Use of midazolam and butorphanol to sedate harbor seal pups (*Phoca vitulina*) undergoing rehabilitation

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Abstract

Between 2012 and 2022, the Vancouver Aquarium Marine Mammal Rescue Centre sedated 110 harbor seal pups for physical examinations, diagnostic procedures, or treatment. A sedation protocol of butorphanol and midazolam (0.1–0.2 mg/kg each) was administered via a single i.v. injection in 171 procedures. Of these, 21 pups were anesthetized only with the injectables, while supplemental isoflurane inhalation anesthesia by mask was provided during 58 procedures; 92 other animals required intubation for respiratory support due to apnea or to achieve a deeper plane of anesthesia to facilitate more invasive procedures. Of the 171 sedations, five were euthanized due to poor prognosis and six failed to recover. Maximum sedation, sufficient for intended procedure or anesthesia induction, was achieved within a mean of 8.5 ± 5.8 min for i.v. injection ($n = 133$). Sedation duration (drug administration to full recovery) without supplemental inhalation anesthesia had a mean of 30.2 min and ranged from 14 to 52 min ($n = 13$). When used in stabilized young harbor seals, administration of injectable butorphanol and midazolam proved to be an effective protocol to obtain safe and reliable sedation for physical examination, minimally invasive diagnostic procedures, or as a premedication for general anesthesia.

1 | INTRODUCTION

Harbor seals are a widely distributed phocid species found along coastal waters of the northern Atlantic and Pacific Oceans, as well as the Baltic and North Seas (Burns, 2009). Phocid seals, like other marine mammal species, have several anatomic and physiologic adaptations which facilitate conservation of oxygen during prolonged diving in their aquatic environment (Panneton, 2013). This dive reflex includes bradycardia and subsequent hypotension, apnea, shunting of blood away from peripheral tissues to prioritize central nervous system and cardiac function, and an overall increased tolerance to carbon dioxide. However, these adaptations may make anesthesia more challenging compared to terrestrial species of similar size as it is suspected that anesthesia can induce an inappropriate dive response, which has been attributed to some anesthetic-related mortalities in pinnipeds (Higgins & Hendrickson, 2013; Lynch et al., 1999; Panneton, 2013). Similar to some domestic species under a light plane of anesthesia, phocids often exhibit apnea following induction (necessitating intubation) or following endotracheal tube placement, necessitating intermittent positive pressure ventilation (Hammond & Elsner, 1977; Higgins & Hendrickson, 2013; Woods et al., 1996). Intubation can be difficult in phocids due to their excessive pharyngeal tissue causing obstruction of the upper airway (Hammond & Elsner, 1977; Higgins & Hendrickson, 2013; Lynch et al., 1999; Pang et al., 2006). The shunting of blood from peripheral tissues can alter thermoregulation as well as complicate peripheral vascular access (Hammond & Elsner, 1977; Higgins & Hendrickson, 2013). Due to these confounding factors, there is an overall higher risk of anesthetic complication in phocids, especially in debilitated animals (Lynch et al., 1999; Pang et al., 2006).

Despite these challenges, numerous phocid species have been safely immobilized either for sampling in the field or medical care in rehabilitation and managed care settings. The exact protocols differ substantially and include injectables and inhalation anesthesia with or without premedication with various injectables (Haulena & Schmitt, 2018). Previously published harbor seal protocols have utilized injectable diazepam and butorphanol alone or in combination as well as propofol infusion (Gulland et al., 1999; Lapierre et al., 2007; Manugian et al., 2014; Tuomi et al., 2000). For longer or more invasive procedures, phocids have been safely maintained on inhalation anesthesia, most commonly with isoflurane or sevoflurane (Gales et al., 2005; Gulland et al., 1999; Huuskonen et al., 2011; Kusagaya & Sato, 2001; Pang et al., 2006). Anesthesia-related complications in phocids have included bradycardia, hypothermia, apnea, and death associated with prolonged apnea causing hypoxemia, often presumed to be associated with the dive response (Gales et al., 2005; Gulland et al., 1999; Higgins & Hendrickson 2013; Lapierre et al., 2007; Lynch et al., 1999; Woods et al., 1996). Hence, there is a need to develop reliable, safe anesthetic protocols for this group of animals.

