Title

Tiletamine/zolazepam immobilisation of adult post-moult southern elephant seal males

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Abstract

Immobilisation and anaesthesia of adult male southern elephant seals (*Mirounga leonina*) is potentially risky for animals and scientists. A tiletamine/zolazepam injection is considered the most appropriate drug combination for field application in this species. Since appropriate dosages are difficult to assess due to uncertainties in weight estimation we used photogrammetry derived weight estimates to ensure precise *post hoc* calculations of dosages. We report on 15 intramuscular tiletamine/zolazepam immobilisations of post-moult males of the upper weight class at King George Island / Isla 25 de Mayo in April 2010. Initial injections were made using blowpipe-syringes. Mean tiletamine/zolazepam combined dosages of 0.71 mg kg^{-1} (SD ± 0.16) ranged between 0.46 and 1.01 mg kg^{-1}. In four cases ketamine was added in dosages between 0.96 and 2.61 mg kg^{-1}. Mean induction periods were 23 min (± 15), and the mean duration of the procedures from first injection to release of the animals required 96 min (± 51). Four seals exhibited periods of apnoea, and one case of an extended, repetitive, and potentially critical apnoea (> 25 and 8 min) required intervention in order to successfully re-initiate spontaneous respiration. All procedures resulted in proper immobilisations allowing for the deployment of the satellite tags on the seals' heads. The fact that even substantial deviations between the initial weight estimates and the photogrammetry derived

weight estimates had no apparent effect on the course of the immobilisation underlines the drugs' wide safety margin in this species.

Keywords: *Mirounga leonina*, anaesthesia, apnoea, moult

Introduction

The injection of a tiletamine/zolazepam combination is reported to provide reliable, practical and safe immobilisation of southern elephant seals (*Mirounga leonina*), regardless of age or sex (Carlini et al. 2009), and independent of the physiological status of the animal during haul out periods ashore, though the aforementioned parameters may have an effect. For example, Field et al. (2002) showed that older seals remained anaesthetised longer than younger ones while McMahon et al. (2000) found a negative relationship between body condition and the duration of anaesthesia - a result confirmed by Field et al. (2002). Adult males of the upper weight class, however, have only been investigated by Carlini et al. (2009), who found that significantly higher tiletamine/zolazepam dosages were required early in the breeding season compared to late in the breeding season, and that both of these dosages were higher than those required early in the moult season. Carlini et al. (2009) did not find significant variation in duration of the anaesthesia at these times, but did not anaesthetize adult males at the end of their annual moult. The loss of body mass during the moulting fast would suggest a similar dose relationship between the beginning and end of the moulting fast as shown by Carlini et al. (2009) for the breeding period. We therefore hypothesized that seals at the end of the moult would remain anaesthetized for longer periods, since they are expected to be in poorer condition compared to the beginning of the moult.

Here we report on 15 successful immobilisations of post-moult adult male southern elephant seals of the upper weight class.

Materials and methods

Immobilisations were carried out using a combination of 250 mg tiletamine and 250 mg zolazepam (Zoletil® 100 vet), occasionally complemented by ketamine hydrochloride (Ketavet® 100 mg ml^{-1}). All procedures were carried out along the beach of the Antarctic Specially Protected Area (ASPA) No. 132 between Mirounga Point and Stranger Point, King George Island / Isla 25 de Mayo (62°14'S, 58°40'W) in April 2010. Individual seals were inspected for condition, i.e. by identifying their status of moult, ensuring the absence of visible injuries, estimating the animals' mass for calculating a proper dose regime, and whether it carried a flipper tag or any other permanent marks. Only seals that had reached the end of the moult and were lying at the outer rim of the occasionally dense and large aggregations of males, were immobilised. Total body weight was estimated before the drugs were prepared. Initially we anticipated a dosage of 0.5 mg Kg^{-1} which was slightly adapted after the first trial. Targeted individuals were drugged via self-evacuating blowpipe-syringes (Teledart®, Germany), delivered with a 1.8 m blowpipe from distances <4 m to remotely inject the drugs into the musculature of the seals' back at the lumbar or pelvic region. The blowpipe syringes allowed for a maximum of 15 ml injection volume. The proper insertion of the 80 x 1,5 mm needle was assessed

and the injection of drugs confirmed by observing the full down-glide of the piston stamp of the pressurized syringe from a distance. Whenever possible, the syringe was removed by hand immediately after injection in order to prevent the seal from possible further nociceptive stimuli from the inserted needle being bent due to movements of the seal or when the syringe is moved by the wind. These stimuli and any other sensations like noise or disturbances due to interactions from neighbouring seals can have negative side effects by prolonging the induction phase, and were therefore minimized whenever possible.

