea events was the response variable (Poisson distribution).

Relationships between vital under sedation and development of diving abilities

To determine relationships and sources of variation among vital signs (RR, HR and apnoea events) during sedation and dive metrics as animals developed greater diving ability, a series of LME were run with restricted maximum likelihood using the glmmTMB package in R.[39,41] Mean RR, minimum RR, mean HR, minimum HR, apnoea (yes/no) (binomial distribution) and apnoea events (number of apnoeas recorded during sedation; Poisson distribution) were response variables. Dive parameters including total time in water (seconds), maximum dive depth (m), maximum dive time (seconds) and the number of dives were predictor variables. A second set of models was run using mean dive depth (m) and mean dive duration (seconds) as predictor variables in place of maxima. Age (weeks) was also included as a predictor variable. Animal ID was included as a random effect to account for repeated measures. Models were ranked using Akaike information criteria (AIC), log likelihoods (logLik) and R^2. The variance explained by the random effect was assessed based on the difference in the marginal (R^2_m; fixed effects only) and conditional (R^2_c; all model variables) R^2 (rsquared, glmm function).

Relationships between maintenance doses, sedation length and individual variation

The relationships between the number of maintenance doses, individual variation and length of sedation (minutes) were investigated using an LME (Poisson distribution). Only sedation procedures for which the initial dose was given IM are included in analysis and summary results.

RESULTS

Over two field seasons, 18 individual Weddell seal pups were sedated for a total of 60 of the 64 planned sedation procedures spanning the neonatal period to weaning (weeks 1–7). One individual in cohort B was removed from the study during the 2019 field season due to a significant injury, and sample sizes for sedation events at weeks 3, 5 and 7 reflect this removal. In addition, one individual in 2017 cohort B was not sedated at week 3 due to signs of stress during metabolic data collection. All individuals were found to be healthy on examination, with no obvious underlying disease, and all were deemed to have an American Society of Anesthesiologists classification of I.[42] A summary of sedation information is provided in Table 1. Cohort A sedation times were significantly longer than cohort B sedation times. Sedation was well tolerated in general, with transient apnoea (breath holds of 10–60 seconds) being the most frequently observed adverse effect (n = 40 events). Of these, cyanosis was observed in nine cases. All animals that exhibited transient apnoea were stimulated manually or position adjustments were made within 60 seconds, and all either responded to these interventions or apnoea resolved spontaneously on its own. Regurgitation was noted in five cases, representing different individuals and age groups. Regurgitation events generally involved small volumes (<100 ml), occurred towards the end of procedures and were associated with a lighter plane of sedation. During regurgitation events, procedures were halted, the oral cavity was wiped clean with gauze and airways were auscultated and determined to be clear prior to resuming the procedure. Body temperatures were well maintained with a total range of 34.7°C–37.4°C across all ages and procedures. RR and HR declined with age (Figure 1a,b), but there were no significant differences in RR or HR between cohorts A and B, despite overall longer sedation times and frequent maintenance doses required in cohort A. The number of transient apnoea events increased with age, consistent with phocid development (Figure 1c). All individuals responded to reversals, administered IM and/or IV, with generally rapid recoveries (Tables 1 and 2).

Sedation

Week 1

For the purposes of this study, sedation data for week 1 animals are included for 2019 only (n = 5). In cohort A, the initial dose of midazolam and butorphanol in combination IM (Table 1) was sufficient to maintain

TABLE 1 Initial sedation doses and associated effects of a combination of midazolam and butorphanol delivered by intramuscular injection in Weddell seal pups between weeks 1 and 7 during the 2017 and 2019 field season in McMurdo Sound, Antarctica

