Effects of age, size and condition of elephant seals (*Mirounga leonina*) on their intravenous anaesthesia with tiletamine and zolazepam

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Southern elephant seals (*Mirounga leonina*) were caught as part of a long-term demographic study on Macquarie Island. Over 18 months, 1033 seals were caught by hand and anaesthetised intravenously with a 1:1 mixture of tiletamine and zolazepam. Assessments were made of the effects of variations in the body condition and age at capture of the seals on the characteristics of their anaesthesia, including induction time and weighted recovery time. The size and condition of the seals were assessed by morphometric and ultrasound measurements. Weighted recovery times decreased as the body condition and age of the seals increased, but there were no residual effects of sex. There were no fatalities, and no periods of apnoea longer than five minutes were recorded. In individual seals there was a significant increase in weighted recovery time with successive captures.

STUDIES of the life history of wild pinnipeds often rely on seals being restrained, either physically or chemically, to obtain information on their diet, physiology and dive behaviour (Slip 1995, Hindell and Lea 1998). The aim of any anaesthetic procedure is to provide a reliable method of immobilisation with predictable responses from the animal, rapid induction of anaesthesia, and a brief recovery time, thereby minimising the disturbance to the animal (Haigh 1978). Many studies have described the successful use of cyclohexamines such as tiletamine in pinnipeds (Geraci 1973, Englehart 1977, Trillmich and Weisner 1979, Gales and Burton 1987a, Woods and others 1989, Shaughnessy 1991, Slip and Woods 1996, McMahon and others 2000), even though this taxon remains difficult to anaesthetise (Geraci 1973, Geraci and others 1981, Parry and others 1981, Mitchell and Burton 1991). Pinnipeds are adapted physiologically to live in extreme environments, and when they are sedated they often suffer side effects such as hypothermia and apnoea, that is, the temporary interruption of normal breathing patterns (Baker and others 1988, Gales 1989, Mitchell and Burton 1991, Woods and others 1994). However, reducing the initial dose rates and administering the anaesthetic intravenously reduces the severity and frequency of such side effects (Slip and Woods 1996, McMahon and others 2000).

Some studies have suggested that the physiological status of southern elephant seals (*Mirounga leonina*) affects their sensitivity and response to anaesthetics (Gales and Burton 1987a, Woods and others 1989, McMahon and others 2000). Woods and others (1989) suggested that the relationship between their physiological state and the effects of sedation is complex and must be considered when preparing to anaesthetise them to minimise the incidence of apnoea and other side effects.

This study investigated the relationships between the physiological status of southern elephant seals, in terms of their age, size and body condition (body-shape index and blubber reserves), and the variation in their individual responses to anaesthesia, and whether their responses to successive captures changed.

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MATERIALS AND METHODS

Between November 1999 and February 2001, 1033 southern elephant seals were anaesthetised intravenously (McMahon and others 2000) as part of a long-term demographic study of a declining population on Macquarie Island (Hindell and others 1994). They were immobilised for safe handling while

their body condition was assessed. Some individuals were fitted with tags for studies of their behaviour at sea (Slip and others 1994, Hindell and others 2000, Irvine and others 2000), stomach lavaged to determine the composition of their diet (Green and Burton 1993, Slip 1995), and had a sample of blubber taken to determine its composition (Iverson and others 1997). The seals ranged in age from 15 months to seven years when they were captured and were in six different phases of their life cycle. Typically, they were caught and immobilised as they returned for breeding, moulting and mid-year haul outs after foraging trips, and some were also caught at the end of these haul outs before they returned to sea

They were caught by hand, had a canvas bag placed over their head, and were injected intravenously, via the lower lumbar region of the extradural vein, with a combined 1:1 mixture of tiletamine and zolazepam (Telazol; Fort Dodge), as described by McMahon and others (2000). The combined dose rates of tiletamine and zolazepam varied between 0.3 and 0.7 mg/kg bodyweight, depending on the level and duration of anaesthesia required. Initial doses were given after their bodyweight had been estimated on the basis of previous experience by field personnel and, after they had been sedated, each seal was weighed to the nearest kg, so that the exact dose rates could be calculated. The restraint and disturbance to the seals was kept to a minimum. The drug induction and recovery times were recorded, together with any periods of apnoea. The induction time (seconds) was defined as the time from the injection of the anaesthetic until the seal failed to respond to head patting and ceased struggling (McMahon and others 2000). The recovery time (minutes) was defined as the time from when the seal was sedated until it could raise its head and maintain it in the raised position (Woods and others 1994). A seal was considered to be apnoeic when it had stopped breathing for longer than five minutes (Slip and Woods 1996). The seals' breathing and capillary refill of the gums were monitored constantly (Woods and others 1994). An endotracheal tube, oxygen, and the respiratory stimulant doxapram, were available in the event of prolonged apnoea or poor capillary refill, but were never required.

