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Inhalant anesthesia for minimally invasive procedures in free-ranging Guadalupe fur seals (*Arctocephalus philippii townsendi*)

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Abstract

Free-ranging otariids are routinely captured for data and sample collection. To achieve this, anesthesia may be used to facilitate handling, decrease stress, and improve human and animal safety. Injectable anesthetics are widely used for such endeavors; however, certain disadvantages to this approach warrant further exploration of alternative anesthetic techniques. Inhalant anesthesia, commonly utilized for otariids in a clinical setting, is used more sparingly in the field, with few studies assessing safety and efficacy in free-ranging otariids. During 2016–2020, 175 Guadalupe fur seals were net-captured and anesthetized with isoflurane and oxygen on Guadalupe Island, Mexico, for satellite telemetry attachment and biological sampling. To contribute to the body of knowledge surrounding the use of inhalants in the field, physiologic and anesthetic parameters (time to induction, total oxygen use, heart rate, respiratory rate, time to recovery, and anesthetic depth) were assessed for effects of biometric and logistical factors (pursuit and holding time, sex, age class, body weight, year, oxygen flow rate, and total anesthesia time). This anesthetic technique provided rapid

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induction and recovery times and rare side effects in Guadalupe fur seals, serving as a practical means of field immobilization for minimally invasive procedures in this imperiled species.

KEYWORDS
anesthesia, anesthetic depth, field immobilization, fur seal, gas anesthesia, Guadalupe fur seal, isoflurane, marine mammal, otariid, pinniped

1 | INTRODUCTION

Anesthesia is often utilized in otariid species to facilitate handling, data and biological sample collection, and telemetry device attachment. Compared to manual restraint alone, anesthesia serves to prevent or mitigate pain perception during sampling, negative impacts from the stress of handling and restraint, and danger to humans. However, especially in a field setting, anesthesia also comes with certain inherent risks, including hypo/hyperthermia, respiratory and/or cardiac depression, injury, and a decreased ability to manage anesthetic complications due to resource limitations (Baylis et al., 2015; Lynch et al., 1999). In otariids, methods to achieve anesthesia in the field are delivery of an injectable induction agent (either remotely via dart gun, or upon capture, via hand injection), delivery of an inhalant anesthetic via face mask (utilizing capture/manual restraint), or a combination (Baylis et al., 2015; Gales & Mattlin, 1998). Remote use of injectable anesthetics alone has traditionally been favored for field immobilization of otariids due to their ease of delivery, minimal equipment requirements, and applicability for animals of all sizes, with the added bonus of not exposing humans and the environment to inhalant gases (Dabin et al., 2002; Gardner et al., 2021; Katz et al., 2019; Sepúlveda et al., 1994). However, despite these positive attributes, certain intrinsic characteristics of remote anesthetic delivery make this method suboptimal for use in some field conditions. These include the potential for incomplete injections (leading to drug failure), the reliance on subjective weight estimations (predisposing to dosing inaccuracy), the potential for traumatic injury or drowning, dose response variability (e.g., induction time, anesthetic depth, and recovery time), and drug-specific side effects (e.g., prolonged respiratory depression, apnea, bradycardia, hypotension, and hypo/hyperthermia; Baylis et al., 2015; Boyd et al., 1990; Dabin et al., 2002; Ferreira & Bester, 1999; Frankfurter et al., 2016; Haulena et al., 2000; Heath et al., 1996; Katz et al., 2019; McKenzie et al., 2013; Sepúlveda et al., 1994). These challenges, as well as advancements in portability of anesthetic units may be why inhalant anesthesia, known for its commendable safety record, has recently gained traction in some field scenarios as both an induction and maintenance agent (Gales & Mattlin, 1998; Heath et al., 1997; Lian et al., 2018). Despite its growing use, minimal literature exists regarding the safety and efficacy of inhalants as sole anesthetic agents in otariids, and no published data exists regarding their use in Guadalupe fur seals (GFS; *Arctocephalus philippii townsendi*). Thus, further evaluating inhalant anesthesia for broader use in otariids and especially in imperiled species such as the Guadalupe fur seal is essential.

The GFS is an elusive otariid species, listed as threatened under the U.S. Endangered Species Act (ESA), and endangered under Mexican Law (NOM-059-SEMARNAT 2010; McCue et al., 2021). During the 19th and 20th centuries, GFS were hunted to near extinction along the west coast of North America (United States and Mexico), reducing their previously abundant population from as many as 200,000, to less than a few dozen individuals (Hernández-Camacho & Trites, 2018; Hubbs, 1956, 1979). Currently, Guadalupe Island, Mexico, is considered their only well-established breeding site, however, rare births are reported at the San Benito Archipelago, a recolonization area located approximately 260 km to the south of Guadalupe Island in the Mexican Pacific (Aurioles-Gamboa et al., 2010; García-Aguilar et al., 2018; Hernández-Camacho & Trites, 2018).

The GFS population has increased during recent years by approximately 8.4% (range 8%–8.8%) annually (between 1991 and 2019), with a population estimate of 63,850 (range 57,199–72,631) in 2019, largely due to protection by the Mexican Government (D'Agnese et al., 2020; Juarez-Ruiz et al., 2022). However, several factors may pose ongoing barriers to full GFS population recovery, including low genetic diversity, a single established breeding site, disease, environmental factors, and anthropogenic threats (Amador-Capitanachi et al., 2020; Barcenas-De la Cruz et al., 2018; D'Agnese et al., 2020; Elorriaga-Verplancken et al., 2016; Gálvez et al., 2020, 2023; Hernández-Camacho & Trites, 2018; Weber et al., 2004; Ziehl-Quirós et al., 2017). Therefore, conservation measures remain imperative for GFS, with ecological and health assessments being fundamental to this effort.