The seals were observed from a distance until the anticipated end of the induction period (about 20 min post-injection). Seals were then approached for the first time, and depth of anaesthesia was assessed by evaluating reactions to stimuli (e.g., noise, touch). As soon as the seals tolerated physical stimuli, the animals' eyes were covered with a towel to protect against solar radiation. The induction period was defined as the time from first injection until physical stimuli were tolerated and the towel could be placed.

The areas of pelage on the seals' heads used to attach the devices were dried and cleaned of oil with acetone and an ARGOS satellite-relay data logger was attached using quick setting Araldite® epoxy resin. After attachment, standard length (in ventral recumbency) was measured, and the weight of the seal was later determined in a *post hoc* calculation via photogrammetry (de Bruyn et al. 2009; see de Bruyn 2010 for data). Finally, each seal was observed from a distance until it recovered. Recovery was defined as the time at which a seal reacted to environmental stimuli sufficiently to defend itself from potential attacks of other seals or harassment by scavenging birds (e.g. South Polar skuas, *Stercorarius maccormicki*), which potentially could lead to corneal damage. During anaesthesia, we monitored heart and respiration rate, rectal temperature, capillary refill time, mucous membrane colour, palpebral reflex, and neurological state (by agitating the whiskers). These assessments were made within the workflow for the attachment of the devices and whenever considered necessary but at least every 5 minutes.

Results

All data and related meta-information are available via the Data Publisher for Earth & Environmental Science PANGAEA (www.pangaea.de; see Bornemann et al. 2010; de Bruyn 2010). Tables 1 and 2 summarize information on mean body lengths, estimated and calculated weights, dosages (target dosage and actual dosage based on calculated weight) (Table 1), and number and type (i.e., drug or drugs used) of injections needed to induce anaesthesia, induction time, recovery time, duration of handling and duration of apnoea events (Table 2).

The seals' standard lengths ranged between 378 and 485 cm (Mean 408 ± SD 286), and mean calculated weight (1,810 ± 406) ranged between 1,241 and 2,694 kg. Tiletamine/zolazepam dosage based on estimated weight was 0.63 (± 0.06) mg kg^{1} (Min: 0.50, Max: 0.69). In 10 cases, we overestimated the weight of the seals relative to the *post hoc* photogrammetry calculations and in five cases weights were underestimated. The median deviation of the estimate from the photogrammetry result was 237 kg (Mean 157 kg ± 412, Min: -794, Max: 759). Actual mean dosage administered (based on calculated weights) was 0.71 (± 0.16) mg kg^{1} (Min: 0.46, Max: 1.01). Despite the fact that most dosages were either higher or lower than intended, induction was successful with a single dose of tiletamine/zolazepam for 11 of 15 seals. Three seals needed a follow-up injection of

ketamine between 0.96 and 1.14 mg kg^{-1}, and one seal required four ketamine follow-up injections of 2.61 mg kg^{-1} in total to induce anaesthesia.

The mean duration of handling from first injection to release of the animal was on average 96 min (± 51 min). The average induction period was 23 min (± 15 min), did not depend on the dosage of tiletamine/zolazepam (see below), and was prolonged in the four animals that required additional ketamine. Excluding these four animals, the mean induction time was 17 min (± 3 min). The recovery is difficult to evaluate when seals are observed from distance in order to minimize disturbances. We defined recovery time as the time between first evidence of awareness (eye reactions, slight head movements) and the time the seal had returned to adequate reaction on environmental stimuli. The observed period for recovery was on average 23 min (± 16 min). Hence the period of complete unconsciousness and immobility between end of induction and onset of recovery ranged between 14 and 208 min with a mean 52 min (± 47 min). With the exception of the relationship between extended induction period and ketamine supplementation, multiple linear regression analyses did not show any relationship between dosages of tiletamine/zolazepam alone or in combination with ketamine supplementation, and induction time, recovery time, handling time or presence/absence of apnoeic events.