Midazolam is an injectable benzodiazepine used primarily as a preoperative medication. It provides sedation, muscle relaxation, and anxiolysis when used in combination with other drugs or general anesthesia. Compared with diazepam, midazolam has a faster onset time, shorter duration of action, and may be delivered by intravenous (i.v.) or intramuscular (i.m.) routes, giving it increased utility (Plumb, 2015). These induction characteristics, in addition to its reversibility with flumazenil, make it a useful component of a multimodal anesthetic combination (Rankin, 2015, Chapter 10). In dogs and cats, the recommended dose of midazolam as a preoperative agent is 0.1–0.3 mg/kg i.v. or i.m. when used in combination with an opioid (Bednarski, 2015, Chapter 44).

Butorphanol is a mu antagonist and kappa agonist opioid drug used in a variety of species as an analgesic, premedication, or antitussive agent (KuKanich & Wiese, 2015, Chapter 11). It has a quick onset, short duration of action, and may be partially reversed with naloxone or naltrexone (KuKanich & Wiese, 2015, Chapter 11).

In domestic species, it is used as an analgesic or in combination with other drugs (such as midazolam) as a sedative at a dose of 0.2–0.4 mg/kg i.v., i.m., or subcutaneous (Bednarski, 2015, Chapter 44). When used together, a midazolam/opioid combination can cause less cardiovascular depression than other drug combinations and has a synergistic effect, creating more predictive sedation for minimally invasive procedures (KuKanich & Wiese, 2015, Chapter 11; Plumb, 2015). Butorphanol and midazolam combinations have been safely used in a wide range of domestic, exotic, and wildlife species (Bush et al., 2012; Plumb, 2015; West et al., 2014; Whoriskey et al., 2022). This study evaluated the use of injectable midazolam and butorphanol to facilitate safe and effective sedation in harbor seal pups undergoing rehabilitation.

2 | MATERIALS AND METHODS

From 2012 to 2020, the Vancouver Aquarium Marine Mammal Rescue Centre (MMRC) performed 174 sedations on 110 harbor seal pups, with 39 seals sedated more than once. The harbor seals were admitted from the along the British Columbia coastline, with the majority of animals coming from large human population centers. The most common cause of pup stranding was maternal separation with associated malnutrition, hypoglycemia, dehydration, and/or hypothermia. Other causes included infectious disease, trauma, and entanglement.

Animals were sedated as medically indicated to permit physical examination, wound debridement and surgery, and/or diagnostic procedures such as radiography, ultrasound, endoscopy, magnetic resonance imaging, or computed tomography. When possible, seals received a full preanesthetic physical examination with bloodwork (complete blood count and serum biochemistry panel) and were stabilized as medically indicated. Four seals were sedated for emergency treatment prior to adequate stabilization. All anesthetic candidates were fasted for at least four hours prior to the procedure. Seals did not have access to water until sufficiently recovered. Thermoregulatory support was administered as needed.

There were minor variations in anesthetic protocols amongst patients as procedures were driven by clinical assessment. Patients were assigned to the following treatment groups for this analysis: injection only (no mask or intubation; i.v. injection route at all dose levels), mask only (in addition to injection, they received inhalant anesthesia by mask) and intubation (seals which were injected and masked to permit intubation, then maintained on inhalant anesthesia). Details of induction protocols are described below.

Records were originally obtained from 174 procedures on 110 individual seals. Single procedures were undertaken on 71 seals, while multiple procedures (2–10) were performed on an additional 39 animals. These 64 repeated trials were generally far enough apart to be considered independent (mean interval 29.3 ± 28.4 days, range 0–123 days) but we removed three points from our data set for procedures that were within 48 hr of the previous one. This left a total of 171 data points from 110 seals, 68 males and 42 females, with an average body mass at time of sedation of 17.8 ± 7.7 kg (range 7.7–36.6 kg).

Under manual restraint, a single injection was delivered i.v. into the epidural intravertebral vein according to standard technique (Barbieri, 2018). Each dose was calculated based on current patient weight at a dosage of 0.1–0.2 mg/kg midazolam (5 mg/ml; Sandoz Inc., Princeton, NJ) and 0.1–0.2 mg/kg butorphanol tartrate (10 mg/ml; Zoetis Canada Inc., Kirkland, Quebec, Canada). The majority of seals ($n = 89$) received 0.15 mg/kg butorphanol and midazolam while 80 received 0.1 mg/kg and two were given 0.2 mg/kg. Injections were administered via a 1 ml or 3 ml syringe and 22 g 1 in. or 1.5 in. needle depending on patient size. When deeper anesthesia was required, seals were subsequently induced with isoflurane via mask with up to 5% isoflurane and oxygen and were maintained via either face mask or endotracheal tube with isoflurane (Fresenius Kabi Canada, Toronto, Ontario, Canada) and oxygen. If a patient became apneic or hypoxic, intermittent positive pressure ventilation was provided.