Historically, GFS have not been well-studied because accessing their single established breeding colony is logistically challenging, but recent increased collaboration between researchers from Mexico and the U.S. has led to myriad telemetry, health, and other conservation-based studies on GFS at Guadalupe Island (Amador-Capitanachi et al., 2020; DeRango et al., 2019; Elorriaga-Verplancken et al., 2016; Garcia-Aguilar et al., 2018; Norris & Elorriaga-Verplancken, 2019, 2020; Tamburin et al., 2020). The need for field immobilization for satellite tag placement, biological sampling, and health assessments afforded an excellent opportunity to gather valuable information on anesthesia in GFS. This study is the first to assess the use of isoflurane inhalant anesthesia as a sole anesthetic agent in free-ranging GFS and to explore statistical comparisons between both biological and logistical parameters, and anesthetic and physiological factors, within a large sample size of fur seals undergoing field immobilization. The knowledge gained from this study is a valuable contribution to GFS and other otariid species' health and safety during anesthetic procedures that are often required for conservation measures.

2 | METHODS

2.1 | Study area and animals

Guadalupe Island is located in the Pacific Ocean, 241 km off the west coast of the Baja California Peninsula, Mexico (29.03°N, 118.28°W). Fieldwork was carried out at Punta Sur, the southern tip of Guadalupe Island, where an abundance of GFS of various age classes are reported (García-Capitanachi et al., 2017). In order to attach satellite telemetry instruments (and secondarily to obtain minimally invasive biological samples), capture and isoflurane inhalant anesthesia were performed on free-ranging GFS during March 2016, April 2017, November 2018, and March 2020. All captures and anesthesias were carried out as a part of other telemetry, health, and trophic ecology studies, while anesthetic-related data were collected and analyzed specifically for this study.

2.2 | Capture, anesthesia, and data collection

Following a visual assessment of body condition, general appearance, respiration (if at rest), and mentation (if awake), individual GFS were net-captured using modified cone-shaped hoop nets (Telaio Clothing, Seattle, WA). Time of disturbance was defined as the time of initial disturbance of the target individual, time of capture as the time the net was placed on an individual, and pursuit time as the time between the two. Following capture, GFS were manually restrained in ventral recumbency by a single restrainer straddling the midsection while using their knees to maintain the fore flippers tucked, and holding behind the base of the skull with two hands. This restraint was performed with the fur seal either inside or outside of the net depending on its size and the restrainer's skill and comfort level. A face mask was held on the muzzle (anesthetic start time), and induction was carried out with 4%–5% isoflurane (MWI, Boise, ID) and oxygen (2–5 L/min). The time between capture and anesthetic start was defined as the holding time, and varied due to time required to move captured individuals to a safe and comfortable anesthesia location, collect any necessary preanesthetic samples/data, and, for instances when more than one individual was captured at the same time (e.g., mom-pup pairs where the mothers were anesthetized first), the waiting time. The total pursuit time plus holding time was defined as the preanesthetic period. A field rebreathing anesthetic machine, manufactured by The Marine Mammal Center was utilized for all anesthesias, and consisted of an isoflurane vaporizer (Datex-Ohmeda, Madison, WI) and oxygen flow meter mounted inside of a Pelican Case (Pelican Products Inc., Torrance, CA), attached to an E cylinder oxygen tank and a rebreathing circuit with a 3–5 L rebreathing bag and a canine induction mask or, for larger individuals, a modified traffic cone. Carbon dioxide absorption was achieved using soda lime (SodaSthesia; VetOne, Boise, ID) and anesthetic waste gas was scavenged to the environment (Figure 1). Time to induction was defined as the time between the mask being placed on the muzzle and the isoflurane level being dropped below 5% by the anesthetist, indicating that the individual was no longer responsive to external stimuli. Once induced, the net, if present, was removed and the mask was placed back over the muzzle (Figure 1). GFS remained in ventral recumbency for the duration of anesthesia. Various data of interest were recorded, as described below.

A brief physical exam was conducted on each individual, including heart and lung auscultation, to assess for signs of disease and other factors that would preclude anesthesia. Biometric data, including sex (female, male) and age class (pup, juvenile, adult) were established based on body size and weight, dentition, overall morphology, and/or if pregnant (based on abdominal palpation of a fetus) or observed with a dependent pup (Allen et al., 2011; Reeves et al., 2002). Standard length (tip of snout to tip of tail) and axillary girth were also recorded. Body weight was measured by hoisting individuals in a sling attached to a digital hanging scale suspended from a wooden beam. During 2016, all GFS were weighed following anesthetic induction, while during subsequent years, most individuals were weighed prior to commencement of anesthesia.

Additional measurements and biological samples collected included canine tooth measurements, a vibrissae pluck, and a nasal swab, all for which the face mask necessitated brief (typically <10 s) removal, as well as genital and rectal swabs and fur clipping. Blood (10–20 ml) was collected from the caudal gluteal vein, or rarely, from the subclavian vein, from all individuals after anesthetic induction. During the 2016 season an initial blood sample was collected from GFS prior to placement of the mask for anesthesia, and at multiple time points throughout anesthesia. Flipper tags were placed on both fore flippers for identification purposes, and in many individuals, satellite tags were attached for subsequent telemetry monitoring (the primary purpose of the fieldwork).

FIGURE 1 Anesthetic set-up for fieldwork with Guadalupe fur seals (*Arctocephalus philippii townsendi*) at Guadalupe Island, Mexico, using a field-portable rebreathing anesthetic machine to deliver inhalant isoflurane in oxygen via face mask.

2.3 | Anesthetic and physiological parameters

Three teams of anesthetists (one team each for the 2016 and 2017 seasons, and one team for both the 2018 and 2020 seasons) carried out the anesthesias for this study. Anesthetic and physiologic parameters, including isoflurane level (%), oxygen flow rate (L/min), total oxygen use ($PSI_{end\ of\ anesthesia} - PSI_{start\ of\ anesthesia}$), heart rate (HR; bpm), respiratory rate (RR; bpm), and capillary refill time (CRT; s), were recorded at 5-min intervals and whenever a parameter was manually changed, throughout anesthesia. In 2016, oxygen saturation (SpO_2; %) was also monitored using a hand-held Masimo Rad-5 pulse oximeter (Masimo, Irvine, CA). GFS were maintained on isoflurane (1%–3%) and oxygen (1–3 L/min); isoflurane and/or oxygen flow rates were adjusted as needed to maintain an appropriate level of anesthesia. During anesthesia if individuals became apneic, demonstrated a decreased RR, or took shallow breaths for a prolonged period, they were intubated and provided with positive pressure ventilation for the remainder of the anesthesia. In those GFS with satellite tags deployed, the length of anesthesia depended on the time it took for the satellite tag adhesive to dry sufficiently, (i.e., not sticky when touched). Once isoflurane was discontinued (anesthetic end time), GFS remained on oxygen until they began moving their heads (range 0–10 min) and were monitored visually until fully recovered. Time to recovery was defined as the time between isoflurane discontinuation and GFS supporting their weight on their fore flippers and moving away from humans.