Discussion

Our standard approach of estimating seal weights visually, resulted in relatively similar dosages for all seals when the amounts of tiletamine/zolazepam are considered relative to the initial weight estimates (0.63 ± 0.06 mg kg^{1}; Min: 0.50, Max: 0.69). Thus the average injection volume (12 ml ± 1) varied only slightly between the lowest and highest dosage given on the basis of the estimates. However, the *post hoc* calculated dosages resulting from the photogrammetry calculation showed a higher mean and a wider range (0.71 ± 0.16 mg kg^{1}, Min: 0.46, Max: 1.01). The photogrammetry based calculated weights have an extremely narrow confidence interval of less than ± 3 % of the computed weight (see de Bruyn 2010), indicating high reliability. Carlini et al. (2009) reported that their method of calculating weights based on length and girth tended to overestimate weight but did not quantify the difference. The equation of Bell et al. (1997) that also took both standard length and girth into account yielded overestimates of 12.5%. Our hypothesis that the dosage will be lower in large males at the end of moult was disproven with respect to the mean values (0.49 ± 0.07 mg kg^{-1}) as presented in Carlini et al. (2009). Though we are unable to compare our calculated weights with that of Carlini et al. (2009) because we were unable to measure girth due to the density of the seal aggregations, the differing dosages are to be attributed to the differing weight calculation regimes leading to less correct and possibly overestimated weights by Carlini et al. (2009).

Anaesthetics and sedatives are more precisely dosed relative to body surface area (BSA) than to body weight, because of better pharmacokinetic scaling as weight is less related to metabolic mass and metabolic processes than BSA (Riviere 1999). Therefore, a dose according to metabolic mass would be more appropriate, particularly in southern elephant seal males as they can lose over 30% of their initial body mass during the fasting periods ashore (Fedak et al. 1994). However, estimating BSA in the field is not practical, although it could be calculated *post hoc* using the photogrammetry method of de Bruyn et al. (2009).

None of the dependent variables i.e. tiletamine/zolazepam alone *vs*. in combination with ketamine, induction time (with the exception of ketamine supplementation), recovery time, handling time, presence or absence of apnoeic events, was associated with dosage in our study. Other factors such as local blood flow at the injection site or inter-individual variability may explain the wide range we observed in some parameters (*cf*. Field et al. 2002). The mean period of 52 min (± 47 min; Table 2) between end of induction and onset of recovery for animals at the end of the annual moult (our study) was longer than the immobilization time measured by Carlini et al. (2009), who found 35 min ± 14 (early breeding n = 22), 34 min ± 12 (late breeding n = 18), 38 min ± 16 (unknown stage of breeding n = 18), and 29 min ± 11 (beginning of moult n = 12). They did not report on significant variation while the number of observations in both studies is comparable. Therefore, our hypothesis that duration of anaesthesia will be longer in large males at the end of moult was proven with respect to the mean values presented in Carlini et al. (2009). Southern elephant seals in our study showed a mean period of unconsciousness and immobility of 52 min (Table 2) after injection with tiletamine and zolazepam. In two cases this period was extended by factor 2 (100 min) and 4 (208 min). We are unable to explain these differences, as they were not related to the dependent variables we measured. The extended anaesthetic period might have been shortened by antagonizing the zolazepam component with the benzodiazepine antagonist flumazenil (Karesh et al. 1997). However, this would have required an injection volume of approximately 250 ml of commercially available flumazenil (0.1 mg ml^{-1}) if average masses and dosages as outlined in Table 1 are considered. Sarmazenil, another benzodiazepine antagonist, is registered for veterinary use and has a tenfold higher concentration. Woods et al. (1995) used sarmazenil (0.5-1.0 mg kg^{-1}) to partially reverse tiletamine/zolazepam anaesthesia in southern elephant seals; they reported a faster recovery but increased muscle tone and tremors attributed to the tiletamine.