Various physiological monitoring took place, although values were not necessarily available for all measures from all 171 procedures. Seals were monitored closely and whenever possible, heart and respiratory rates were recorded every 5 min from drug administration to recovery. Monitoring was performed via direct auscultation, visualization of

thoracic wall movements, and/or electrocardiography. Sedation efficacy was characterized by mortality, recovery time, minimum heart rate, and minimum respiratory rate. In cases where seals were placed on intermittent positive pressure ventilation, the minimum reported respiratory rate used in analysis was taken prior to ventilation initiation. Differences between treatment groups were tested using Kruskall-Wallace H test via Prism 9 software (GraphPad Software, Boston, MA). Although dosage differed during the trials, we did not explicitly test for differences given uneven sample sizes.

Of the 171 anesthetic procedures, reversal agents flumazenil at 0.01 mg/kg i.m. and naltrexone at 0.05–0.15 mg/kg i.m. were administered in 135 cases based on clinical need. Supplemental treatment, such as fluids, antibiotics, etc. were given as medically indicated. Two animals received emergency treatment due to compromised recoveries.

3 | RESULTS

Treatment scenarios and anesthetic monitoring results are summarized in Table 1. Of the 171 sedations, 21 animals received injection only, 58 were masked and 92 were intubated.

There was a significant difference among treatment groups in minimum spontaneous respiration rates (RRmin) recorded before any ventilation intervention (Kruskall-Wallace $H = 12.28$, $p = .002$). Mean RRmin was significantly greater for the injection only (11.6 ± 9.9, $n = 20$) compared to either those masked only (5.4 ± 5.6, $n = 52$) or intubated (5.0 ± 3.6, $n = 77$) groups.

We found no statistical difference in minimum heart rate (Hrmin; Kruskall-Wallace $H = 4.3$, $p = .11$) between the injection only (82.4 ± 15.2 BPM, $n = 21$), masked only group (78.8 ± 24.7 BPM, $n = 55$), or intubated seals (85.0 ± 24.4 BPM, $n = 91$) treatment groups.

Adequate sedation was achieved in all cases using just the injection (regardless of dose or eventual sedation treatment) sufficient to permit gentle handling or subsequent application of mask for delivery of inhalation anesthesia. Maximum sedation was achieved within a mean of 8.5 ± 5.8 min ($n = 133$).

Sedation duration (drug administration to full recovery) without supplemental inhalation anesthesia had a mean of 30.2 min and ranged from 14 to 52 min ($n = 13$). In this study, only one seal received injectables without receiving a reversal (recovery time = 50 min). Across all treatments, duration time was much shorter for animals receiving reversal agents (47.3 versus 66.6 min) primarily due to changes in recovery time. Overall, recovery time after being administered reversals was predictably rapid, and was significantly affected by anesthetic regime (Kruskall-Wallace $H = 23.5$, $p < .001$). Recovery times were most rapid for seals only administered injectables (3.4 ± 2.6 min, $n = 12$),

TABLE 1 Number of procedures carried out using one of three treatment scenarios: combined butorphanol and midazolam injection alone (regardless of dose or route of administration), injection followed by isoflurane administered via a face mask, and injection followed by mask and intubation (maintained on inhalant). The number of individual seals for each treatment (some seals had multiple repeated or different treatments), the number of unexpected mortalities, minimum heart rate, and minimum respiratory rate are also provided.

	Injection alone	Injection + inhalant (mask)	Injection + inhalant (intubation)
Number of procedures	25	58	94
Number of seals	24	48	71
Number of mortalities	1	0	5
Heart rate, minimum (BPM)	91.9 ± 29.0 (25)	78.8 ± 24.7 (55)	85.2 ± 24.2 (93)
Respiratory rate, minimum (BPM)	11.6 ± 9.9 (20)	5.4 ± 5.6 (52)	5.0 ± 3.6 (79)

followed by those masked with isoflurane (6.0 ± 4.0 min, $n = 22$) and those that were intubated (11.0 ± 7.6, $n = 59$). Not surprisingly, time to recovery was more prolonged when reversals were not administered. In these cases, recovery time from injection only was longest (50.0 min, $n = 1$), and almost identical for cases where they were only masked (55.0 ± 18.4, $n = 6$) or intubated (54.8 ± 42.2, $n = 8$). Not surprising given the small sample sizes, we found no significant difference between the masked and intubated treatments from each other (Mann–Whitney U test, $p = .95$). There were two procedures (not included in statistical analysis) with prolonged recoveries which necessitated emergency medications and/or repeat reversal medications.