Anesthetic depth was retrospectively assigned a score using two factors: (1) the number of times the isoflurane level was increased from any previous level after initially being decreased below 5% (i.e., during the maintenance phase of anesthesia), and (2) the number of times an individual was reportedly “light” (increased jaw tone, return of palpebral reflexes), “moving,” or otherwise “responding” to a stimulus, as noted on its anesthesia record (such notations varied somewhat based on the anesthetic team present since there was no prospective standardized anesthetic depth assessment used in this study). The mean of these two factors was calculated to provide an anesthetic depth score (0–2.5; 0 = deepest anesthesia, 2.5 = least deep anesthesia) for each individual.

2.4 | Statistical analyses

Statistical analysis of anesthetic and physiologic parameters was performed in R using the package “brms,” data preparation was done using the package “dplyr,” and figures were created using the packages “ggplot2” and “patchwork” (Bürkner, 2017; Pederson, 2022; R Core Team, 2022; Wickham, 2016; Wickham et al., 2023). The correlation between response variables of interest (time to induction, total oxygen use, HR, RR, time to recovery, and anesthetic depth) and explanatory variables (pursuit time, holding time, sex, age class, body weight, year, oxygen flow rate, total anesthesia time, and anesthetic depth) were analyzed using regression models, with statistical significance of specific variables estimated using likelihood ratio testing of models with and without the explanatory variable of interest. Models included the covariates preanesthetic period, sex, age class, year, oxygen flow rate, RR, and total anesthesia time to account for potential confounding effects, where necessary. For induction-associated assessments, only the oxygen flow rate during induction was utilized, while the overall oxygen flow rate was used for all other comparisons. Intubated individuals were excluded from analyses.

All mean values are reported with standard error (*SE*). Models with time to induction, total oxygen use, and time to recovery as response variables were fitted using a Poisson error distribution and log link function. Models with HR and RR as response variables were fitted using a normal error distribution. Models with anesthetic depth as a response variable were fitted using a quasi-Poisson error distribution and log link function. In order to avoid reporting of statistical results based solely on an arbitrary cutoff *p* value of .05, statistical significance is reported in terms of statistical support based on *p* values, where “no” support indicates *p* values much higher than .05, “weak” support is used for *p* values close to .05 (roughly .01–.1), “moderate” support is used for *p* values that are clearly smaller than .05 (roughly .001–.01), and “strong” support is used for *p* values much smaller than .05 (roughly < .001). This follows current practices in statistical reporting (Muff et al., 2022).

3 | RESULTS

3.1 | Animal capture, anesthesia, and data collection

Inhalation anesthesia with isoflurane was performed on 175 free-ranging GFS at Guadalupe Island, Mexico as described under Methods (Table 1). Prior to capture all GFS were visually assessed to be in good to excellent body condition, have no visible wounds, injuries, or nasal or other discharge, and be fully ambulatory. All individuals resisted capture, demonstrated appropriate mentation and respiratory character, and upon physical examination, revealed no signs of disease or other abnormalities that might negatively impact anesthesia. In the March 2016, April 2017, and March 2020 field seasons, 3/10, 8/11, and 15/15 adult female GFS, respectively, were pregnant according to palpation of a fetus on physical examination. Seven adult female GFS in 2016 and one adult female GFS in 2017 did not have a notation in their record regarding pregnancy and so were categorized as unknown. None of the 15 adult females in 2018 (November) were palpably pregnant, which is consistent with early pregnancy typical for this species during this time of year (Riedman, 1990).

3.2 | Statistical analyses of anesthetic and physiological parameters

In most cases, time of disturbance was the same or nearly the same as time of capture, however, in six cases, such as with individuals that were more wary or in less accessible locations, pursuit time, ranging from 0 to 19 min (1 ± 0.2 min), was noteworthy (>5 min). Likewise, lengthy holding times were rare, ranging from 1 to 74 min (13 ± 1.1 min). Time to induction ranged between 1 and 21 min (7 ± 0.3 min). For every minute increase in time from initial disturbance until the start of anesthesia (the preanesthetic period), the time to induction lengthened by 0.7% ($SE = 0.3\%$, $p = .007$); however, this was mainly observed in pups. Among all GFS, there was strong statistical support for a shorter time to induction for pups compared to older individuals (4.4 ± 0.3 vs. 7.5 ± 0.3 min; $p < .0001$), which held true even with oxygen flow rate and RR accounted for in the model (Figure 2). Sex, body weight, year, and oxygen flow rate did not correlate significantly with time to induction (no statistical support).

In GFS, total oxygen use ranged from 50 to 950 PSI (274.6 ± 10.4 PSI) and there was strong statistical support for correlations with age class, body weight, and year. Less oxygen was utilized for pups than for individuals of older age classes (237.5 ± 15.2 vs. 282.4 ± 12.1 PSI; $p < .0001$), even when taking body weight, year, oxygen flow rate, and total anesthesia time into account (Figure 3). Overall, for every increase in weight by 1 kg, the total oxygen use increased by 0.4% ($SE = 0.06\%$, $p < .0001$), even when accounting for sex, age class, year, oxygen flow rate, and total anesthesia time. Oxygen use, while controlling for age class and total anesthesia time, differed significantly among years, with an average of 310 ± 38.8 PSI in 2016, 306 ± 22.4 PSI in 2017, 227 ± 11.5 PSI in 2018, and 245 ± 10.2 PSI used in 2020 ($p < .0001$). Similarly, there was strong statistical support for a correlation between oxygen flow rate and year, with 2016 having significantly lower mean oxygen flow rates (1.80 ± 0.08 L/min) than 2017

TABLE 1 Mean (SE) and number of Guadalupe fur seals (*Arctocephalus philippii townsendi*) anesthetized by sex, and age class, at Guadalupe Island, Mexico.