	Week 1	Week 3	Week 5	Week 7
Cohort A	$n = 5$ (2019 only)	$n = 9$	$n = 9$	$n = 9$
Midazolam (mg/kg)	0.2	0.2	0.2–0.4	0.2–0.3
Butorphanol (mg/kg)	0.1	0.1	0.1–0.15	0.15–0.2
Mean time to first effects (minutes)	9.6 ± 10.5	7.5 ± 5.36 ($n = 5$)	4.57 ± 2.07 ($n = 5$)	4.89 ± 4.01 ($n = 5$)
Mean time to immobility (minutes)	11.4 ± 9.0	15.8 ± 7.7 ($n = 5$)	9.4 ± 7.2 ($n = 5$)	11.8 ± 6.9 ($n = 5$)
Mean sedation time (minutes)	134.0 ± 7.9	132.0 ± 11.8 ($n = 6$)	132.0 ± 18.3	115 ± 22
Mean time to recovery (minutes)	5.2 ± 3.4	3.4 ± 1.1 ($n = 5$)	10.8 ± 11.8 ($n = 5$)	4.6 ± 4.8 ($n = 5$)
Number of individuals that required maintenance doses	2	6	4	4
Mean time to first maintenance dose (minutes)	43.5 ± 29	51 ± 36.8	82.5 ± 47.9	30 ± 13.5
Mean RR (breaths/minute), range	27 ± 4 (15–40)	23 ± 8 (8–40)	17 ± 3 (4–40)	18 ± 5 (1–40)
Mean HR (beats/minute), range	127 ± 6 (80–120)	114 ± 6 (96–160)	107 ± 11 (76–130)	98 ± 12 (54–120)
Number of individuals with apnoea events	2	6	5	4
Individuals with apnoea and cyanosis	1	2	0	2
Regurgitation events	0	1	1	1
Cohort B	$n = 1$ (2019)	$n = 7$	$n = 8$	$n = 8$
Midazolam (mg/kg)	0.2	0.1–0.2	0.2	0.2
Butorphanol (mg/kg)	0.1	0.075–0.15	0.075–0.15	0.15
Mean time to first effects (minutes)	3	4.5 ± 1.05 ($n = 4$)	4.14 ± 1.95 ($n = 4$)	4.50 ± 2.51 ($n = 4$)
Mean time to immobility (minutes)		5.25 ± 0.5 ($n = 4$)	8.3 ± 3.4 ($n = 4$)	12.0 ± 9.9 ($n = 4$)
Mean sedation time (minutes)	76	65.3 ± 10.1	52.3 ± 4.5	46.10 ± 8.11
Mean time to recovery (minutes)	6	5.0 ± 1.0 ($n = 4$)	9.5 ± 2.4 ($n = 4$)	7.7 ± 5.0 ($n = 4$)
Number of individuals that required maintenance doses	0	0	0	0
RR (breaths/minute), mean (total range)	20 (14–28)	20 ± 6 (4–40)	19 ± 6 (1–40)	18 ± 4 (4–40)
HR (beats/minute), mean (total range)	120 (100–170)	110 ± 5 (96–130)	96 ± 5 (80–190)	92 ± 11 (30–120)
Number of individuals with apnoea events	0	5	7	7
Individuals with apnoea and cyanosis	0	1	2	1
Regurgitation events	0	1	1	0

Note: All means are reported with 1 standard deviation. Flumazenil (0.7–8 μg/kg) and naltrexone (0.1 mg/kg) were given at the end of procedures to reverse sedative effects.
Abbreviations: HR, heart rate; RR, respiratory rate.

sedation for three of the five procedures, with two individuals requiring a maintenance dose. One individual received two additional doses of midazolam (0.1 mg/kg) and butorphanol (0.05 mg/kg) IM, given 64 and 75 minutes after the induction dose. Another individual received one maintenance dose of midazolam (0.1 mg/kg) and butorphanol (0.05 mg/kg) IV 23 minutes after the induction dose. Transient apnoea events occurred in two cases; one was associated with induction and the other was associated with a period of light sedation at the end of the procedure. Cyanosis also developed in the latter individual.

