



Field anesthesia of juvenile Steller sea lions (*Eumetopias jubatus*) using inhalation anesthesia

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ABSTRACT

Between 1998 and 2008, 621 Steller sea lions (*Eumetopias jubatus*, SSL) were captured underwater by SCUBA divers and anesthetized with isoflurane ($n = 602$) or sevoflurane ($n = 19$). We found significantly faster induction time ($\bar{x} \pm SD$) for sevoflurane (11 ± 6 min) compared to isoflurane (14 ± 6 min), as well as an interaction between anesthetists using the isoflurane protocol. Severe hypothermia with temperatures $<35^{\circ}\text{C}$ were measured in 22% of all animals, and had significant associations with month, length of anesthesia, and sex. Mortality rate was low (0.33%). We conclude that both isoflurane and sevoflurane anesthesia were effective for field anesthesia to safely handle and sample SSL.

Key words: anesthesia, hypothermia, isoflurane, sevoflurane, Steller sea lion, *Eumetopias jubatus*.

The Steller sea lion (*Eumetopias jubatus*, SSL) is a marine apex predator inhabiting the Alaskan coastline (Castellini *et al.* 2012). Two genetically distinct population segments (DPS) of SSL are recognized; the western DPS found west of 144°W and the eastern DPS found east of 144°W . The National Marine Fisheries Service listed the western DPS as endangered under the US Endangered Species Act (ESA), whereas the eastern DPS was recently delisted (NMFS 2013). The reasons for this population decline, and subsequent failure of some regions in the western DPS to recover are still uncertain (Beckmen *et al.* 2002, Burek *et al.* 2005, Atkinson *et al.* 2008, Castellini *et al.* 2012, Rea *et al.* 2013). Several management-oriented research programs have

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sought to understand why segments of the western DPS population have failed to recover, and biological samples and specimens are routinely collected (Burek *et al.* 2005; Pitcher *et al.* 2005; Rehberg and Burns 2008; Castellini *et al.* 2012; Rea *et al.* 2013, 2016; Correa *et al.* 2014; Lander *et al.* 2014; Spitz *et al.* 2015).

Field anesthesia is needed for sampling and satellite tagging wild pinnipeds (Haulena 2014), thus development of safe anesthesia protocols is of paramount importance. Remote drug delivery with a dart projector carries the risk that the animal will escape to the water after darting and possibly drown once anesthetized (Heath *et al.* 1996, Haulena 2014). Darting is also considered a less efficient capture technique, because only one animal can be captured per rookery or haul-out per event, as the remaining animals are disturbed and escape to water. Older young of the year and yearling animals (juveniles) can occasionally be physically restrained onshore using a net, but with a lower success rate, due to the difficulty of approach and safe handling of these animals which range between 50 and 184 kg in mass (Haulena 2014). Juvenile pinnipeds are curious and playful animals, and will often approach recreational divers. This behavior allowed development of an underwater capture technique for SSL (Raum-Suryan *et al.* 2004).

In addition to risks associated with capturing wild SSLs, anesthetizing pinnipeds presents many challenges. Pinnipeds have a strong dive response that can be triggered by anesthesia and lead to breath-holding, apnea, and bradycardia (Haulena and Heath 2001, Haulena 2014). Additionally, field conditions without sophisticated monitoring equipment and difficulties obtaining intravenous access also pose challenges for anesthetizing pinnipeds in the field. To address these challenges, a protocol for inhalation anesthesia to replace remote delivery of tiletamine-zolazepam, was developed using a portable field anesthesia machine (Heath *et al.* 1996, 1997).

In this study, our goal was to describe the anesthetic drug protocols used for juvenile SSL live captured under water by SCUBA divers. Here we describe the use of isoflurane for field anesthesia of this species over a 10 yr research period (1998–2008). We also provide a comparison of induction and recovery times and document vital signs in a subset of animals that were induced under sevoflurane during the first 2 yr of protocol development.