	Adult	Juvenile	Pup	Total
Females	51	33	27	111
Males	0	33	31	64
Total	51	66	58	175
Mean weight (kg)	48.2 ± 1.6	30.9 ± 1.3	19.3 ± 0.5	32.1 ± 1.1
Weight range (kg)	25.6–78.9	18.0–80.2	12.0–30.8	12.0–80.2

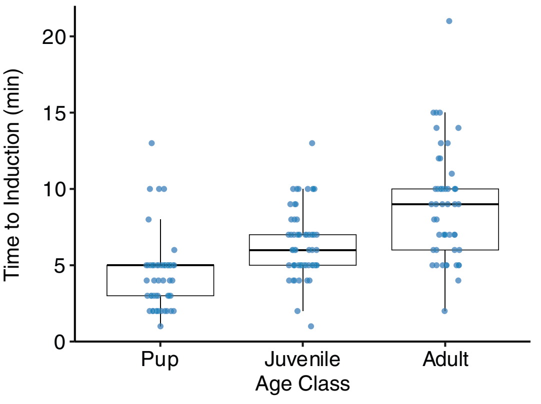

FIGURE 2 Time to induction of anesthesia across age class in free-ranging Guadalupe fur seals (*Arctocephalus philippii townsendi*) at Guadalupe Island, Mexico. Boxplots indicate quantiles (50%: horizontal bold line, 25% and 75%: horizontal normal lines, 5% and 95%: ends of vertical lines). Data points (blue) are jittered for visibility.

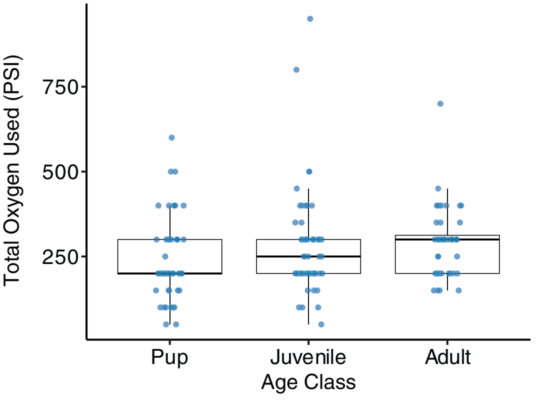

FIGURE 3 Total oxygen use among anesthetized Guadalupe fur seals (*Arctocephalus philippii townsendi*) across age class at Guadalupe Island, Mexico. Boxplots indicate quantiles (50%: horizontal bold line, 25% and 75%: horizontal normal lines, 5% and 95%: ends of vertical lines). Data points (blue) are jittered for visibility.

(3.22 ± 0.11 L/min), 2018 (2.60 ± 0.05 L/min), and 2020 (2.57 ± 0.04 L/min), even when controlling for sex, body weight, and RR ($p < .0001$).

GFS heart rate (HR) under anesthesia ranged from 80 to 164 beats per minute (118 ± 11.9 bpm). There was moderate statistical support for females having higher HR than males (119 ± 1 vs. 116 ± 1 bpm; $p = .002$), when controlling for age class, and strong statistical support for pups having higher HR than older individuals (121 ± 1.3 vs. 117 ± 0.8 bpm; $p = .001$), when controlling for sex (Figure 4). Additional parameters including body weight, year, oxygen flow rate, and total anesthesia time were not found to be correlated with HR (no statistical support).

In GFS under anesthesia, respiratory rate (RR) ranged from 1 to 24 breaths per minute (8.0 ± 3.3 bpm). There was weak statistical support for females having higher RR than males (8.0 ± 0.2 vs. 7.5 ± 0.3 bpm; $p = .011$), and strong statistical support for pups having higher RR than older individuals (9.0 ± 0.3 vs. 7.2 ± 0.2 bpm; $p < .0001$; Figure 5). Likewise, there was weak statistical support for a negative correlation between body weight and RR (even after correcting for age class), where for every 20 kg increase in GFS weight, RR decreased by 1 bpm ($SE = 0.4$, $p = .006$). This effect was apparent overall but was strongest in juveniles and adult females. Oxygen flow rate was

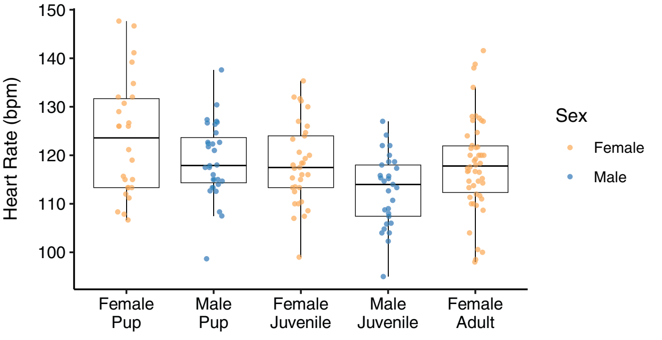

FIGURE 4 Heart rate among anesthetized Guadalupe fur seals (*Arctocephalus philippii townsendi*) across sex and age class at Guadalupe Island, Mexico (female = orange; male = blue). Boxplots indicate quantiles (50%: horizontal bold line, 25% and 75%: horizontal normal lines, 5% and 95%: ends of vertical lines). Data points (orange and blue) are jittered for visibility.