After they had been anaesthetised, the seals were measured to the nearest 10 mm and morphometric measurements (m) were made to calculate indices of body shape and volume (Fig 1). Relative measures of blubber thickness were obtained by using an ultrasound backfat depth system (A-Scan Plus; Sis-Pro). The total seal volume (TSV) was calculated by modifying the method of Gales and Burton (1987b). This method

assumed that the seals were circular in cross-section, with the diameter at any cross-section being equal to their height at that section. It was assumed that all the blubber lay in the hypodermis and over the whole body and that the flippers contained insignificant amounts of subcutaneous fat (Bryden 1967). The seal's body was divided into seven sections (C1 to C7) (Fig 1), with the head and hips to the base of the tail forming cones and the rest of the body sections forming truncated cones. The girth measurements (G) were used as basal circumferences in the calculation of the full and truncated cones; G1 and G6 were used for the circumferences of the head and hips to the end of the tail cones (C1 and C7). The height (K) of these cones was calculated by subtracting the G1 to G6 measurement from the standard length and then halving this value. For the other body sections, it was assumed that the larger of the girths formed the base of the cone, and the height (H) of the cone was either half the distance between G1 and G3 for the anterior cones C2 and C3 (Ha), or onethird the distance between G3 and G6 for the posterior cones C4, C5 and C6 (Hp). The volume of the anterior part of the seal, sections C1 and C3, was calculated as follows:

$$V_{anterior} = \sum_{i=2}^{3} \left(\frac{\pi H_a}{3} \left(\left(\frac{G_i}{2\pi} \right)^2 + \left(\frac{G_i G_{i+1}}{\pi^2} \right) + \left(\frac{G_{i+1}}{2\pi} \right)^2 \right) \right) + \frac{\pi K}{3} \left(\frac{G_1}{2\pi} \right)^2$$

where G_i is the girth at the base of the cone and G_{i+1} is the girth at the top of the cone. The volume of the posterior part of the seal, sections C4 to C7, was calculated as follows:

$$V_{posterior} = \sum_{i=3}^{6} \left(\frac{\pi H_{p}}{3} \left(\left(\frac{G_{i}}{2\pi} \right)^{2} + \left(\frac{G_{i}G_{i+1}}{\pi^{2}} \right) + \left(\frac{G_{i+1}}{2\pi} \right)^{2} \right) \right) + \frac{\pi K}{3} \left(\frac{G_{6}}{2\pi} \right)^{2}$$

The two volumes were summed to calculate the TSV. Blubber thickness (b) was measured at each of the girth sites along the seal's dorsal side (Gales and Burton 1987b, Slip and others 1992). The blubber depth was assumed to be constant around the seal's girth and was subtracted from the radii of the cones to calculate the volume of the inner cone (Fig 1). The total volume of these cones was calculated as for the TSV, and was assumed to represent the total volume of lean tissue (TLV). The total blubber volume (TBV) was calculated by subtracting TLV from TSV.

Body condition was assessed by two methods. First, the proportion of blubber by volume (BV) was calculated as TBV/TSV. However, BV could be calculated for only the 553 seals

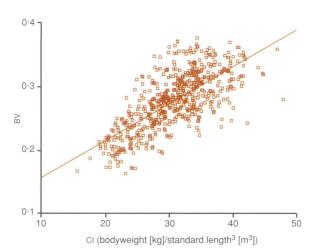


FIG 2: Relationship between the proportion of blubber by volume (BV) and the body condition index (CI) ($r^2=0.493$, P<0.001)