METHODS

Study Area

A total of 622 free-ranging Steller sea lions aged 2–45 mo with a body mass of 73 ± 6 kg ($\bar{x} \pm$ SD; range 20–232 kg) were live-captured by SCUBA divers near rookeries and haul-outs over a 10 yr period from 1998 to 2008 in coastal Alaska. Sea lions were captured and studied in southeast Alaska (SEA, $n = 279$), Prince Williams Sound (PWS, $n = 311$), central Gulf of Alaska ($n = 13$), eastern Aleutian Islands (EAI, $n = 5$), and central Aleutian Islands (CAI, $n = 14$) (Fig. 1). More males ($n = 364$) were captured than females ($n = 256$), and for two animals the sex was not recorded.

Anesthesia

Juvenile SSLs were captured by SCUBA divers interacting with inquisitive animals underwater to place a lasso (or “noose”) around the sea lion’s neck (Raum Suryan *et al.*

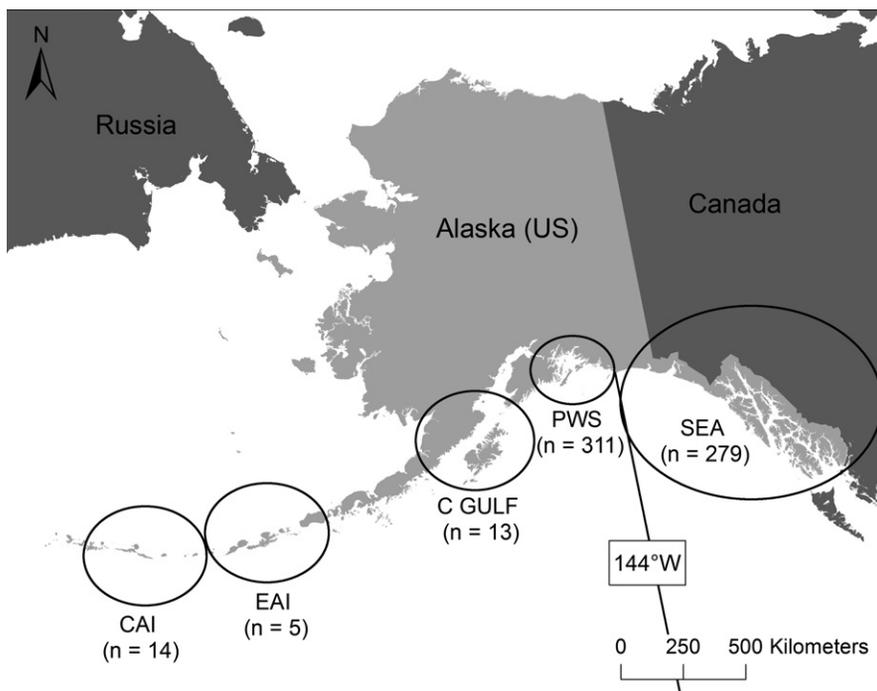


Figure 1. Free-ranging Steller sea lions (*Eumetopias jubatus*) were live captured by divers in the following locations: southeast Alaska (SEA, $n = 279$), Prince Williams Sound (PWS, $n = 311$), central Gulf of Alaska (C GULF, $n = 13$), eastern Aleutian Islands (EAI, $n = 5$) and central Aleutian Islands (CAI, $n = 14$), for a total of 622 SSL captures.

2004). The distal end of this capture line was attached to a floating buoy that could be retrieved from the water follow underwater connection to a SSL, or in calm weather was connected directly to the capture skiff for faster retrieval of the captured animal. The lasso portion of the capture line had a corroding pin, which would dissolve over time in the event that an animal escaped the capture crew.

The capture team manually placed the animal into a capture box in a nearby skiff. The capture box containing the animal was then transported to and loaded onto a research vessel and weighed as described in Rea *et al.* (2016). Animals were allowed to rest before being anesthetized. The rest time between capture and anesthesia varied from <1 h in some of the early years, to up to several hours for some animals.

