## ORIGINAL PAPER

# Immobilization of adult male southern elephant seals (*Mirounga leonina*) during the breeding and molting periods using a tiletamine/zolazepam mixture and ketamine

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Abstract Seventy-seven immobilizations were carried out on adult male southern elephant seals at Stranger Point, Isla 25 de Mayo (King George Island) using a combination of Zoletil<sup>®</sup> (tiletamine and zolazepam) and ketamine in order to obtain biological samples. During 2006/2007, 22 males were immobilized at the beginning of their breeding period (EB), 19 of which were recaptured at the end of breeding (LB). Four were given only once at an unknown stage of breeding (USB) and 18 males were immobilized at the beginning of molting (BM). During 2007/2008, 14 adult males were immobilized at an USB. Zoletil® was administered using an automatic discharge device, whereas ketamine was injected directly with a syringe, and was used only when the initial sedation was not enough to carry out the programmed sampling. The initial mean dose of Zoletil<sup>®</sup> was  $1,387 \pm 304$  mg, which represented  $0.60 \pm 0.14$  mg/kg, range 0.36–1.05, n = 77. In 47 procedures, an average dose of  $1.04 \pm 0.66$  mg/kg of ketamine was added. Mean immobilization time was

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 $34 \pm 14$  min. In 25 out of the 77 procedures, males showed apnea, which lasted  $8 \pm 4$  min (range 2–15 min). The necessary doses of Zoletil<sup>®</sup> and ketamine to attain immobilization differed between stages. For animals taken twice, doses (mg/kg) of Zoletil<sup>®</sup> and ketamine were significantly higher at the beginning than at the end of breeding. During molting, the doses of Zoletil<sup>®</sup> given were significantly lower than those used during breeding, although the proportion of animals that required ketamine during molting was significantly higher than during breeding. Zoletil<sup>®</sup> proved to be a safe immobilizing agent for field work on adult males of this species, given the wide range of doses used without any serious consequences. Furthermore, the addition of ketamine was useful when the initial sedation was not satisfactory or for prolonging the immobilization period in a practical and reliable way.

**Keywords** Anesthesia · Southern elephant seal · Tiletamine–zolazepam · Chemical restraint · Ketamine

## Introduction

Southern elephant seals (*Mirounga leonina*) have usually been immobilized with ketamine combined with diazepam or xylazine (e.g. Gales and Burton 1987; Baker et al. 1988; Bester 1988; Gales 1989; Woods et al. 1989, 1994, 1995; Mitchell and Burton 1991; Slip and Woods 1996). Fewer reports are available on the use of a tiletamine–zolazepam mixture (Baker et al. 1990; Mitchell and Burton 1991; Woods et al. 1994, 1995; McMahon et al. 2000; Field et al. 2002) and the use of medetomidine alone or combined with ketamine was only reported by Woods et al. (1996). Although adult males were immobilized in some of these studies, the principal target was females, and juvenile and sub-adult males. There is only one study which specifically reports drug effects on adult males, in which etorphine–acepromacine and ketamine were used for sedation (Ramdohr et al. 2001).

Male southern elephant seals spend most of their annual cycle at sea, going ashore twice a year, once during the breeding period (September-November) and once during the molting period (January-March). Throughout breeding, adult males actively defend their positions within or on the periphery of harems. During molting, they form groups with other animals of similar size. Because of their large size (4.5 m long and 1,500-4,000 kg) and their aggressiveness during breeding, it may be dangerous to obtain samples without appropriate sedation. Due to the densely packed aggregations they form on land during molting, it is difficult to estimate their weight by eye in order to calculate the initial drug dose to be administered. Finally, physiological status, which is an important factor affecting drug response (Woods et al. 1989, 1999; McMahon et al. 2000; Field et al. 2002), changes markedly during their annual cycle, as adult males do not feed while ashore and can lose over 30% of their initial body mass, mostly from fat reserves (Fedak et al. 1994).