Within the cyclohexamines, tiletamine is more potent than ketamine but both have very similar effects. The benzodiazepine zolazepam in turn is a potent central muscle relaxant. The disposition and elimination of both components can be quite different on species level (Semple et al. 2000; Lin et al. 1993). Thus, some species display increased muscle tone and spasms during late stage of anaesthesia because of the predominance of tiletamine effects, others ataxia because tiletamine is eliminated faster and thus zolazepam mediated muscle relaxation continues to have an effect. The latter might play a role in southern elephant seals, though cyclohexamine pharmacokinetics are investigated only for ketamine, showing a mean plasma elimination half-life of 46 min (Min: 17, Max: 108) for post-moult adult females. Even though physiological status affects drug response (e.g. Field et al. 2002; McMahon et al. 2000; Woods et al. 1989), the pharmacokinetics of ketamine were independent from physiological state in the Woods et al. (1999) study. However, physiological state might be relevant for adult males of higher age if we consider a lower metabolism and hepatic clearance in this age class and less fat volume will be available for redistribution at the end of the moult when animals are lean (Field et al. 2002). Hepatic clearance might also be compromised within the moulting fast due to high concentrations of ketone bodies, hyperlipemia as a result of lipid catabolism, and high metabolic demand. With the current set up it cannot be differentiated whether redistribution or metabolism and elimination were the major determinant of the duration of anaesthesia.

Four seals exhibited periods of apnoea (Table 2). Three of these fell within the range reported for adult male northern elephant seals during natural breathing patterns (Blackwell and Le Boeuf 1993, Castellini 1994). One case of an extended, repetitive, and critical apnoea (> 25 min and 8 min)

required intervention in order to re-initiate spontaneous respiration. In this case, the seal was lying with its head facing downward due to the uneven substrate on which it was resting. Since the seal opened one of its nostrils but could not take a breath, we considered an obstruction of the upper respiratory tract the primary cause of the apnoea, and thus a critical situation. When the mucosal colour became cyanotic, and palpebral reflex and the reaction to whisker stimulus ceased, we moved the seal onto its side and put ice blocks under the belly and head in order to lift the head to the same level as the body. After 25 min in this position the seal breathed only once before another apnoeic period of 8 min. We lifted the seal's head up in line with its neck and it managed to breathe again, however breathing remained shallow throughout the handling period and mucous membranes remained cyanotic. Following equations presented in Slip and Woods (1996), the calculated aerobic dive limit (cADL) for this particular animal was 40 min, and the cumulative times of apnoeas recorded match this figure closely. Thus, we considered this case as critical, and not typical of routine sleep apnoeas. However, the event did not appear to have short term survival implications for this seals, as it was tracked at sea for at least 9 months after tagging.

Protective reflexes and laryngeal tone are maintained with cyclohexamines such as tiletamine and ketamine (Riviere and Papich 2009) and they induce only minor respiratory depression, but they produce an apneustic breathing pattern with irregular breathing and long breath holds. This is believed to be mediated by chemical dissociation of thalamoneocortical areas from ventral hippocampal structures (Weingarten 1972). Apneustic breathing can be seen after head injury and is considered a symptom for decerebration and absence of cortical modulation of respiration (Glasser et al. 1966). Mitchel and Burton (1991) observed an extended period of apnoea >26 min in a 1,500 kg elephant seal male after tiletamine/zolazepam injection (though at a higher dosage), also with unproductive opening of nostrils without inhalation. We attribute the prolonged apnoea we observed to mechanical obstruction of the glottis by peripharyngeal tissue or the flaccid soft palate as suggested by Haulena and Heath (2001) following Phelan and Green (1992) and Lynch et al. (1999). Re-opening of the obstructed airways by moving or stretching the neck to relieve the obstruction (this study) or placing an endotracheal tube to open the airway allows for continuation of spontaneous respiration.

Our study has shown that tiletamine/zolazepam is an appropriate combination for immobilisation of post-moult adult male southern elephant seals of the upper weight class. The fact that even substantial deviations between the initial weight estimates and the photogrammetry based weight calculations had no apparent effect on the course of the immobilisation underlines the drugs' wide safety margin in this species.