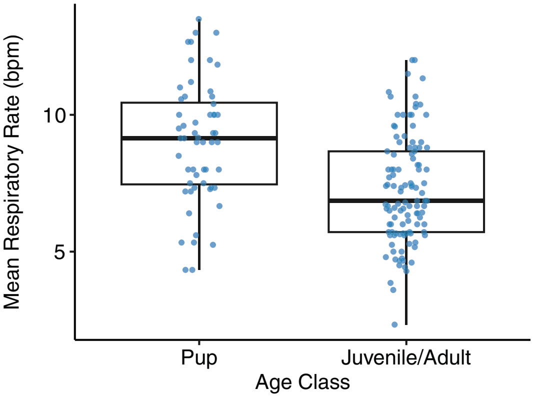

FIGURE 5 Respiratory rate under anesthesia across age class of Guadalupe fur seals (*Arctocephalus philippii townsendi*) at Guadalupe Island, Mexico. Boxplots indicate quantiles (50%: horizontal bold line, 25% and 75%: horizontal normal lines, 5% and 95%: ends of vertical lines). Data points (blue) are jittered for visibility.

also inversely correlated with RR; for every L/min increase in oxygen flow rate, the RR decreased by 1.01 ± 0.33 bpm ($p = .003$). Year and total anesthesia time had no statistical support for a correlation with RR.

Total anesthesia time was 14–60 min (31.5 ± 0.6 min) and was largely dependent on time needed for biological sampling (2016) or satellite tag placement (2017, 2018, 2020). The total anesthesia time varied among years, with 2016 having the longest anesthesia times (46.1 ± 2.1 min) and 2017, 2018, and 2020 having similar total anesthesia times (29.2 ± 1.1, 30.5 ± 0.7, and 30.8 ± 0.6 min, respectively). Time to recovery ranged from 1 to 24 min (6.1 ± 0.3 min). There was strong statistical support for a correlation between year and time to recovery ($p < .0001$), with 2018 having the shortest time to recovery (3.2 ± 0.2 min) and 2020 having the longest (7.9 ± 0.4 min), even when taking sex and age class into account to control for variation in sample population characteristics among the different years. There was no statistical support for sex, age class, body weight, or total anesthesia time to be correlated with recovery time, nor with isoflurane levels.

Anesthetic depth was sufficient to carry out minimally invasive sample collection in free-ranging GFS, including maintaining individuals anesthetized during brief periods with the face mask removed. Seventy-five individuals (43%) were reported to be "light" or "moving" in response to stimuli or had their isoflurane level increased at least once during the maintenance phase of anesthesia. There was moderate statistical support for correlations between anesthetic depth score and total anesthesia time, body weight, year, and time to recovery. For every 1-min increase in anesthesia duration, the anesthetic depth score increased by 6.0% (i.e., GFS were less deep during longer anesthesias; $SE = 1.6\%$, $p = .0002$). For every 1-kg increase in body weight, the anesthetic depth score increased by 3.3% (i.e., heavier individuals were less deep under anesthesia; $SE = 1.0\%$, $p = .004$). The year 2020 was associated with the deepest anesthesias while the 2016 GFS were the least deep under anesthesia, and, for every increase in anesthetic depth score by 1, time to recovery shortened by 12.9% ($SE = 5.2\%$, $p = .004$) (Figure 6). There was no statistical support for anesthetic depth to be correlated with sex, age class, or oxygen flow rate.

Few side effects were observed, including mild muscle tremors ($n = 4$), nystagmus ($n = 3$), and apnea and/or shallow breathing requiring intubation and positive pressure ventilation ($n = 6$). While most individuals had CRTs (capillary refill times) of 2 s or lower throughout anesthesia, few ($n = 9$) had a reported CRT of 3 s during one or two time points; however, none of these were individuals that necessitated intubation. For the 15 GFS for which SpO_2 was monitored, saturation was 98% or higher at every reading. All 175 GFS in this study recovered fully from anesthesia and several were sighted on subsequent days, some nursing their pups in the rookery. Satellite tags were placed on 143 individuals; transmission data from these tags demonstrated 100% survival ≥7 days postanesthesia, and at least 98% survival 2 weeks postanesthesia.

4 | DISCUSSION

In free-ranging GFS, net-capture followed by manual restraint and inhalant isoflurane anesthesia provided rapid induction and recovery times with no major anesthetic complications, and proved a safe and effective means of anesthesia for a wide range of GFS in a field setting. The induction times in our study were in the middle of the range of published induction times for other otariid species undergoing isoflurane inhalant anesthesia (Table 2). Variation among studies could be due to differences in species/sex/age class demographics and/or anesthetic technique, including induction isoflurane levels and oxygen flow rates (Gales & Mattlin, 1998; Heath et al., 1997; Kreeger et al., 2002; Lian et al., 2018; Yamaya et al., 2006). Our data indicated that a longer preanesthetic period was associated with longer induction times, a correlation which we expect was caused by an acute stress response. When animals experience acute stress through handling or sampling, the resultant catecholamine release leads to activation of the autonomic nervous system, inciting physiologic changes that may ultimately require increased drug doses and

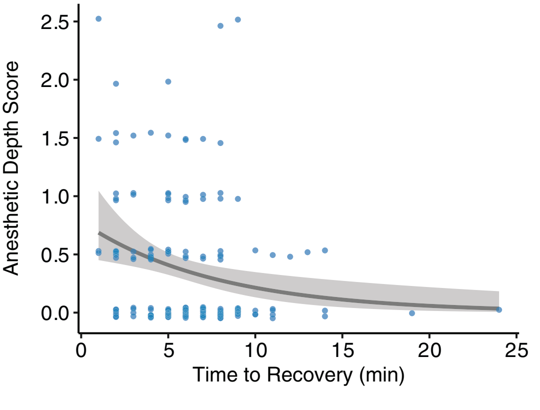

FIGURE 6 Anesthetic depth score versus time to recovery in anesthetized Guadalupe fur seals (*Arctocephalus philippii townsendi*) at Guadalupe Island, Mexico. The gray line shows the fitted regression with light gray standard error bands. Data points (blue) are jittered for visibility.

TABLE 2 Otariid isoflurane inhalant anesthesia study comparison data from available literature.