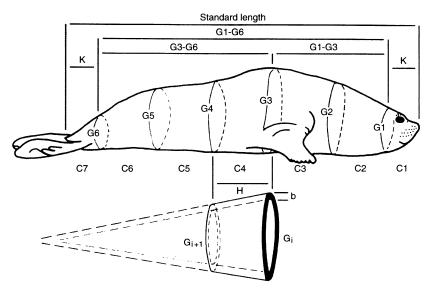


FIG 1: Morphometric measurements used in the calculation of blubber volume. G1 to G6 represent the circumference of the body at sites 1 to 6, creating seven cones, comprising five truncated and two terminal cones (C1 to C7). The depth of blubber (b) was assessed at the dorsal surface of all six sites and used to calculate total blubber volume. H Height of cones C2 to C6, K Height of cones C1 and C7, G_i Girth at the base of the cone, G_{i+1} Girth at the top of the cone

from which six measurements of dorsal blubber thickness were taken. For the other seals, an index of body shape was used as a substitute index of condition, for which the condition index (CI) was calculated as bodyweight (kg) + standard length³ (m³) (Virgl and Messier 1993, Chabot 1994). Although BV was considered to be a better measurement of body condition, CI was found to be a reasonable index of blubber content (r²=0·493, P<0·001) (Fig 2). For the analyses for which there were few seals with estimates of BV, a second analysis was included in which all the seals with an estimate of CI were used.

Anaesthetic induction times and recovery times were analysed in relation to the dose rate, sex, age and condition of the seals. The recovery times and weighted recovery times (see below) were \log_e transformed to normalise the data and to homogenise the variances among factor levels (Sokal and Rohlf 1981). These transformed variables were used for all subsequent analyses. The bodyweight-specific dose rate accounted for more than 50 per cent of the variation in recovery time (r^2 =0·502, P<0·001) (Fig 3), so for all subsequent analyses the recovery times were weighted by the reciprocal of the dose rate. Henceforth, 'weighted recovery time' refers to this weighted measure of recovery time. This weighting also

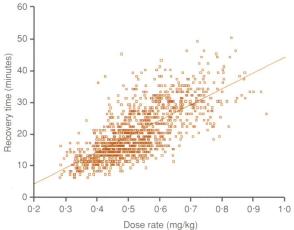


FIG 3: Relationship between weightspecific dose rates and recovery time (r²=0·502, P<0·001)

TABLE 1: Mean (se) values of the body condition of seals of different ages and at the

Age (years)	BV Beginning of haul out	End of haul out	CI Beginning of haul out	End of haul out
1	0.318 (0.005)	0.227 (0.006)	36-1 (0-30)	29-8 (0-81)
2	0.321 (0.003)	0.252 (0.007)	35-1 (0-40)	26·7 (0·55)
3	0.303 (0.003)	0.242 (0.006)	32.9 (0.27)	25.5 (0.49)
4	0.283 (0.003)	0.256 (0.011)	32.2 (0.52)	25·3 (1·03)
5	0.273 (0.011)	<u>`</u> '	30.9 (0.53)	24·3 (0·61)
6	0-261 (0-002)	0.224 (0.005)	30·7 (O·47)	22·3 (0·81)
7	0.276 (0.003)	0.218 (0.004)	31·0 (0·56)	22·6 (0·37)

BV Proportion of blubber by volume, CI Index of body shape, that is, bodyweight (kg)/standard length³ (m³)

controls for the size of the seal and the level of immobilisation required. Age was calculated as the age of the seal to the nearest month when it was captured.

To test for sex differences in blubber volume, the data from the seals aged one to four years, that is, the seals from which data on both males and females were collected, were combined and a one-way, unbalanced, general factorial general linear model (GLM) was run (SPSS for Windows version 7.5.1; SPSS Inc.) of the form:

Proportion blubber by volume = sex + error

in which the sex term was designated as a random term, and the error term represents the unexplained variation in the response variable, the proportion of blubber by volume.

Unbalanced, general factorial GLMs were used to describe the relationships between age, sex and condition of the times of induction and recovery. The models tested the effect of all the main factors (age, sex and condition) and their two- and three-way interactions on the response variables, induction time and weighted recovery time. For example, the saturated model was:

and all the models considered included all the combinations of terms presented in the saturated model. Here, age was designated as a fixed term, sex as a random term, and cond (condition) as a covariate. An asterisk between factors indicates an interaction term.

There was no a priori reason to assume a single model to describe the contribution of the terms and their interactions to the response variables, so a form of model selection with Akaike's Information Criteria (AIC) was used to select the most parsimonious model (Lebreton and others 1992); AIC was calculated as follows:

$$AIC = 2LL + 2p$$

where LL is –2log likelihood calculated from the mean squared error (MSE) and the type III sum of squares error (SSE) for each general factorial GLM, and p is the number of model parameters. The AIC values were then ranked on a relative scale from 0 (poor) to 1 (good), that is, the model weight. Thus, the 'best-fit' model had the lowest AIC value and the highest model weight (Lebreton and others 1992).