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316

317 **Table captions**

318 Table 1:

319 Information on mean lengths, estimated and calculated masses, dosages referring to
320 estimated/calculated masses of 15 adult male southern elephant seals immobilised at King George
321 Island / Isla 25 de Mayo between March and April 2010

322

323 Table 2:

324 Information on number of injections to complete induction, durations of induction and recovery,
325 duration of full procedures, and events of apnoeas of 15 adult male southern elephant seals
326 immobilised at King George Island / Isla 25 de Mayo between March and April 2010

Table 1

Seal	Standard length [cm]	Estimated mass [kg]	Calculated mass [kg]	Deviation from estimate [kg]	Deviation from estimate [%]	Zoletil Dose ref. to est. [mg/kg]	Zoletil Dose ref. to cal. [mg/kg]	Ketamine Dose ref. cal. [mg/kg]
JUB2010_sel_a_m_01	386	2000	1913	87	5	0.50	0.52	2.61
JUB2010_sel_a_m_02	390	2000	1241	759	61	0.63	1.01	
JUB2010_sel_a_m_03	381	2000	1325	675	51	0.63	0.94	
JUB2010_sel_a_m_04	409	2200	1950	250	13	0.57	0.64	1.03
JUB2010_sel_a_m_05	380	1800	1316	484	37	0.56	0.76	1.14
JUB2010_sel_a_m_06	403	2200	1935	265	14	0.57	0.65	
JUB2010_sel_a_m_07	430	2200	1967	233	12	0.59	0.66	
JUB2010_sel_a_m_08	378	1800	1465	335	23	0.69	0.85	
JUB2010_sel_a_m_09	400	2000	1462	538	37	0.65	0.89	
JUB2010_sel_a_m_10	421	1900	2064	-164	-8	0.68	0.63	
JUB2010_sel_a_m_11		1900	2295	-395	-17	0.66	0.54	
JUB2010_sel_a_m_12	426	1900	2010	-110	-5	0.66	0.62	
JUB2010_sel_a_m_13	485	1900	2694	-794	-29	0.66	0.46	
JUB2010_sel_a_m_14	400	1800	1563	237	15	0.69	0.80	0.96
JUB2010_sel_a_m_15	426	1900	1949	-49	-3	0.66	0.64	
Mean	**408**	**1967**	**1810**	**157**	**14**	**0.63**	**0.71**	**1.43**
SD	286	140	406	412	25	0.06	0.16	0.77
Median	403	1900	1935	237	13	0.65	0.65	
Min	378	1800	1241	-794	-29	0.50	0.46	0.96
Max	485	2200	2694	759	61	0.69	1.01	2.61

Table 2

Seal	Injections to complete induction	Time between injections [min]	Duration of induction [min]	Duration of recovery [min]	Period between ind. and rec. [min]	Duration of total procedure [min]	Apnoea [min]
JUB2010_sel_a_m_01	4	55	70		35	105	
JUB2010_sel_a_m_02	1		20	4	14	38	
JUB2010_sel_a_m_03	1		20	35	57	112	
JUB2010_sel_a_m_04	2	30	35	39	28	102	
JUB2010_sel_a_m_05	2	25	30	45	100	175	10
JUB2010_sel_a_m_06	1		15	10	208	233	
JUB2010_sel_a_m_07	1		14	25	20	59	
JUB2010_sel_a_m_08	1		15	17	74	106	> 25 plus 8
JUB2010_sel_a_m_09	1		15	8	30	53	21
JUB2010_sel_a_m_10	1		24	18	52	94	
JUB2010_sel_a_m_11	1		15	10	35	60	
JUB2010_sel_a_m_12	1		15	10	37	62	
JUB2010_sel_a_m_13	1		15	55	35	105	
JUB2010_sel_a_m_14	2	20	28	27	27	82	
JUB2010_sel_a_m_15	1		15	14	26	55	11
Mean		**32**	**23**	**23**	**52**	**96**	
SD		16	15	16	47	51	
Median	1		15	18	35	94	
Min	1	20	14	4	14	38	
Max	4	55	70	55	208	233	