There were six deaths either during the procedure or within 24 hr of recovery. Two of these mortalities occurred among severely debilitated patients not stabilized prior to sedation due to having significant trauma that necessitated emergent medical attention. Necropsy results from those two individuals as well as two additional mortalities confirmed severe comorbidities, such as sepsis or herpesvirus infection. Of the remaining two losses, one animal had received sedation to permit imaging while the other underwent bilateral cataract surgery; both expired despite reversals and emergency treatment. Necropsy of those two individuals did not identify any notable cause of death, suggesting possible anesthesia related losses. Five additional animals were euthanized under sedation due to poor prognosis. After removing the five euthanized individuals, a mortality rate of 3.6% (6/169) was determined.

4 | DISCUSSION

In this study, a low dose combination of injectable butorphanol and midazolam produced safe and reliable sedation to facilitate restraint for noninvasive procedures or as a premedication for general anesthesia in harbor seals undergoing rehabilitation. In contrast to previous diazepam studies which were only used in healthy animals, the butorphanol/midazolam protocol boasted a margin of safety for application in mildly compromised patients ($n = 171$) with a dose ranging from 0.1 to 0.15 mg/kg of each sedative with a low mortality rate. The predictive sedation achieved in the dosing range permits flexibility when evaluating patient health status or in times when an accurate weight is not available. Two additional seals were sedated safely with 0.2 mg/kg of each sedative; additional observation may be warranted to ensure higher dosing is well tolerated on a larger sample set. The sedation cocktail was also given safely to three patients i.m. giving added utility to the protocol in patients where venous access is inaccessible. The extended patient observation postprocedure in this study also boasts its safety margin compared to previous harbor seal studies when patients were immediately released and limitedly monitored postsedation.

The primary complication observed in this study was apnea, observed in patients with and without additional isoflurane, which was mitigated through intubation and intermittent positive pressure ventilation. Although pups were stabilized prior to anesthesia, there likely were comorbidities impacting the animal's physiologic health status, which may have influenced level and duration of sedative effect of some individuals. Our case series had six mortalities, four of which occurred among animals with ongoing comorbidities confirmed by necropsy and histologic exam, and two that may have been more directly related to anesthesia, which resulted in a low mortality rate of 3.6%. The severely compromised individuals likely were poor anesthetic candidates and their underlying conditions contributed to their loss under anesthesia.

The multiroute administration and partial reversibility of this sedation protocol have added utility for applications in individuals of various phocid species which may be difficult to restrain in both wild and managed care settings. Sedative and reversal medications can also be obtained in concentrated formulations that allow for smaller volumes to be used, even in larger patients.

In conclusion, when used in healthy harbor seal pups, administration of low-dose injectable butorphanol and midazolam at a dose ranging from 0.1 mg/kg to 0.15 mg/kg each proved to be an effective protocol to obtain safe and reliable sedation, which facilitated physical examination, diagnostic testing, or induction of general anesthesia. For future cases, the authors recommend routine administration of reversal agents flumazenil at 0.01 mg/kg i.m. and

naltrexone at 0.05–0.15 mg/kg i.m., especially in patients undergoing general anesthesia, to aide in recovery and mitigate potential patient loss associated with known or unknown comorbidities. As with all anesthetic procedures, the clinician should always be prepared for intubation and intermittent positive pressure ventilation, regardless of intended procedure duration, due to the likelihood for developing apnea in phocids.

AUTHOR CONTRIBUTIONS

Chelsea E. Anderson: Conceptualization; data curation; investigation; methodology; project administration; resources; writing – original draft; writing – review and editing. **Karisa Tang:** Data curation; writing – review and editing. **Courtney Pace:** Data curation; writing – review and editing. **David A. S. Rosen:** Formal analysis; resources; software; writing – review and editing. **Martin Haulena:** Conceptualization; methodology; resources; supervision; writing – review and editing.

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