Furthermore, a repeated-measures GLM was used to test for within-seal responses to the model terms. In these analyses, only CI was used as a measure of condition because of the smaller numbers of animals (n=553) for which the index of BV was calculated. The results are reported as means (se).

Differences in weighted recovery times between the different haul out sessions, that is, start of breeding, start of moult and start of mid-year, were tested by using a GLM of the form:

 $log_e(recovery rate) = haul out period + error$

in which haul out period was designated as a random class term

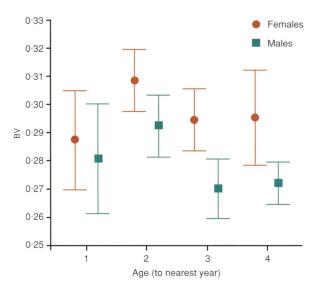


FIG 4: Proportion of blubber by volume (BV) for male and female seals between one and four years of age

The relationship between the number of captures experienced by a seal during the course of the study to its recovery time at the final capture was also investigated. Few animals were caught more than five times, and a Monte Carlo randomisation (Manly 1997) was therefore used to examine the relationship between the weighted recovery time and number of previous captures of the 211 seals which were captured more than once. This method involved randomising the order of the weighted recovery times relative to the capture rates 10,000 times, and assessing the squared difference between the recovery rate and number of captures per individual. For each iteration, the sum of the squared difference (ss_{rand}) between these values was compared with the sum derived from the true order (ss_{true}). The number of times ss_{rand} was less than ss_{true} in the 10,000 iterations gave the probability $(P_{10,000})$ that the relationship, if any, was due to chance. This method was applied because of the heterogeneity of the variances among seals captured different numbers of times. An examination of the data indicated that a loge transformation was incapable of homogenising the variances or normalising the weighted recovery times. Thus, the distribution-free randomisation approach to examine the effects of repeated captures and periods of anaesthesia provided results which did not violate parametric modelling assumptions (Manly 1997).

RESULTS

The mean (se) dose rate was 0.533 (0.003) mg/kg, resulting in a mean induction time of 36.5 (0.39) seconds and a mean duration of 20.6 (0.26) minutes in the 1033 southern elephant seals. There were no fatalities or periods of apnoea and all the seals were later re-sighted at Macquarie Island. None of the adult females caught during the breeding season deserted their pup after they had recovered from anaesthesia.

On average, the female seals had a greater proportion of blubber than males between one and four years of age (P<0.001) (Fig 4). In terms of both CL and BV, there were significant changes in body condition between when the seals hauled out and when they returned to sea at the end of the haul out (Table 1).

Induction times

The model selection by AIC failed to detect a single, best-fit model for induction times, and none of the models explained

TABLE 2: Mean (se) induction times (seconds) for seals of different ages at the beginning and end of periods of haul out

Age (years)	Beginning of haul out	End of haul out
1	35.7 (0.70)	38-1 (1-51)
2	35.5 (0.79)	37·8 (1·22)
3	34.5 (0.76)	36·4 (1·03)
4	35·5 (1·01)	38·9 (1·92)
5	36.8 (2.23)	36-0 (6-34)
6	39-8 (1-18)	39.5 (2.70)
7	41·3 (1·74)	45.0 (2.72)

a significant amount of variation in induction time. Thus, there were no detectable effects of sex, age or condition on induction times (Fig 5, Table 2).

Recovery times

Using CI, model selection by AIC failed to detect a single, best-fit model. However, the model including CI alone had the highest AIC weight (0·137). The model showed a weak, but significantly negative effect of condition (P<0·001), but accounted for only 4·4 per cent of the variation in recovery time. This model showed that seals in poorer condition had longer recovery times. The next best model was for age and CI and had an AIC weight of 0·121; it showed that age had a slight positive influence on recovery time (P<0·002) (Fig 5).

Using BV, the most parsimonious model included BV alone and had an AIC weight of 0.138; there was a small, negative effect on recovery times (P=0.048). The next best model using BV was for sex only, with an AIC weight of 0.129, but this effect can be described adequately by the difference in proportions of blubber between the sexes (Fig 4).

The most parsimonious models for both condition measures failed to identify an effect of sex on recovery time. For all the indices of body condition there was a weak, but significantly negative effect of condition, such that seals in poorer condition remained anaesthetised for longer periods. There was also a slight positive effect of age, with older animals taking longer to recover when using the model containing the age and CI terms. There was no effect of the time of year when the seals hauled out on their recovery time that could not be explained by differences in body condition.