As part of a study on breeding biology and feeding ecology, southern elephant seal males were immobilized at Stranger Point, Isla 25 de Mayo (King George Island) during the 2006/2007 and 2007/2008 seasons. This paper reports the use of a tiletamine–zolazepam mixture and ketamine to immobilize adult male southern elephant seals during the breeding and molting periods.

#### Materials and methods

Seventy-seven immobilizations of adult males were carried out using a combination of 250 mg tiletamine and 250 mg zolazepam, commercially available as Zoletil<sup>®</sup>100 (Virbac, Australia, Pty Ltd.), and ketamine, at Stranger Point, Isla 25 de Mayo/King George Island (62°14′S, 58°40′W).

In 2006/2007, 22 males were immobilized at an early stage of the breeding period (EB), 19 of which were recaptured at a late stage of the breeding period (LB). Four were given once at an unknown stage of the breeding period (USB) and 18 were immobilized at the beginning of the molting period (BM). In 2007/2008, 14 adult males were immobilized at an USB.

The males in the EB group, sampled from 6 October to 28 October, were anesthetized within a period of up to 20 days after arrival. Of these, males recaptured during breeding were grouped as LB (sampled from 6 November to 17 November), the mean period between immobilizations being 26 days. The males with unknown arrival dates

were grouped as USB and were immobilized only once between 16 October and 22 November.

Of all the animals immobilized at breeding, 4 were never observed associated with a harem, 16 were dominant males and 20 subordinate males. This classification was made on a temporal basis, the dominant male being that which was observed twice or more as harem bull in any harem throughout the breeding period, while the subordinate male was never observed, or was observed only once, as harem bull during the censuses (Carlini et al. 2006). Males were also classified based on their distance from the nearest border of the harem on the day when they were immobilized as harem bull (inside the female groups), peripheral 1 (0-10 m from the harem), peripheral 2 (11-20 m from the harem) or solitary (more than 20 m from the harem). Since males changed their position frequently, we recorded one position per immobilization during the breeding period. Following this classification, immobilizations were carried out in males occupying a harem bull position on 16 occasions, a peripheral 1 position on 18 occasions, a peripheral 2 position on 7 occasions, and solitary in 11 cases. In seven procedures, males could not be classified because they were immobilized early during the breeding period, at a time when no or very few females had arrived at the colony. To determine male social position, censuses were carried out daily throughout the breeding seasons to record male presence and status. Zoletil<sup>®</sup> was administered intramuscularly in the pelvic region using an automatic discharge device inserted manually. The device was made by gluing two syringes back to back. The upper syringe acts as an air chamber and the lower one holds the drug. This syringe is connected to a needle of  $2 \times 150$  mm by means a 12 cm plastic canula. This canula prevents the device from coming off when the seal moves after injection. Following the initial sedation, ketamine (50 mg/ml) was administered when the animal was not adequately sedated via an intramuscular injection by syringes (1-5 injections), throughout the immobilization procedure. We preferred to add ketamine instead of reinjecting Zoletil<sup>®</sup> because the metabolism and elimination rates of tiletamine and zolazepam are different, which could be a disadvantage when animals are injected repeatedly (Woods et al. 1994). The aim was to find the necessary dose rates to produce a level 4 of chemical restraint as defined in Woods et al. (1994).

Initially, each animal's weight was estimated by eye and doses of Zoletil<sup>®</sup> slightly lower than those usually used in females at Stranger Point (0.7–1.0 mg/kg) (A.R. Carlini, unpublished data) were administered. After they had been immobilized, animals were measured (standard length and axillary girth) and their weight were reestimated to calculate the actual dose given. Male weight was estimated using the following regression equation, obtained from

female elephant seals weighed and measured in the same study area (A.R. Carlini, unpublished data):

weight (kg) = 
$$26.4 + 44.8(SL \times AG^2)$$
,  
 $F_{(1,249)} = 3,567$ ,  $P < 0.0001$ ,  $R^2 = 0.93$  (1)

where SL is the standard length measured as a straight line from the tip of the nose to the tip of the tail with the animal in ventral recumbency (American Society of Mammalogists 1967) and AG is the axillary girth.