SP	C/FR	NUM	Sex	Age	CAPT METH	IND METH	IND ISO (%)	IND O_2 (L/min)	Mean Time to IND (min)	INT?	MAINT ISO (%)	MAINT O_2 (L/min)	Mean HR (bpm)	Mean RR (bpm)	Mean REC time (min)	Reference
GFS	FR	175	B	P/J/A	NET	MR + FM	5	2–5	7 ± 0.3	N	1–3	1–3	118 ± 11.9	8 ± 3.25	6.1 ± 0.3	This study
SASL	FR	13	F	A	HC + PH	SC + FM	5	5–10	14 ± 7	Y	0.75–1.5	3–5	104 ± 10	NR	NR	Dassis et al., 2016
NZSL	FR	29	F	A	NET	MR + FM	4	10–15	2.7 ± 1.4	N	0.8–4	6–10	NR	7.3 ± 1.5	8.2 ± 4.9	Gales & Mattlin, 1998
NZFS	FR	5	F	A	NET	MR + FM	4	10–15	2.5 ± 0.8	N	1.2–2	6–10	NR	6.4 ± 0.6	5.3 ± 4.3	Gales & Mattlin, 1998
CSL	FR	115	B	P	HC + PH	MR + FM	2.5–3	1–2	7.1 ± 2.7	Y	0.75–1.5	0.8–1	116.6	28.2	7.3 ± 4.8	Heath et al., 1997
SSL	FR	602	B	J	L	CB + FM	4–5	5–10	14 ± 6	Y	1–2	2–3	109 ± 15	7 ± 5	5 ± 3	Lian et al., 2018
SSL	C	8	F	A	N/A	FM	5	NR	NR	NR	1.5–2.5	NR	56.5 ± 3.7	NR	NR	Storlund et al., 2021
NFS	C	5	F	A	N/A	FM	5	NR	NR	NR	1.5–2.5	NR	113 ± 15.5	NR	NR	Storlund et al., 2021
SASL	C	1	M	A	N/A	CI	2	30	≤15	Y	2	10–15	97 ± 6	13 ± 2	NR	Yamaya et al., 2006
CSL	C	3	F	A	N/A	CI	2	30	≤15	Y	2	10–15	97 ± 6	13 ± 2	NR	Yamaya et al., 2006

Note: A = adult; B = both sexes; C = captive; CAPT = capture; CB = capture box; CI = chamber induction; CSL = California sea lion (*Zalophus californianus*); F = female; FM = face mask; FR = free-ranging; HC = hand-capture; IND = induction; INT = intubated; ISO = isoflurane; J = juvenile; L = lassoed; M = male; MAINT = maintenance; METH = method; MR = manual restraint; N = No; N/A = not applicable; NET = netted; NFS = Northern fur seal; NR = not reported; NUM = number of individuals; NZFS = New Zealand fur seal (*Arctocephalus forsteri*); NZSL = New Zealand sea lion (*Phocarctos hookeri*); O_2 = oxygen; P = pup; PH = prolonged holding; REC = recovery; REF = reference; SASL = South American sea lion (*Otaria flavescens*); SC = squeeze cage; SP = species; SSL = Steller sea lion; Y = yes.

thus longer induction periods (DeRango et al., 2019; Lacourt & Tarrant, 1985). While the modest effect of a longer preanesthetic period would only become clinically relevant during prolonged holding times (and only if this association remained linear), knowledge of the potential for prolonged induction times associated with acute stress may be clinically useful in some scenarios. Examples include rookeries, where numerous fur seals may be captured during a single capture event and maintained in nets or enclosures for short or extended periods prior to anesthesia, and when extensive preanesthetic sampling is necessary.

In our study, pups displayed nearly 50% shorter induction times than individuals of older age classes, which has not been reported in other studies but was considered clinically relevant. In humans, there is an age-related effect on isoflurane minimum alveolar concentration (MAC) requirements, with older individuals having lower MAC values and neonates having the highest; however, this should lead to an increased anesthetic requirement for induction in young animals (and therefore longer induction times), which was in direct contrast to our findings in GFS (Aranke et al., 2013). Similarly, most adult females in our study were known or assumed to be pregnant, and therefore should experience a reduction in MAC as well as an increase in maternal oxygen consumption, both leading to faster induction times (Bedson & Riccoboni, 2014; Neuman & Koren, 2013; Robertson, 2016; Wlody et al., 2012). In rats and piglets, isoflurane MAC requirement is reduced as body temperature decreases (Satas et al., 1996; Vitez et al., 1974). Body temperature was not measured in our study, however, in northern fur seals (*Callorhinus ursinus*), the mean temperature of pups handled briefly for rectal temperature determination was only 0.5°C lower than intrathoracic temperatures obtained from freshly (within 10–15 s) killed adults under similar, moderate environmental conditions (Bartholomew & Wilke, 1956). Perhaps the biggest factors outweighing the influences of age class and pregnancy on MAC and leading to shorter induction times in pups were a better fit of the mask and reduced breath-holding by pups. Many adult GFS in our study were anesthetized using modified traffic cones, which lack a rubber diaphragm and thus may have a reduced ability to create a snug fit and prevent anesthetic leakage compared to the canine anesthetic masks used for GFS pups. Additionally, reduced breath-holding in pups, which have poorer diving capacity than older individuals (associated with limited oxygen storage capacity), may have had the largest impact on the association between age class and time to induction (Richmond et al., 2006). Anecdotally, pups tended to breath-hold during induction much less than their adult counterparts. Further investigation of the interactions among these factors would enhance understanding of the various influences on induction speed in otariids undergoing anesthesia.

Notably, induction times in GFS in the current study were comparable to, if not generally shorter than, what has traditionally been reported for injectable anesthetics (Dabin et al., 2002; Ferreira & Bester, 1999; Melin et al., 2013; Sepúlveda et al., 1994; Spelman, 2004). In addition, the risk of drowning during the preanesthetic period is eliminated due to having the animal in hand; however, limitations include safely capturing and manually restraining larger adult (i.e., male) otariids for anesthetic induction.