In cohort B, midazolam IV was initially administered alone ($n = 4$). No apnoea events were recorded following the administration of midazolam IV. As a single agent, midazolam IV did not provide adequate immobilisation for procedures in any pup, and butorphanol was subsequently administered IV between 15 and 30 minutes after the initial injection of midazolam (Table 2). The addition of butorphanol provided a moderate level of sedation for the remainder of the procedure. There were four transient apnoea events among three of the four individuals. Three of these apnoea events occurred immediately following the administration of butorphanol IV. No apnoea events were presented with cyanosis. Given the insufficient sedation achieved by midazolam IV alone and the frequency of apnoea events associated with IV butorphanol, the administration of sedatives IV for induction was discontinued. The fifth pup in cohort B received midazolam and butorphanol in combination administered IM, consistent with the protocol

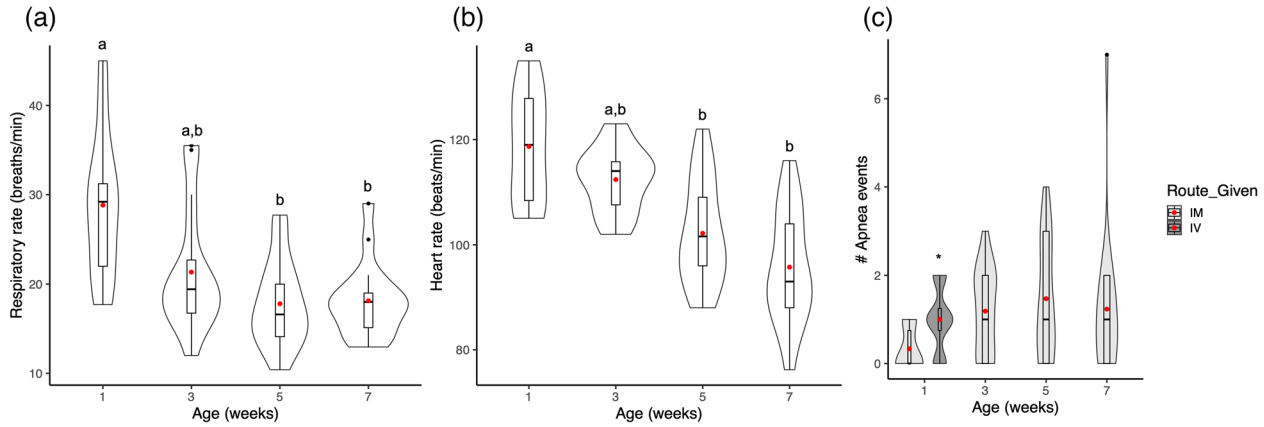

FIGURE 1 (a) Respiratory rate (breaths/minute), (b) heart rate (beats/minute) and (c) number of transient apnea events during sedation procedures across ages for Weddell seal pups sedated with a combination of midazolam and butorphanol. Data were combined for cohorts A and B. Mean (red circle), median (hash), outliers (solid circle) and quartiles (whiskers) of data are shown. Different letters above each plot indicate significant differences among age groups ($p < 0.05$). A significant difference ($p < 0.05$) existed between intramuscular (IM) (light grey) and intravenous (IV) (dark grey) administration and the number of apnoea events experienced by week 1 pups, indicated by an asterisk (*).

TABLE 2 Initial sedation doses and associated effects of midazolam administered alone by intravenous (IV) injection, followed by butorphanol IV in Weddell seal pups at week 1 in cohort B ($n = 4$), during the 2019 field season in McMurdo Sound, Antarctica

	Week 1			
Cohort B (2019)	**Pup #1**[a]	**Pup #2**	**Pup #3**	**Pup #4**
Initial midazolam IV (mg/kg)	0.2	0.3	0.3	0.3
Effect of initial midazolam	Ambulatory with stimulation	Moderate sedation for 17 minutes, then light sedation for 16 minutes	Transient moderate sedation—ambulatory and vocal in 9 minutes	Transient moderate sedation (<20 minutes)
Butorphanol IV (mg/kg)	0.1	0.1	0.1	0.1
Time from initial dose (minutes)	27	29	16	20
Length of sedation (minutes)	68	86	90	54
Time to recovery (minutes)	4	8	3	4
Mean RR (breaths/minute), range	32.5 (20–40)	17 (16–20)	38 (30–50)	45 (40–50)
Mean HR (beats/minute), range	105 (100–120)	111 (108–120)	107 (100–110)	107 (100–120)
Number of apnoea events	1	0	2	1
Onset of apnoea (in minutes following butorphanol IV)	Immediate	NA	1, 64	1
Cyanosis events	0	0	0	0
Regurgitation events	0	0	0	0