A second mass estimation was made from Eq. 6 provided by Bell et al. (1997), which used the combined data obtained from southern elephant seal adult females, young males and females, and yearlings (both sexes). Induction time was defined as the time from injection (Zoletil<sup>®</sup> in all cases) until the animal failed to raise its head when a researcher approached. Duration of immobilization was defined as the time from induction until the animal could raise its head. In both cases, a researcher approached the animal at regular periods beginning 5 min after the first injection. Males were considered apnoetic when they stopped breathing for more than 1 min. Convulsions and tremors were also recorded throughout the immobilization procedures. Animals were monitored from first injection until they could raise their head and move toward the researcher when he/she approached.

#### Statistical analyses

Values are given as mean  $\pm$  SD except where otherwise indicated and results were considered to be significant at the P < 0.05 level. Differences in frequencies were tested with Chi-square analysis. Student's *t* tests were conducted to analyze differences between means of independent samples and Student's paired *t* tests to analyze dependent samples, while the Pearson product moment correlation was used to measure the strength of associations between pairs of variables.

The Kolmogorov–Smirnov one sample test for normality was used to determine whether the data was normally distributed and the Levene median test to confirm homogeneity of variances.

## Results

Overall mass estimation from Eq. 1 was  $12.5 \pm 2.2\%$ higher than that obtained from Eq. 6 provided by Bell et al. (1997). The differences were similar for all the stages considered (EB:  $13.9 \pm 2.3\%$ , LB:  $11.3 \pm 1.7\%$ , USB:  $11.3 \pm 2.6\%$ , BM:  $11.8 \pm 1.3\%$ ). For this reason, although the estimated absolute values of the doses injected are different depending on the equation chosen, the statistic tests we applied below yielded similar results using any of these mass estimations. The mean masses (Eq. 1), dose rates of anesthetics, induction times, duration of sedation and occasions on which apnea was observed are summarized for each group considered in Table 1.

The initial dose of Zoletil<sup>®</sup> for all the immobilization procedures was  $1,387 \pm 304$  mg, representing an estimated dose of 0.60  $\pm$  0.13 mg/kg (range 0.36–1.05 mg/kg, n = 77) (Table 1). Ketamine was given in addition to Zoletil<sup>®</sup> in 47 cases to achieve a better degree of immobilization. The total dose of ketamine injected was  $1.04 \pm 0.66$  mg/kg (range 0.28–2.60 mg/kg) administered in 1 up to 5 different injections throughout the immobilization procedure. In general, the dose of Zoletil<sup>®</sup> in those cases in which it was not necessary to administer ketamine was significantly higher than when ketamine was required to attain a sufficient degree of immobilization  $(0.67 \pm 0.13 \text{ mg/kg}, n = 30 \text{ vs.} 0.56 \pm 0.12 \text{ mg/kg},$ n = 47; t test, P < 0.005). The above tendency is also observed when the stages are treated separately although, because of the sample size, differences were not significant at P < 0.05 (EB: 0.71 ± 0.10 mg/kg, n = 8 vs.  $0.62 \pm 0.13$  mg/kg, n = 14; LB:  $0.62 \pm 0.09$  mg/kg, n = 11 vs.  $0.55 \pm 0.08$  mg/kg, n = 8; USB: 0.71  $\pm$ 0.18 mg/kg, n = 9 vs. 0.63  $\pm$  0.14 mg/kg, n = 9; BM: 0.54 mg/kg, n = 2 vs. 0.49  $\pm$  0.07 mg/kg, n = 16).