The correlation between year and oxygen flow rate is likely explained by anesthetists' manipulation of the flow rate, in this case based on estimated tidal volume and the residual volume in the rebreathing bag. In human medicine, pregnant females demonstrate an increased tidal volume and minute ventilation compared to nonpregnant females (Bedson & Riccoboni, 2014; Neuman & Koren, 2013; Robertson, 2016). Similarly, poor nutritional condition and health deficits (e.g., respiratory disease) can lead to poor respiratory muscle function, reduced control of ventilation, and alterations in tidal volume (Ghignone & Quintin, 1986). It is unlikely that GFS with nutritional or health issues leading to reduced respiratory function were anesthetized in our study (because all individuals were screened prior to capture and/or at induction), but health anomalies in free-ranging otariids are an important concern when collecting individuals for anesthesia for field sampling and instrumentation.

Young animals have a lower tidal volume compared to adults, and thus it was not surprising that pups (and lighter-weight individuals) in our study utilized less oxygen, even despite higher RRs. Because age class and total anesthesia time were accounted for in the model assessing total oxygen use, the discrepancy among years was likely due to interanesthetist variability, although contributions by differences in pregnancy, or less likely, nutritional, or health status of GFS over the years cannot be excluded (Fowler, 1995; Kreeger et al., 2002). It is important to keep in mind that individuals in this study were not intubated; thus, total oxygen use only represents the amount depleted from the tank and does not necessarily represent nor correlate to the amount of oxygen actually utilized by the individual. In our study, a model was developed to estimate total oxygen use based on a fur seal's weight. Such information may be useful for planning purposes to predict the quantity of oxygen to bring into a field anesthesia scenario based on the size, number, and total anesthesia time requirements of the target population.

Heart rate was comparable to but slightly above the means reported for other otariids induced and maintained with isoflurane (Table 2). Like humans, in which females have a smaller heart size and an intrinsically faster heart rhythm, female GFS in our study demonstrated higher mean HR than males (Prabhavathi et al., 2014). It should also be noted that most females in our study were known or suspected to be pregnant, which in canines and humans has been shown to increase HR and thus may have confounded our findings (Desai et al., 2004; Olsson et al., 2003). The higher HR in younger GFS in our study was similar to what has been reported in canine puppies and Weddell seal pups (*Leptonychotes weddellii*), presumably due to advances in cardiovascular function and development of diving capacity during ontogeny (Navarette et al., 2021; Whoriskey et al., 2022). Although interesting, differences in HR among sex and age classes were small and not clinically relevant. Compared to otariids anesthetized with injectable drugs, GFS in the current study had average HRs within those same reported ranges (Dennison et al., 2008; Haulena et al., 2000; Katz et al., 2019; Sepúlveda et al., 1994).

The mean RRs for GFS in this study were similar to those reported during maintenance anesthesia in other otariids anesthetized with isoflurane gas (Table 2), as well as to otariids induced with injectable anesthesia (Dennison et al., 2008; Gales & Mattlin 1998; Haulena et al., 2000; Heath et al., 1997; Katz et al., 2019; Lian et al., 2018; Sepúlveda et al., 1994; Yamaya et al., 2006). The inverse association between RR and age class has not been reported in otariids before. Although studies in canines did not reveal any differences in RR among ages, in humans, RR decreases with increasing age and body weight, presumably starting off higher in younger individuals to improve minute ventilation in support of their greater metabolic demands (Fleming et al., 2011; Gagliardi & Rusconi, 1997; Herbert et al., 2020; Navarette et al., 2021; Rishniw et al., 2012). Despite admittedly small variations, the model developed to estimate RR in GFS based on weight could be useful clinically when anesthetizing individuals with known weights, if the association remains linear. In humans, males have higher RR than females due to a higher metabolism per unit body weight; however, in our study, females had higher RR than males (Gagliardi & Rusconi, 1997). It is possible that pregnancy distorted both the age and sex-associated effects on RR (in humans, pregnancy is associated with an increase in RR; however, this increase is typically only minor), and further investigation of the effects of pregnancy on anesthetic factors in otariids is warranted (Soma-Pillay et al., 2016). The association between RR and oxygen flow rate is likely caused by anesthetist influence on flow rates in response to RR. Overall, differences in RR among the various groups in our study, although statistically significant, would be difficult to tease apart for use clinically.

Recovery times in GFS were similar to and in the middle of the range of reports for other otariids anesthetized with isoflurane (Table 2; Gales & Mattlin, 1998; Heath et al., 1997; Lian et al., 2018). The lack of difference in recovery times among body size and age class groups was unexpected. Despite the propensity for a higher MAC in young compared to older humans, GFS pups in our study did not demonstrate faster recovery times as would have been predicted (Aranke et al., 2013). Likewise, adult female GFS in our study, most of which were pregnant, did not demonstrate longer recovery times even though in humans, pregnancy substantially decreases MAC as early as the first trimester, leading to a diminished anesthetic requirement (Wlody et al., 2012). Since smaller individuals should be more prone to hypothermia during anesthesia based on their lower body size to surface area ratio, it could be postulated that differences in body temperature under anesthesia may have offset the effects of age class and pregnancy status on MAC (Pottie et al., 2007; Redondo et al., 2012; Satas et al., 1996; Vitez et al., 1974). However, in a study of juvenile Steller sea lions (*Eumetopias jubatus*), nearly 22% experienced hypothermia under inhalant anesthesia, yet hypothermia was not found to be associated with body weight (weight range of 20–232 kg; Lian et al., 2018). Likewise, in canines undergoing anesthesia for MRIs, body temperature at any given body mass (weight range of 2–83 kg) changed only marginally and was rarely considered clinically significant (Paul & Alef, 2023).

Because the years with the longest and shortest times to recovery had the same anesthetic team, the correlation between year and recovery time in this study may have been due to environmental variance (e.g., temperature), or less likely, variation in nutritional or health status. In contrast to reports on inhalant anesthesia in humans and rats, total anesthesia time was not found to be associated with recovery time. This could have been due to differences in anesthetic depth, drug solubility or concentration variations among individuals, or the anesthesia team's adeptness at predicting the end of anesthesia and weaning isoflurane levels in advance (Eger & Johnson, 1987; Frost, 2014). A more controlled study and standardized anesthetic protocol (if feasible in such a setting) would facilitate investigation of these effects. In comparison to recovery times for otariids induced with injectable anesthetic agents both with and without reversal (range 2–39 min and 20–120 min, respectively), time to recovery in GFS in our study was generally quicker (Dabin et al., 2002; Dennison et al., 2008; Melin et al., 2013; Sepúlveda et al., 1994; Spelman, 2004). Recovery time discrepancies among otariid studies could be attributed to differences in anesthetic employed, or due to variations in species, environmental conditions (i.e., temperature), anesthetic technique, definition of recovery, or physiological differences among individuals in the various studies (sex, age class, pregnancy status, or nutritional or health status; Bedson & Riccoboni, 2014; Fowler, 1995; Kreeger et al., 2002; Pottie et al., 2007; West et al., 2007).