Note: All pups were appropriately sedated for procedures following administration of butorphanol IV. All pups were administered with flumazenil (4 μg/kg IM) and naltrexone (0.1 mg/kg IM) to reverse effects at the end of procedures.
Abbreviations: HR, heart rate; RR, respiratory rate.
[a]Pup #1 received a second dose of midazolam (0.1 mg/kg) 11 minutes after the initial dose for a total of 0.3 mg/kg midazolam.

for all other age groups. In this fifth pup, the initial dose provided sufficient sedation for the length of the procedure with no observed adverse effects (Table 1).

Week 3

In cohort A, the initial dose of midazolam and butorphanol in combination IM (Table 1) provided sufficient sedation for the length of the procedure in three of the nine pups. Four individuals received one maintenance dose of midazolam (0.1 mg/kg) and butorphanol (0.05 mg/kg) IV. Two individuals received two maintenance doses of midazolam (0.1 mg/kg) and butorphanol (0.05 mg/kg) IV at 28 and 96 minutes, and 51 and 78 minutes after the initial doses, respectively. Two individuals regurgitated once during the procedure.

In cohort B, initial sedation doses of midazolam and butorphanol in combination IM were sufficient for all procedures, with a maximum procedure length of 90 minutes (Table 1).

Week 5

In cohort A, four of nine pups required maintenance doses (Table 1); two pups received one dose of either midazolam (0.1 mg/kg) IV or butorphanol (0.05 mg/kg) IV 1.5 hours into the procedure. Two

pups required two maintenance doses each. One received midazolam (0.1 mg/kg) and butorphanol (0.075 mg/kg) IM 20 minutes after the initial dose, followed by a second dose of midazolam (0.1 mg/kg) and butorphanol (0.075 mg/kg) IV 40 minutes after the initial dose. One pup received midazolam (0.1 mg/kg) and butorphanol (0.075 mg/kg) IV 70 minutes after the initial dose, and a repeat of this same dose 80 minutes after the initial dose. One individual regurgitated once during the procedure.

In cohort B, the initial sedative combination provided sufficient sedation for the length of all procedures, with a maximum procedure length of 57 minutes (Table 1). One individual regurgitated once during the procedure.

Week 7

In cohort A, four of the nine individuals required maintenance doses (Table 1); two received one dose each of midazolam (0.1 mg/kg) and butorphanol (0.05 mg/kg) IV administered 25 minutes after the initial dose, and two individuals required two maintenance doses each. One individual was particularly refractory to sedation in prior sampling weeks; this pup received a higher initial dose of midazolam (0.3 mg/kg) and butorphanol (0.2 mg/kg) IM. Despite the higher dose, this individual required two maintenance doses; the first dose of midazolam (0.15 mg/kg) and butorphanol (0.1 mg/kg) IV was administered 20 minutes after the induction dose, and the second dose of midazolam (0.15 mg/kg) and butorphanol (0.1 mg/kg) IV was administered 69 minutes after the induction dose. A second individual received two maintenance doses of midazolam (0.1 mg/kg) and butorphanol (0.05 mg/kg) IV at 50 and 92 minutes after the induction dose. One individual regurgitated several times during the procedure, and the procedure was terminated after 63 minutes.

In cohort B, the initial dose of sedation was sufficient for all procedures, with a maximum procedure length of 55 minutes (Table 1).

Recovery

Recovery times and doses are presented in Tables 1 and 2. All pups were successfully reversed at the end of procedures, and recovery was defined as alert and fully ambulatory with purposeful movement. Naltrexone was administered at 0.1 mg/kg across all ages and procedures.