The mean time between immobilizations for males anesthetized twice during the breeding period was  $26 \pm 4$  days. The estimated weight loss for this period was  $420 \pm 200$  kg  $(16 \pm 7 \text{ kg/day})$ , representing  $16 \pm 6\%$ of their initial weight. The doses of Zoletil<sup>®</sup> used for these animals at early breeding were significantly higher than those given at late breeding  $(0.66 \pm 0.11 \text{ and} 0.60 \pm 0.09 \text{ mg/kg}, n = 19$ , respectively; paired *t* test, P < 0.01). Furthermore, during early breeding, a greater number of males required ketamine injections and the mean doses used were higher than at late breeding  $(1.13 \pm 0.78 \text{ mg/kg}, n = 11 \text{ vs}. 0.60 \pm 0.26 \text{ mg/kg},$ n = 8; one tail *t* test, P < 0.05). Induction time or immobilization time was not significantly different between procedures (*t* test, P > 0.5 for both cases).

The doses of Zoletil<sup>®</sup> given at the beginning of molting were significantly lower than those used during breeding (EB, LB and USB, data combined) ( $0.49 \pm 0.07$ , n = 18 vs.  $0.64 \pm 0.13$ , n = 59, respectively; t test, P < 0.0001). However, the average number of animals that required ketamine during molting was significantly greater than during breeding (molting: 16/18 males, breeding: 31/59 males) (Chi-square = 7.66, P < 0.01). When the breeding (EB, LB and USB, data combined) and molting subgroups in which ketamine was required are compared, the doses of Zoletil<sup>®</sup> used during molting were significantly lower than those used during breeding ( $0.49 \pm 0.07$ , n = 16 vs.  $0.61 \pm 0.12$ , n = 31, respectively; t test, P < 0.0001).

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	Early breeding (EB)	Late breeding (LB)	Unknown stage of breeding (USB)	Beginning of molting (MB)	All procedures
Estimated weight (kg)	$2,632 \pm 587 \ (n = 22)$	$2,152 \pm 393 \ (n = 19)$	$2,460 \pm 560 \ (n = 18)$	$2,056 \pm 235 \ (n = 18)$	$2,339 \pm 517 \ (n = 77)$
Zoletil <sup>®</sup> (mg/kg)	$0.65 \pm 0.13 \ (n = 22)$	$0.60 \pm 0.09 \ (n = 19)$	$0.67 \pm 0.16 \ (n = 18)$	$0.49 \pm 0.07 \ (n = 18)$	$0.60 \pm 0.13 \ (n = 77)$
Ketamine (mg/kg)	$1.02 \pm 0.71 \ (n = 14)$	$0.60 \pm 0.26 \ (n = 8)$	$1.14 \pm 0.85 \ (n = 9)$	$1.20 \pm 0.55 \ (n = 16)$	$1.04 \pm 0.66 \ (n = 47)$
Induction time <sup>a</sup> (min)	$12 \pm 4 \ (n = 8)$	$15 \pm 4 \ (n = 11)$	$16 \pm 13 \ (n = 9)$	17 $(n = 2)$	$14 \pm 6 \ (n = 30)$
Induction time <sup>b</sup> (min)	$29 \pm 19 \ (n = 14)$	$26 \pm 7 \ (n = 7)$	$42 \pm 31 \ (n = 9)$	$42 \pm 16 \ (n = 10)$	$34 \pm 21 \ (n = 40)$
Immobilization time (min)	$35 \pm 14 \ (n = 22)$	$34 \pm 12 \ (n = 18)$	$38 \pm 16 \ (n = 18)$	$29 \pm 11 \ (n = 12)$	$34 \pm 14 \ (n = 70)$
Apnea (min)	$9 \pm 5 \ (n = 10)$	$8 \pm 4 \ (n = 9)$	$8 \pm 2 \ (n = 5)$	9 ( $n = 1$ )	$8 \pm 4 \ (n = 25)$

Table 1 Mean masses, dose rates of anesthetics, induction times, duration of sedation and occasion on which apnoea was observed in male southern elephant seals at Isla 25 de Mayo

Induction time and immobilization time were not recorded for one animal at the end of breeding (LB) and for six animals at the beginning of molting (BM)

<sup>a</sup> For animals which were given Zoletil<sup>®</sup> only

<sup>b</sup> For animals which required an added dose of ketamine. In these cases, induction time was recorded from the initial injection of Zoletil<sup>®</sup> until induction was achieved after the ketamine injection

However, the doses of ketamine given in the molting period were higher at a non-significant level (1.20  $\pm$  0.55, n = 16 vs. 0.94  $\pm$  0.69, n = 31, molting and breeding, respectively; *t* test, P > 0.05).