A reliable anesthetic depth is essential to personnel and animal safety as well as efficient animal processing and sample collection, and isoflurane anesthesia provided a consistent and easily titratable anesthetic depth in GFS in our study. Depth of anesthesia is rarely discussed in detail in otariid studies and a standardized depth assessment scale is not utilized, precluding meaningful comparison of our study with others (Gales & Mattlin, 1998; Haulena et al., 2000; Heath et al., 1997; Lian et al., 2018; Storlund et al., 2021; Yamaya et al., 2006). Deeper levels of anesthesia correlated directly with shorter total anesthesia time, presumably due to the ability to process deeply anesthetized GFS more quickly. Deeper levels of anesthesia also correlated directly with lower body weight. The basis for this is unknown, however one possibility is that indicators of reduced anesthetic depth (e.g., movement) in smaller sized individuals were less hindering to processing and thus less likely to be recorded on their anesthetic records. As expected, anesthetic depth was inversely associated with recovery duration (individuals that were less deep recovered more quickly). The association between year and anesthetic depth could be a result of variations in the anesthesia teams' diligence in recording episodes of GFS being "light" or "moving" in response to stimuli during the various years or a result of the lack of a standardized anesthetic depth assessment among anesthesia teams in our study. In remotely immobilized otariids, regardless of drug combination, the depth of anesthesia induced is often reported as highly variable, whether due to individual differences in dose–response, underestimation of dose, incomplete injection, or just not reaching a level of anesthesia suitable for performing the desired diagnostics (Baylis et al., 2015; Boyd et al., 1990; Dabin et al., 2002; Dennison et al., 2008; McKenzie et al., 2013; Melin et al., 2013). Overall, despite the seemingly high proportion of GFS reported as having a light plane of anesthesia during the study, most movements and responses to stimuli were minimal and only rarely resulted in brief discontinuation of sampling or other procedures.

Similar to other reports of gas anesthesia in otariids, few adverse side effects were observed in GFS in this study, no severe consequences resulted, and satellite tag data confirmed survival in all individuals beyond the perianesthetic period. In the available literature regarding isoflurane anesthesia in otariids (781 total anesthesias), side effects, if any, were generally infrequent and mild (Dassis et al., 2016; Gales & Mattlin, 1998; Heath et al., 1997, Storlund et al., 2021; Yamaya et al., 2006). However, one study reported occasional apnea which resolved with the use of doxapram, two individuals that experienced respiratory arrest and were resuscitated successfully with atropine and epinephrine, and one death (accounting for 0.13% of the 781 isoflurane anesthesias reported in the literature) due to asphyxiation from aspiration of gastric contents (Lian et al., 2018). In contrast, all studies reporting on injectable field immobilization of otariids noted adverse side effects, including hypo/hyperthermia, vomiting, retching, brady/tachycardia, and/or tremors/convulsions, and nearly all reported mortalities of one or more individuals (Baylis et al., 2015; Boyd et al., 1990; Dabin et al., 2002; Ferreira & Bester, 1999; Frankfurter et al., 2016; Gardner et al., 2021; Heath et al., 1996; Katz et al., 2019; McKenzie et al., 2013; Melin et al., 2013; Sepúlveda et al., 1994).

4.1 | Conclusion

Overall, isoflurane and oxygen proved an exceptional method of immobilization for free-ranging GFS during fieldwork at Guadalupe Island, including producing reliable and efficient induction and recovery times, adequate anesthetic depth for minimally invasive procedures, and a low risk of injury and medical complications during the anesthetic and perianesthetic periods. Information on anesthesia of free-ranging GFS is still limited, and therefore it is necessary to increase knowledge regarding concurrent clinical conditions (e.g., nutritional and health status) as well as side effects of isoflurane anesthesia (e.g., apnea) in GFS and other otariid species. Additionally, pregnancy in otariids may be underacknowledged in its effects on physiologic parameters under anesthesia, and future studies could serve to elucidate these influences. Finally, utilizing a standardized (and ideally prospective) anesthetic depth assessment would enhance analysis of the anesthetic employed.

Gas anesthesia offers many benefits, including quick induction times, reliable anesthetic depth control, rapid recovery times, and infrequent adverse side effects (Gales & Mattlin, 1998; Geschke & Chilvers, 2009; Haulena & Heath, 2001; Heath et al., 1997; Lian et al., 2018; McKenzie et al., 2013). However, this technique may be limiting in that it requires heavy equipment, net- or hand-capture and manual restraint of animals (thus making it unsuitable for adult male otariids), and lack of a quick reversal mechanism. In contrast, injectable anesthesia is ideal in some scenarios due to streamlined equipment needs, the ability to immobilize animals of various sizes and from various distances, and the option to reverse anesthetic drugs when necessary (Baylis et al., 2015). Researchers must take such characteristics into consideration when assessing the species and habitat utilized for fieldwork and making a determination of which anesthetic technique to employ in order to optimize animal health and safety.

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AUTHOR CONTRIBUTIONS

Lorraine Barbosa: Conceptualization; project administration; writing – original draft. **Benny Borremans:** Formal analysis. **Alissa Deming:** Methodology; writing – review and editing. **Casandra Galvez:** Methodology; writing – review and editing. **Tenaya Norris:** Methodology; supervision; writing – review and editing. **Sarah Pattison:** Methodology; writing – review and editing. **Sophie Whoriskey:** Methodology; writing – review and editing. **Fernando Elorriaga-Verplanken:** Methodology; resources; supervision; writing – review and editing.

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