A repeated measures GLM was used on individual seals which were caught at the start and again at the end of a haul out period to test the effects of sex, age and condition on recovery times more effectively by removing individual variation (Table 3). This analysis demonstrated a weak, but significant effect of body condition (P<0.008), but no effect of

TABLE 3: Mean (se) recovery time of seals of different ages at the beginning and end of periods of haul out

Age (years)	Recovery time (Beginning of haul out		Weighted recovery ti Beginning of haul out	
1	16.0 (0.41)	23.5 (1.10)	33-3 (0-59)	39-3 (1-30)
2	20-1 (0-61)	28·1 (1·00)	36·7 (O·84)	42·0 (1·20)
3	20-0 (0-58)	26.3 (0.93)	39·2 (O·96)	45·1 (1·40)
4	21.3 (0.90)	18·2 (1·31)	40·8 (1·35)	35·4 (2·83)
5	15·2 (O·52)	16·0 (1·08)	33·3 (1·20)	31.0 (4.56)
6	22.3 (0.99)	21·2 (1·66)	42·2 (1·72)	31.0 (2.22)
7	22.5 (0.97)	31.7 (1.53)	41-3 (1-42)	45.8 (1.66)

age. However, when the differences in condition among the individual seals were considered, the effect of condition was lost and the effect of age became significant (P<0.026).

Differences between haul outs for different age groups

There were significant differences between the different haul out periods in weighted recovery time (P<0.001) (Fig 6). Seals caught at the start of the annual moult recovered more quickly than seals caught during the breeding or mid-year haul outs. However, since only adult females were present during the breeding season and weighted recovery time varies with age, this relationship was re-examined for adult females (comparing breeding and moult haul outs) and for juvenile seals aged less than five years (comparing moult and mid-year haul outs). There was no difference in the weighted recovery time of adult females at the start of the breeding haul outs and at the start of the moult haul out. However, juvenile seals caught at the beginning of the moult still recovered more quickly (P<0.001), even though they were in poorer condition (P=0.011) than those caught at the start of the mid-year haul out.

Number of previous captures

There was a small but significant increase in weighted recovery time with the number of times a seal was caught and immobilised (Fig 7). The Monte Carlo randomisation showed a significant positive relationship ($P_{10,000} = 0.0206$).

DISCUSSION

Past studies have shown that the size or bodyweight of a seal is the most important consideration when planning its anaesthesia, and that the use of a weight-specific dose provides a safe and reliable procedure for the capture and handling of elephant seals (Baker and others 1988, Gales 1989,

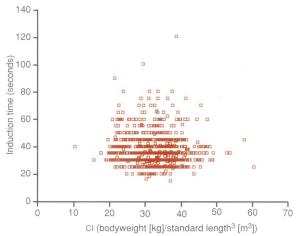


FIG 5: Induction time as a function of body condition index (CI)

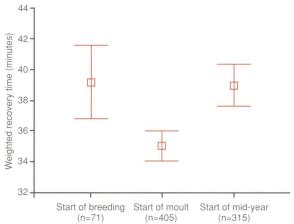


FIG 6: Recovery rate (95 per cent confidence intervals) at the beginning of each haul out period

Haul out period

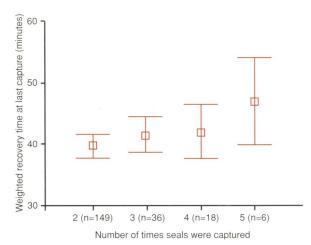


FIG 7: Recovery rate and 95 per cent confidence intervals relative to the number of captures experienced by individual seals

Woods and others 1989, 1994, Slip and Woods 1996, McMahon and others 2000). Many authors have described variation between species of seals and between individuals of one species during anaesthesia, the reasons for which are poorly understood, and which may carry an increased risk and an increased likelihood of side effects. The method of drug administration accounts for most of the variation observed during anaesthesia. Slip and Woods (1996) showed that the variation can be reduced by administering drugs intravenously rather than intramuscularly. The intravenous administration of smaller doses of tiletamine and zolazepam resulted in more rapid induction and a shorter period of sedation, thus avoiding many of the potential problems associated with the sedation of pinnipeds, such as apnoea, hypothermia and death (Slip and Woods 1996, McMahon and others 2000).