Induction time for animals anesthetized with Zoletil<sup>®</sup> was  $14 \pm 6$  min, whereas induction time for males that required an added dose of ketamine was  $34 \pm 21$  min. (Table 1). When the injection of ketamine was necessary, induction time was much longer because there was a delay to see whether the Zoletil<sup>®</sup> dose was producing an effect.

In 25 out of the 77 immobilizations, males exhibited apnea, which lasted  $8 \pm 4 \min$  (range 2 15 min) (Table 1). Respiratory stimulation was not used in any of the cases as southern elephant seals may tolerate approximately 20 min of apnea owing to their diving adaptations (Castellini 1994). The doses of Zoletil<sup>®</sup> were significantly higher for apnoeic animals only at the EB stage  $(0.72 \pm 0.10, n = 10 \text{ vs.})$  $0.59 \pm 0.11$ , n = 12; t test, P < 0.05), but there were no significant differences during the LB or USB stage. The BM stage was not checked, since only one male exhibited apnea at this stage. There was no correlation between apnea duration and the doses of Zoletil<sup>®</sup> used, either for all the animals (r = 0.0, P > 0.5, n = 25) or for the subgroup anesthetized with Zoletil<sup>®</sup> only (r = 0.0, P > 0.5, n = 16). Only nine animals exhibited apnea longer than 10 min. There was no significant difference in the Zelazol doses injected in this subgroup in relation to the doses given to the other males  $(0.64 \pm 0.13, n = 9 \text{ vs. } 0.60 \pm 0.13, n = 68;$ t test, P > 0.05). Slight muscular tremors were observed in three animals, two of which had been administered only Zoletil<sup>®</sup>. All these animals also exhibited apnea. Convulsions were not observed in any of the animals.

All the males that were immobilized twice remained on the beach throughout the breeding season after the procedures. Only 1 of the 18 animals immobilized once (USB group) was not observed in the area again. On two occasions, a harem bull was challenged by a peripheral male before he had totally recovered from anesthesia. On one of these occasions, the harem bull was defeated due to the effects of the immobilization procedure. This happened at the end of the breeding period, when female groups were small. The defeated male subsequently reached a dominant position in a similarly sized harem nearby.

# Discussion

The tiletamine-zolazepam mixture, with added ketamine when necessary, was highly successful in the immobilization of adult male southern elephant seals. For the first injection, the maximum dose of Zoletil<sup>®</sup> used (1,900 mg) was diluted in 19 ml of distilled water and for most of the procedures in less than 15 ml, which can be useful for remote drug injection. Moreover, Zoletil® showed a wide drug safety margin at the dose rate used, since the highest initial dose was almost three times the lowest one, and although the quantity of drug injected had an effect on the initial sedation achieved (see "Results"), even lower doses of Zoletil<sup>®</sup> allowed enough sedation to inject ketamine and reach a good degree of immobilization. However, the addition of ketamine implies an increase in the induction time because of the methodology applied (see "Results"). Potential ways to reduce induction time, including the injection of higher doses, the delivery of the main drug in a medium that facilitates its uptake or the addition of sedative drugs, have been described for large ungulates by McMahon and Bradshaw (2008) although they have not been tried in this study. Finally, there were no fatalities throughout the procedures and very few animals showed side effects such as tremors. Convulsions were not observed and apneas, although frequent (25 out of 77 procedures), were in general well below 20 min, a breath holding period exhibited by the animals while sleeping on land (Castellini 1994).