Inhalation anesthesia was administered using a portable field anesthesia machine² delivering either isoflurane ($n = 602$) or sevoflurane ($n = 19$) *via* a mask. The mask was a plastic or rubber traffic cone modified to snugly fit a range of SSL head diameters and fitted to the anesthesia machine (Fig. 2). Prior to anesthesia, bars and wedges were used to provide safe restraint within the capture box and safe access to the head during mask induction. Animals were intubated with an endotracheal tube (9–16 mm for animals <100 kg and 14–24 mm for animals >100 kg) after induction. Gas anesthetic was delivered at 4%–5% vaporizer setting with 5–10 L/min oxygen flow

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Figure 2. A juvenile Steller sea lion receives isoflurane anesthesia through a mask, which is a modified traffic cone fitted to the portable anesthesia unit.

rate. The concentration of the gas anesthetic was reduced to 1%–2% with 2–3 L/min oxygen flow to maintain anesthesia after induction (loss of swallow reflex and following intubation). The waste gas tube was either vented to the environment outside of the working area or a canister scavenger system was used. During colder periods the vaporizer was heated with external heat sources. The vaporizer was kept in an insulated cover with chemical hand warmers enclosed in contact, and during the winter electric heaters were kept nearby in the work area to maintain appropriate working temperatures for the equipment, animals, and people. The heat sources (*i.e.*, heat lamps, or high voltage heaters) were positioned at a distance from the vaporizer unit, to avoid affecting delivery rates of anesthetic.

Time variables recorded included induction time (defined as the time from applying the mask to intubation), recovery time (time from when the vaporizer was turned off to extubation), and length of anesthesia (time from applying the mask to regaining all reflexes and extubation). Most animals underwent a lighter initial anesthesia event using mask only after the resting period to allow for collection of minimal blood samples and intramuscular (IM) injection of deuterium oxide for estimation of body fat content (Rea *et al.* 2016). Data for these anesthesia events were not included

in this study as animals were not fully anesthetized, and were not intubated. We monitored vital signs throughout the anesthetic period, measuring core body temperature with an esophageal digital thermometer, heart rate with a pulse oximeter (Nellcor 20P; Nellcor Inc., Pleasanton, CA) or an esophageal stethoscope, and respiratory rate by counting thoracic excursions or rebreathing bag contractions. Apnea was defined as halted respiration (no visible breaths) >1 min. Capillary refill time (CRT) was measured by applying pressure to the oral mucosa and counting the seconds until the mucosa was perfused again (returned to pinkish color). The color of the mucus membrane and CRT was assessed throughout the anesthetic period. Hypothermic (<36.5°C) animals were actively heated with artificial heat sources including wraps, insulating pads, warm water, and hot water bottles. Severe hypothermia was defined as <35.0°C (Ko and Krimins 2014).

A dose of 0.3 mg/kg diazepam (Diazepam injection USP 5 mg/mL; Hospira, Inc., Lake Forest, IL) was administered IM for sedation in 11 animals that were too aggressive for safe handling in the capture box. We removed these animals from the analysis for induction time and recovery time. In cases of respiratory depression or severe apnea, the sea lion was manually ventilated and/or administered 1 mg/kg doxapram (Dopram Injection 20 mg/mL; West-Ward Pharmaceuticals, Eatontown, NJ) IM or sublingually to stimulate respiration. For resuscitation from respiratory arrest, a dose of 0.02 mg/kg atropine (Atropine Sulfate Injection 1/120 Grain, 0.54 mg/mL; Med-Pharmex Inc., Pomona, CA) and 0.08 mg/kg epinephrine (Epinephrine Injection USP, 1 mg/mL; International Medication Systems Ltd. South El Monte, CA), were administered IM or intratracheally through the endotracheal tube, in addition to mechanical assistance with respirations and doxapram.