Due to a supply shortage in 2017, flumazenil doses were lower to conserve inventory. Doses ranged between 0.7 and 4 µg/kg. In all but one case, flumazenil was administered at a dose of 0.75 or 1.5 µg/kg. One individual received a flumazenil dose of 4 µg/kg due to minimal response observed with 1.5 µg/kg. Naltrexone and flumazenil were administered IV ($n = 4$), IM ($n = 12$) or a combination of IV and IM ($n = 4$), depending on environmental conditions and individual reactions under sedation. The route of administration was not recorded in three procedures. One individual in 2017 required two doses of each reversal at weeks 3, 5 and 7 for full recovery, with week 7 requiring three doses of naltrexone and an increased dose of flumazenil on second administration (4 µg/kg). Mean recovery times ranged between 0 and 12 minutes when recovery time was recorded ($n = 17$). Recovery data for 2017 are excluded from Table 1, as the same recovery timepoints were not consistently recorded in this year and there was greater variation in routes of administration and doses, making comparisons difficult.

In 2019, the flumazenil dose was increased to 4 µg/kg and standardised across procedures, except for pups in cohort B at weeks 5 and 7, which received an increased dose of flumazenil (8 µg/kg) to speed recovery. This higher dose of flumazenil resulted in higher volumes of drug, and the dose was given in two injections, half IM and the other half IV (approximately 4 ml each). In two individuals, one at week 1 and one at week 3, two doses of each reversal were required for full recovery.

Changes in vital signs and apnoea events with age (weeks 1, 3, 5 and 7)

Mean RR was significantly different across age ($F_{3,56} = 8.698$; $p < 0.05$), with significantly lower RR at week 5 compared to week 1 ($p = 0.013$) and significantly lower RR at week 7 compared to week 1 ($p = 0.016$; Figure 1a). No other ages were significantly different from each other for RR. There was a significant effect of age on mean HR ($F_{3,56} = 15.924$; $p < 0.05$), and post hoc analysis showed that mean HR was significantly higher at week 1 than at week 5 ($p = 0.013$) and week 7 ($p = 0.016$). No other ages were significantly different from each other for HR (Figure 1b). Apnoea events were not significantly different among ages ($\chi^2 = 6.2623$; $p = 0.09$), though week 1 pups tended to have fewer apnoea events than older pups on average (Figure 1c). Additionally, there was no relationship between apnoea events and the initial IM dose of either midazolam or butorphanol. A significant difference ($p < 0.05$) was present between IM and IV administration and the number of apnoea events experienced by week 1 pups, such that week 1 pups experienced significantly more apnoea events under IV administration (Figure 1c).

Correlations between the number of apnoea events and length of sedation were investigated, and no relationship was identified (Figure 2); therefore, models were not corrected for variation in sedation length.

Relationships between vital signs under sedation and development of diving abilities

The top models for each response variable are presented in Table 3. The top ranked model for mean

TABLE 3 Linear mixed model results examining relationships among respiratory rate (RR), heart rate (HR), apnoea events and development of diving in Weddell seal pups

Response	Model	logLik	AIC	ΔAIC	R^2_m; R^2_c
Mean RR	35.04 – 0.0112 (number of dives) + (age)	–194.993	406.5	0.95	0.14; 0.46
	35.01 – 0.003 (maximum dive depth) + (age)	–195.470	407.4	3.24	0.14; 0.52
Mean HR	122.8 + 0.078(number of dives) + (age)	–174.922	366.3	2.6	0.55; 0.79
Number of top up doses	–6.65 + 0.04546 (length of sedation [minutes])	–32.162	70.8	16.05	0.59; 0.71

Note: ΔAIC is the difference between the top model and next ranked model.
Abbreviations: AIC, Akaike information criteria; logLik, log likelihoods.