The tiletamine-zolazepam mixture injected intramuscularly was successfully used in southern elephant seals (mainly females and younger animals) by Baker et al. (1990) at an average dose of  $(0.95 \pm 0.22 \text{ mg/kg})$  when only one injection was necessary. Moreover, Zoletil<sup>®</sup> was used by Woods et al. (1994) in premolting females at a mean dose of 0.96 (range 0.72-1.2). This drug combination was also administered intravenously in young females and males at a mean dose of  $0.46 \pm 0.08$  mg/kg (McMahon et al. 2000) and in southern elephant seals of different ages and body conditions at a mean dose of 0.53 mg/kg by Field et al. (2002). In those studies where the drug was administered intramuscularly, the mean dose reported is higher than in our study,  $0.67 \pm 0.13$  mg/kg (for animals immobilized with Zoletil<sup>®</sup> only). This could be explained by an overestimation of the male's weight and/or a real difference in the sensitivity of the male to the anesthetic. We used a standard lengthaxillary girth relationship derived from females weighed at Stranger Point to estimate male mass, since the length-mass relationship does not take into account the physiological status of the animal, which is of overriding importance in animals immobilized twice while fasting. The length-mass relationship provided by Ling and Bryden (1981) tends to underestimate mass (Woods et al. 1989), and a further relationship developed by Gales and Burton (1987) yields weights that are approximately 15% higher. However, our weight estimation was on average 28% higher than that calculated with the Gales and Burton (1987) equation and around 9% higher than that calculated from Bryden (1972). More recently, Bell et al. (1997) developed an equation in which both standard length and axillary girth were taken into account, and compared with this equation, our estimation was 12.5% higher (see "Results"). The above comparisons imply that an overestimation of male mass could have taken place in our study, resulting in an underestimation of the average dose injected. However, southern elephant seal adult males could be more sensitive to Zoletil<sup>®</sup> than females due to their greater body mass and lower mass-specific metabolic rate, as was observed by Boyd et al. (1990) in adult male and female Antarctic fur seals (Arctocephallus gazella), a species with a three- to fourfold difference in body mass between sexes such as southern elephant seals.

There was a difference in the doses used at the beginning and end of breeding for males immobilized twice. The dose of Zoletil<sup>®</sup>, the number of animals in which ketamine had to be added and the mean dose of ketamine injected were higher at the beginning of breeding. During the breeding period, males fast while fighting for a high rank in the social hierarchy and competing for mates. The metabolic cost of fasting will be mostly supported from fat reserves, which supply around 95% of the energy costs of fasting in pinnipeds (Adams and Costa 1993). This implies that males will have a different body composition between the immobilization procedures, having a higher proportion of lean tissue at the end of breeding. For instance, an average male sampled twice in this study weighing 2,572 kg, having 39% body fat in the first procedure and losing the average mass estimated here (420 kg), will have around 30% body fat in the second procedure, assuming a loss of 87% adipose tissue and 13% lean tissue by mass (Deutsh et al. 1990). Woods et al. (1999) found that the kinetics of ketamine when administered with pethidine are similar in premolting, postmolting and postpartum female southern elephant seals with different proportions of blubber at these stages, and suggested that blubber is not involved in the initial distribution of ketamine. They suggested that it could be more useful to base initial doses on lean tissue instead of total body mass. Our calculation of drug dose was made on the basis of total mass. Although males taken twice in our study changed their total body mass markedly, lean body mass is expected to have changed only fractionally, since the mass changes will have occurred mostly through fat loss. So, if we had used lean tissue instead of total body mass to calculate the amount of Zoletil<sup>®</sup> injected, the differences in drug dose (between procedures) in males taken twice during breeding would have been even greater than we reported, since differences in lean tissue between procedures are smaller than those calculated in total body mass. It is not clear why the drug dose necessary to obtain a satisfactory level of immobilization was greater at the beginning of breeding. However, the result observed was surprising, since it is usually accepted that fat animals will require smaller doses of anesthetics than what they would require when in a leaner state. Woods et al. (1989) suggested that there could be differences in the distribution of anesthetics and/or inherent differences in drug-receptor tissue sensitivity related to different physiological states, which may account for differences in the duration of sedation in southern elephant seals. It is possible that males in the second procedure, after a considerable fasting period while fighting for mates, were subjected to considerable physiological stress due to their negative energetic balance, which might explain the smaller dose necessary to attain a satisfactory sedation. This does not necessarily mean that fatter animals will need more drug, but that the time of the annual cycle itself (beginning or end of each fasting period) could be more important than an animal's fatness when calculating drug doses. McMahon et al. (2000) and Field et al. (2002) found that thinner animals remained immobilized for longer than