After extubation the animals were kept unrestrained on the rear deck of the ship for visual monitoring and undisturbed recovery. Recovery qualities were subjectively assessed by the anesthetists. When the sea lions were assessed to be fully recovered, they were allowed to leave the ship under their own power.

Statistical Analysis

All statistical analyses were performed as linear models in the statistical software R, version 3.1.1 (R Development Core Team 2014). Variables analyzed included drug type (isoflurane and sevoflurane), induction time, recovery time, body temperature, anesthetist, year, month, region, sex, body weight, time from capture to anesthesia, and duration of anesthesia. We tested for normal distribution of data using a Shapiro-Wilks test and, if necessary, log transformed to achieve normality.

The difference between the drug types was tested with a two-tailed *t*-test. Due to the large difference in sample size between the two drug types for induction and recovery time and body temperature, the analysis was run twice; once with all the animals ($n = 19$ sevoflurane, $n = 602$ isoflurane), and again with a random subsample of 19 individuals in the same age and weight class, anesthetized in the same region (SEA), and equal number of individuals from the same seasons (March, May, and November) receiving the isoflurane protocol (only from the first 2 yr, when sevoflurane also was used and with the same anesthetist performing the anesthesia for both protocols) to have equal sample sizes. Variables including year, month, region, sex, body weight, time from capture to anesthesia, and duration of anesthesia were tested for association with body temperature using linear regression and ANOVA. Interaction between the variables was tested with an ANOVA. Nine anesthetists were involved in the project during the 10 yr period, with two anesthetists present for

most captures. Each anesthetist anesthetized at least 20 animals. The difference in induction time and recovery time between anesthetists (for isoflurane protocol only) was compared using an ANOVA. The interaction between induction time, recovery time, and anesthetists was also tested with an ANOVA.

For all statistical analysis, we considered P -values < 0.05 significant. Additionally, summary statistics were calculated for all measured vital signs and are reported as $\bar{x} \pm SD$ (range) in the results section. The complete data set included records of 621 anesthesia events, but due to some lost or unrecorded data, there is periodically unequal sample sizes in results presented.

RESULTS

Capture Technique and Drug Combinations

Of the 621 juvenile SSL that were captured and anesthetized with either isoflurane or sevoflurane, there was a significantly faster induction time for sevoflurane than isoflurane ($t = -2.6$, $P = 0.0182$), but no difference in recovery time (Table 1). When equal sample sizes from the two drug types were compared, there was still a significantly faster induction time ($t = -2.2$, $P = 0.038$) for sevoflurane and no difference in recovery time between the two protocols (Table 1).

The mean induction and recovery times ranged from 9 to 18 min and 2 to 10 min, respectively, among the anesthetists. We found a significant difference in induction ($F_{7,582} = 4.5$, $P < 0.001$) and recovery ($F_{7,485} = 8.6$, $P < 0.001$) between anesthetists, with a significant interaction ($F_{7,461} = 33.0$, $P < 0.001$). The rest time between capture and anesthesia was 111 ± 62 (17–558) min. The anesthesia time was 61 ± 18 (22–147) min for isoflurane and 75 ± 22 (46–117) for sevoflurane (Table 1).

Vital Signs

The most prevalent complication of isoflurane and sevoflurane anesthesia was hypothermia, while apnea occurred during some isoflurane events. Severe hypothermia with temperatures $< 35^\circ\text{C}$ were measured in 132 (21.9%) of all animals. Hypothermia was significantly associated with month ($r^2 = 0.29$, $F_{9,455} = 21.3$, $P < 0.01$), with the majority of events occurring in February and March. Hypothermia also had a significant but weak association with length of anesthesia (slope =

Table 1. Comparison of induction times and recovery times in minutes between isoflurane ($n = 602$) and sevoflurane ($n = 19$) anesthesia for free-ranging Steller sea lions, after being live captured by divers in the Gulf of Alaska and near the Aleutian Islands. Values are presented as $\bar{x} \pm SD$ (range).