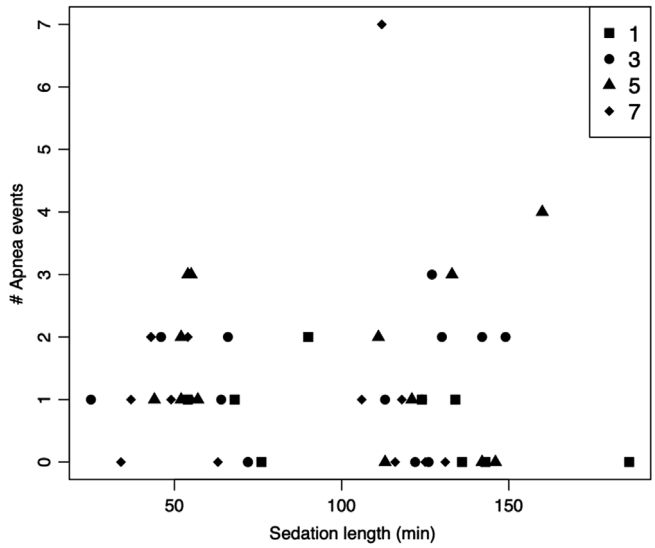

FIGURE 2 No relationship was apparent between the length of the sedation (in minutes) for each procedure and the number of apnoea events experienced by Weddell seal pups ($n = 18$). Symbols represent different ages at handling: week 1 (squares), week 3 (circles), week 5 (triangles) and week 7 (diamonds)

RR showed that there was a significant relationship between mean RR and the number of dives and age (Table 3); although this model was not significantly different from the second ranked model (AIC < 2), for which maximum dive depth and age were both significant contributors to variation in mean RR (Table 3). In both models, the effects of the dive parameters were small. Combined, individual variation and age accounted for 54% of the variation in mean RR (R^2_m = 0.14; R^2_c = 0.54). Mean HR was significantly related to the number of dives and age. Together, individual variation, number of dives and age accounted for 79% of the variation in mean HR, with individual ID accounting for 24% of the variation (R^2_m = 0.55; R^2_c = 0.79). Neither apnoea nor the number of apnoea events during a procedure were related to dive parameters or age of the pup in any model.

Relationships between maintenance doses, sedation length and individual variation

For procedures in which midazolam and butorphanol were given in combination IM, maintenance doses (given at half the initial dose) were needed in 16 of the procedures, all of which were in cohort A (where mean procedure length was longer and sampling procedures were more invasive). Sedation duration was not recorded in 10 procedures. No more than two maintenance doses were needed for any procedure. The LME showed that there was a significant effect of sedation length on the number of maintenance doses (ΔAIC = 16.05 from null model; R^2_m = 0.59); however, individual variation accounted for 11% of the variation in dose requirements (R^2_c = 0.70) (Table 3). Of the 18 individuals in this study, seven needed maintenance doses. In six procedures, two maintenance doses were required to maintain an adequate level of sedation, and all six procedures occurred in the same two individuals.

DISCUSSION

The combination of midazolam and butorphanol was safe and effective in producing adequate sedation and muscle relaxation for the sampling objectives. No morbidity or mortality was observed secondary to administration of sedative agents, regardless of route, and doses were scalable to suit individual and procedural needs. As animal age increased, an increase in butorphanol dosage was required to achieve adequate sedation. As expected, maintenance doses were more common when procedure lengths were longer. Rather than sedation length or initial dose, individual variation was the best predictor of whether a maintenance dose would be necessary. Minimum effective doses are always recommended to minimise the likelihood of adverse effects,[43] and future applications of this protocol should consider individual response in addition to all other study goals when selecting initial doses.

In 1-week-old pups, IV midazolam (0.2–0.3 mg/kg) was ineffective in providing sedation and muscle relaxation for longer than 15 minutes and was insufficient for achieving full immobilisation (Table 2). Whether midazolam could be effective alone at higher doses and in older animals is unknown. Midazolam at doses as high as 0.5 mg/kg has been sufficient for moderate to heavy sedation in other phocid species[26]; and in a closely related phocid, the crabeater seal (*Lobodon carcinophaga*), an average dose of 0.55 mg/kg was effective in producing moderate sedation for

induction of inhalant anaesthesia.[44] Depending on sampling requirements, midazolam alone may be effective for shorter duration handlings without invasive sampling procedures and as a pre-induction agent for general anaesthesia.