fatter ones despite receiving similar doses of Zoletil<sup>®</sup> and that there was a negative relationship between degree of fatness and immobilization time (although it only explained less than 10% of the variation observed). They proposed that as tiletamine and zolazepam are lipophilic, some are absorbed in fatty tissue during their redistribution. This implies that fatter animals would be expected to have less drug available to prolong the anesthesia (Field et al. 2002). Since we injected the Zoletil<sup>®</sup> intramuscularly, drug accumulation in fatty tissue could also be a plausible explanation for the variation observed during breeding in animals taken twice.

The doses of Zoletil<sup>®</sup> used during molting were lower than those used during breeding. However, 16 of the 18 animals immobilized at the beginning of molting had to be given ketamine to attain a sufficient degree of immobilization (Table 1). Because molting females exhibited apnea more frequently than breeding females (A.R. Carlini, unpublished data), we were concerned about the effect of Zoletil<sup>®</sup> on molting males. For this reason, lower initial doses of Zoletil<sup>®</sup> were chosen, adding ketamine to achieve appropriate sedation. Our procedure during this stage could explain why only 1 out of 18 males exhibited apnea for a short time, although induction time was increased (Table 1) due to the procedure chosen.

The tendency to undergo longer apnea periods after chemical immobilization in phocid seals is common, and can result in fatalities if apnea persists (Gales 1989; Mitchell and Burton 1991). Apnea periods of up to 10 min while sleeping on land are usual in southern elephant seals, but periods longer than 10 min after chemical immobilization are considered as a drug side effect (Woods et al. 1994). Overall, in our study, animals showed apnea in 32%of the immobilization procedures, although the percentage decreases to 12% if we consider only the cases where animals were apnoetic for more than 10 min. Although the relation between the occurrence of apnea and the drug dosage is not totally clear, a lower Zoletil® dose was related to the absence of apnea in the species (McMahon et al. 2000). There was some evidence that higher doses of Zoletil<sup>®</sup> were related to apnea since apnoetic animals in the EB group received significantly higher Zoletil<sup>®</sup> doses than those that did not show apnea and only one animal in the BM group became apnoetic. However, there was no relationship between the dose of Zoletil® injected and the duration of apnea, and the average Zoletil<sup>®</sup> dose injected was not significantly different between apnoetic animals and those that did not exhibit apnea in the LB or USB group. None of the animals immobilized showed periods of apnea longer than 15 min, which is well below the theoretical aerobic dive limit, estimated at around 25 min in female southern elephant seals (Hindell and Lea 1998).

In conclusion, we consider that initial doses of Zoletil<sup>®</sup> at rates of 0.7 mg/kg during early breeding, 0.6 mg/kg during late breeding and 0.5 mg/kg during molting provide a safe initial dosage for adult male southern elephant seals, and allow us to inject ketamine when necessary. Although special care must be taken when harem bulls are immobilized during breeding because of the presence of peripheral males which can challenge them before total recovery, the fact that all but one of the adult males remained in their original harems after the procedure suggests that Zoletil<sup>®</sup> does not appear to have any serious effects on their behavior.

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