Time variable	Isoflurane	Sevoflurane	P	t	df
Induction time	14 ± 6 (3–70)	11 ± 6 (5–226)	0.018	-2.60	18.66
Recovery time	5 ± 3 (1–42)	4 ± 2 (0–9)	0.638	-0.48	20.86
Anesthesia time	61 ± 18 (22–147)	75 ± 22 (46–117)	—	—	—
Induction time	15 ± 8 (5–38) $n = 19$	11 ± 6 (5–26) $n = 19$	0.038	-2.20	35.88
Recovery time	4 ± 2 (0–8) $n = 19$	4 ± 2 (0–9) $n = 19$	0.323	1.00	30.60

-0.02, $r^2 = 0.02$, $F_{1,439} = 8.4$, $P < 0.01$) and sex ($F_{2,462} = 8.6$, $P < 0.01$), but no association with year, region, body weight, and time spent resting from capture to start of anesthesia. The mean body temperature was 36.4°C for isoflurane and 35.0°C for sevoflurane (Table 2). There was a significant difference between the two drug types for body temperature when all animals were compared ($t = 4.06$, $P < 0.001$) with warmer animals on the isoflurane protocol; however, this difference was not apparent when equal sample sizes from the two drug types were compared ($t = 1.42$, $P = 0.17$). The mean respiratory rate throughout the anesthesia period was 7 breaths/min for both drug types (Table 2). Apnea occurred occasionally at the time of intubation or at the end of the procedure after turning off the vaporizer but before extubation. Some of these animals needed mechanical assistance with respirations. Additionally, doxapram was used to stimulate respirations in 21 animals (3%). Two animals experienced respiratory arrest during anesthesia. These animals were resuscitated successfully with atropine and epinephrine. The average heart rates measured in beats per minute were 109 ± 15 (55–170) for isoflurane and 102 ± 11 (87–121) for sevoflurane (Table 2).

Mortalities

We had two mortalities (0.33%). One 7-mo-old female died during recovery following a sevoflurane procedure. The animal was found dead in the capture box with its head twisted and nose pressed into a corner. Necropsy confirmed asphyxiation as cause of death. A 23-mo-old female died during initial masking with isoflurane anesthesia. The necropsy confirmed asphyxiation due to aspiration of gastric contents. As a part of other studies satellite data recorders were deployed on a majority of the animals. No mortalities were reported postcapture (Raum-Suryan *et al.* 2004; Pitcher *et al.* 2005; Call *et al.* 2007; Rehberg and Burns 2008; Lander *et al.* 2010, 2014; Hui *et al.* 2015; Spitz *et al.* 2015; Rea *et al.* 2016).

DISCUSSION

Capture Method and Anesthesia Protocols

Here we present data for a novel procedure describing successful gas anesthesia after underwater captures of juvenile SSLs. During a span of 10 yr, 621 animals were anesthetized in the field, with a very low mortality rate. These results demonstrate an

Table 2. Vital signs recorded for free-ranging Steller sea lions during isoflurane and sevoflurane anesthesia, after being live captured by divers in the Gulf of Alaska and near the Aleutian Islands. Values are presented as $\bar{x} \pm SD$ (range).

Variable	Unit	<i>n</i>	Isoflurane	<i>n</i>	Sevoflurane
Body temperature	°C	582	36.4 ± 2.6 (29.4–40.5)	17	35.0 ± 1.0 (33.3–37.7)
Heart rate	beats/min	588	109 ± 15 (55–170)	17	102 ± 11 (87–121)
Respiratory rate	breaths/min	589	7 ± 5 (0–40)	17	7 ± 2 (2–11)

effective and reliable capture method and field anesthesia for SSL, which resulted in limited disturbance to other animals on the haul-out.