Although considered to be minimally invasive, muscle biopsy was anticipated to elicit some pain, and the combination of butorphanol with intradermal and subcutaneous lidocaine was sufficient in greatly reducing or eliminating a pain response in these individuals. Studies with greater anticipated pain should carefully consider the level of analgesia required and whether this protocol will be sufficient. While a pain response during sample collection was reduced during this procedure, both analgesics used were short term and likely did not provide analgesia for more than a few hours after sample collection.[12,45] There is limited information on pharmacokinetics of other analgesics, such as non-steroidal anti-inflammatories (NSAIDs), in phocid species, and combined with the low level of pain expected following initial sample collection, these medications were not considered for this study. However, NSAIDs have been used regularly in captive and rehabilitation pinnipeds, and could be considered for additional analgesic support.[46]

Given the suspected increased sensitivity of neonatal animals to anaesthetic drugs, a species-specific, safe and reversible sedation protocol was desired for this age class. Reversible sedation protocols facilitate a rapid recovery and provide agonistic effects under emergency situations. A fast recovery was particularly important in this study as vocal recognition ('contact' calls) is a key mother–pup reunification behaviour,[47,48] and a quick return of normal alert and vocal behaviours was thus highly desired. Reversibility was also desired in this extreme environment where unpredictable weather conditions may change rapidly, necessitating a quick recovery for both human and animal safety. The protocol used in this study had successfully been used in the sedation of neonatal harbour seals[19] and was modified for Weddell seal pups. The most recently developed and commonly applied sedation protocol for adult Weddell seals includes the non-reversible drug ketamine,[9,10] with observed recovery times between 37 and 44 minutes.[9] All pups in this study were administered reversals at the conclusion of sampling, and all clinically recovered quickly (0–12 minutes post-administration) and were fully capable of safely navigating around the rookery and entering the water. While the study protocol produced the desired sedation and reversal effects, it is worth noting that the pharmacology of both the sedatives used in this study and the reversal agents have not been studied in this species. Therefore, there are limitations in our understanding of the underlying mechanisms by which these medications produced the observed effects.

The adverse effects associated with sedation in this study included transient apnoea and, infrequently, regurgitation. An unknown factor in evaluating regurgitation in these patients was the timing of feeding events prior to procedures. In both human and veterinary medicine, fasting is recommended for at least 2 hours prior to anaesthesia to reduce the risk of regurgitation and aspiration.[49,50] It is possible that recent feeding events predisposed these pups to regurgitation under sedation, and this is an important consideration when working with dependent pups. Butorphanol is a respiratory suppressant[23,51,52] and was likely responsible for inducing the apnoea observed in these individuals. This is supported by the minimal effects observed with midazolam administered alone and the rapid onset of apnoea observed in 1-week-old pups following IV butorphanol administration. Individuals that received the combination induction dose also experienced transient apnoea, although the timing of onset was more variable. The apnoea observed in all individuals was transient and resolved with minimal or no intervention. In some cases, patients were alert with eyes open during breath-hold events. Transient apnoea has been observed in one obese harbour seal, which received butorphanol and a similar benzodiazepine, diazepam.[53] The observed apnoea in this study suggests that close respiratory monitoring is warranted, especially with IV administration of butorphanol, and that individual variation and age likely play a role in the occurrence of apnoea. Dose-dependent relationships to adverse effects are well known in the veterinary profession, though interestingly, no relationship between dose and apnoea events was observed for the range of doses administered in this study. Regurgitation occurred infrequently and association with dose was not investigated. In previous studies of Weddell seals using other immobilisation agents, poor sedation results and adverse effects (apnoea, death) have been linked to inaccurate estimations of an individual's mass, or possibly unintended IV administration.[9,10] Pups in this study were all weighed prior to sedation, allowing for precise dose calculations, but variations in administration, including accidental IV or subcutaneous injection, may have played a role in the observed effects. Appropriate interventions, including medications and intubation supplies, should be available to protect individuals from respiratory compromise. In addition, the sample size in this study is relatively small when considering all potential individual reactions to a novel medication, and the observed responses may not encompass all possible adverse reactions.