The significantly faster induction time for sevoflurane was most likely attributed to blood solubility qualities and thereby potency. Potency of inhalation gases is related to their minimal alveolar concentration (MAC) and blood solubility that affects gas availability to blood and thus to the brain. A low MAC and blood solubility induces a rapid anesthetic effect on the central nervous system. Both sevoflurane and isoflurane have a low solubility, with <0.2% metabolized, while sevoflurane is less soluble than isoflurane (Steffey 2001). Isoflurane has a lower MAC than sevoflurane (Steffey and Mama 2007), but the lower solubility of sevoflurane likely accounted for faster induction times *via* faster delivery to the brain. The same characteristics should also lead to a faster recovery for sevoflurane. The relatively fast mean recovery times of 5 and 4 min, respectively, for isoflurane and sevoflurane in our study were not significantly different. However, sevoflurane recoveries were subjectively assessed as resulting in more alert animals at the time of extubation. Isoflurane and sevoflurane have very similar characteristics and similar actions on the cardiovascular and respiratory systems, but isoflurane is more potent with a lower MAC (Steffey 2001). Since MAC was not titrated, we did not compare heart and respiratory rates statistically between the two protocols. When we compared body temperature, although animals appeared warmer on the isoflurane protocol, the difference was not significant. Given our findings of stable heart and respiratory rates for both protocols, higher cost associated with sevoflurane at the time of the study outweighed the benefit of slightly shorter induction times for sevoflurane over isoflurane.

The significant difference between anesthetists, both for induction and recovery times, were probably related to different levels of experience and comfort for having animals in deeper anesthetic planes. These small differences are inconsequential for research outcome and animal safety; however, for research studies evaluating or optimizing anesthetic protocols, the human operator should be accounted for and included as an explanatory variable.

Vital Signs

Normal body temperature in SSL has been established to 36°C–38.2°C (Horning and Mellish 2014). The low temperatures in the current study were more pronounced from January to March, most likely due to seasonably lower ambient temperatures during winter. There was also a correlation with longer anesthesia times and hypothermia, with temperatures dropping below 35°C after 110 min of anesthesia time. These low body temperatures could arise from isoflurane-induced vasodilation (Steffey 2001, Haulena 2014), which would continue to decrease the body temperature of the animal the longer vasodilation exists. Despite no difference in body fat between the sexes (Rea *et al.* 2016) we found that females had higher average body temperature under anesthesia than males. There was also no association with body weight, which was expected to make smaller animals more susceptible to heat loss and hypothermia (Ko and Krimins 2014). This could have been mitigated by the attending anesthetist, providing extra external heating to smaller animals, which probably prevented hypothermia-related mortalities.

In addition to hypothermia, apnea was another severe side effect. Isoflurane is a respiratory depressant (Steffey 2001), which likely led to apnea. The controlled field environment with endotracheal intubation and manual positive pressure anesthesia

allowed for management of these respiratory emergencies and may have prevented additional mortalities (McDonnell and Kerr 2015).

Field Anesthesia and Mortality Rates

This study had an exceptionally low mortality rate (0.33%), nearing the mortality rate for cats and dogs undergoing anesthetic procedures in veterinary hospitals (Muir 2007). The general recommendation for field anesthesia is that mortality rates above 2% are unacceptable (Arnemo *et al.* 2006, Kreeger and Arnemo 2012). Our results show that the capture method and anesthesia protocol are well below this benchmark, although we recognize that capture myopathy and related mortality can occur up to 4 wk postcapture. A study based on animals represented in our study investigated mortality up to 14 d postcapture with telemetry tracking, and found an estimated mortality rate of $\leq 1.6\%$ (Fadely *et al.* 2008). No direct mortalities were reported from animals with satellite data recorders.

Conclusion

Safe capture and anesthesia procedures are essential to assure safe handling in the field. If capture and anesthesia are required during the colder months of January through March, increased risk of hypothermia during anesthesia should be considered and mitigated. The capture method and field anesthesia protocol with either isoflurane or sevoflurane can be used by researchers and managers to continue research efforts to conserve this species.

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