This study provided a unique opportunity to investigate correlations between observed clinical effects of sedation and stage of diving development. RR generally decreased with age, and individual variation accounted for most of the variability, though a weak relationship between RR and dive parameters was present. HR was significantly different across the ages examined, and overall mean HR decreased over the course of the study. This effect was best explained by age, individual variation and number of dives (Table 3). The decline in HR as the animals increased in cardiovascular fitness and diving capabilities is not surprising. Transient apnoea events

observed in this study became more common as animals aged and began diving (week 3 and older). Dive depth and number of dives performed did not appear to influence whether apnoea was present (Table 3). These observed responses complement previous literature in elephant seals and harbour seals on the physiological changes in cardiorespiratory patterns in relation to apnoea.[54–56] Young pups exhibit a variable HR response with voluntary apnoea that becomes more regular as they age, ultimately leading to lower HRs during apnoea events. [54–58] The sample size in this study is statistically insufficient to fully describe the underlying relationships between the measured vital signs under sedation and dive parameters, particularly with the large contribution of individual variation observed in Weddell pups of this age.[37,38,59] Other underlying mechanisms that were not examined in this study may play a role in the observed results. Despite this, the observed variations in RR and HR as animals aged are likely partially due to the physiological changes associated with this period of development. This observation has important implications for monitoring responses to sedative agents, particularly where a physiological change is occurring. Age and physiological state should be taken into consideration when immobilising phocids in the field.

CONCLUSIONS

A combination of midazolam (0.2–0.3 mg/kg) and butorphanol (0.1–0.2 mg/kg) provided safe, effective and reversible sedation for Weddell seal pups between the ages of 1 and 7 weeks in a remote field setting, including during the sensitive neonatal period. Naltrexone (0.1 mg/kg) and flumazenil (4–8 µg/kg) are recommended for reversal of the primary sedative agents. Dose and route of administration should be considered with future applications of this protocol. Appropriate emergency interventions to support respiration should be available, including the ability to intubate and ventilate. Individual response to sedation varies, and the stage of development likely plays a role in observed effects of sedative agents. When using this protocol in the field, researchers should consider the individual and stage of development when estimating and interpreting the clinical effects of sedation.

AUTHOR CONTRIBUTIONS

Project planning, project implementation, data analysis and interpretation, manuscript writing and editing and manuscript submission: Sophie Whoriskey. *Project planning, project implementation, data analysis and interpretation and manuscript writing and editing*: Linnea E. Pearson. *Project planning, project implementation, data interpretation and manuscript editing*: Heather S. Harris. *Project planning, project implementation, data interpretation and manuscript editing*: Emily R. Whitmer. *Project planning, project implementation, data interpretation and manuscript editing*: Heather E.M. Liwanag. Project planning, *Project implementation and manuscript editing*: Erin Brodie. *Project planning, project implementation, data interpretation and manuscript editing*: Shawn Johnson.

ACKNOWLEDGEMENTS

The authors would like to thank field team members Lars Tomanek, Bridget Ward and Emma Weitzner for helping in data collection, and Liz Eby for assisting in data management. They would also like to thank Cara Field for contributions to protocol refinement. Additional thanks to everyone at McMurdo Station including, but not limited to, Science Cargo, Crary Lab and the Galley crew. A special thanks to B-009 (PI: Jay Rotella) for help in identifying study animals. This research was funded by the National Science Foundation (ANT-1543539 to H.L., S.J. and L.P.), with field support by the United States Antarctic Program, Leidos ASC, and conducted under NOAA permit #21006-01 and ACA 2018-013 M#1, and Cal Poly IACUC #1605 and 1904.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data are available through the US Antarctic Program Data Center at www.usap-dc.org.

ETHICS STATEMENT

This study was conducted with ethical approval from NOAA Fisheries under the Marine Mammal Protection Act (permit # 21006-01), the Antarctic Conservation Act (permit # 2018-013 M#1) and the California Polytechnic University Institutional Animal Care and Use Committee (#1605 and 1904).

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How to cite this article: Whoriskey S, Pearson LE, Harris HS, Whitmer ER, Liwanag HEM, Brodie E, et al. Using a combination of midazolam and butorphanol is a safe and effective reversible field sedation protocol for Weddell seal (*Leptonychotes weddellii*) pups. Vet Rec. 2022;e2238.
https://doi.org/10.1002/vetr.2238