Induction times were unaffected by body condition, most probably as a result of the rapid uptake of the anaesthetic when it was injected intravenously. The single bolus of anaesthetic would be transported rapidly to the brain, and the concentration required to induce sedation would be reached quickly (Woods and others 1999). There is also little opportunity for the drug to be redistributed to the muscles and blubber, so that variations in body composition would be expected to have little effect.

McMahon and others (2000) showed that the recovery time was related positively to the dose rate ($r^2=0.245$). However, little has been done to explain the wide variation in the response of seals to anaesthesia, and few authors have suggested possible causes (Trillmich and Weisner 1979, Loughlin and Spraker 1989, Woods and others 1989). However, they have all ascribed some of the variation to the physiological demands associated with breeding and moulting, to the absorption rates of the anaesthetic agents, and to the seal's level of activity before it was anaesthetised. McMahon and others (2000) found a weak but negative relationship between the seal's condition and the duration of the period of anaesthesia. In the present study, there was a greater range in the age and condition of the seals, and in the dose rates administered to them and, hence, greater statistical power, and the same relationship was observed. For the repeated-measures models, the differences between the results for within- and between-individual responses to anaesthesia were to be expected because first, all the seals were losing blubber while they were hauled out, and secondly, the rate of blubber loss appears to vary with age. However, although there was a relationship between body condition and duration of anaesthesia, even the precisely measured indices of body condition still accounted for only a small proportion of the variations observed. Most of the variation (after accounting for dose rates) still resulted from differences among individual seals.

An intravenous injection results in a drug reaching the brain more quickly than after an intravenous injection, and the recovery times are therefore quicker (Rowland and Tozer 1995a). Tiletamine and zolazepam are lipophilic and accumulate more rapidly in fatty than in lean tissue (Rowland and Tozer 1995b). When the drugs are redistributed, some of them are absorbed into the fatty tissue. As a result, fatter seals would be expected to have less free drug available to prolong the anaesthesia, and to have shorter recovery times. This knowledge can be applied to reduce dose rates further and predict the seals' responses to weight-specific dose rates.

Two studies have associated physiological 'stresses' during the breeding season to longer durations of anaesthesia (Trillmich and Weisner 1979, Woods and others 1989). If so, one would expect that the responses to anaesthesia would be different at different times of the year, for example, when the seals are breeding or moulting. However, no such differences were observed between postpartum and premoult females. There was evidence to suggest that the time of haul out (moulting versus the mid-year) had an effect on the weighted recovery times of juvenile seals, despite the fact that they were on average, fatter during the mid-year haul out. This effect was most probably due to differences in the metabolic rates of the juvenile seals, which may have a lower metabolic rate during the mid-year haul out than during the moult.

Older seals remained anaesthetised longer than younger seals for the same relative dose of drug administered, possibly because as seals mature, their proportion of blubber decreases, as does their metabolic rate (Boily and Lavigne 1997), resulting in a slower metabolism of the anaesthetic agent. For pinnipeds in general, metabolic rate decreases as the juvenile approaches breeding age. It is thought that this change coincides with the increase in their capacity to dive deep and with their ability to fast while ashore for breeding and moulting (Kooyman 1985, Guppy and others 1986, Hochachka and Guppy 1987, Hindell and others 1991, Boily 1996, Boily and Lavigne 1997). Another possibility is that the rate of clearance of the benzocyclohexamines by the liver decreases with age, as has been observed in human beings (Rowland and Tozer 1995a). This hepatic metabolism may also be depressed by reduced enzyme production in the liver, leaving more free drug to affect the depth and duration of anaesthesia. However, this phenomenon has yet to be tested quantitatively in animals.

In individual seals there was a positive relationship between the number of times they were captured and their weighted recovery time; however, this result must be treated cautiously because it is based on very few data for animals caught more than four times. Nonetheless, there are two plausible explanations for this observation. First, the serially captured animals were becoming habituated to being restrained, and were becoming less stressed; they may have had a lower heart rate and the vascular distribution of the drugs may have been slower than in seals which were more stressed when they were captured. Secondly, the seals caught more often may not have been as efficient at breaking down the drug because the pathways used in the metabolism of the drugs may have been affected. A similar relationship has been described in cats tranquilised with diazepam (Valium; Roche Products) (Levy and others 1994, Center and others 1996).

In some phocid species, simple inhalation anaesthesia has been used as an effective alternative to the intravenous injections of drugs (Kusagaya and Sato 2001); however, intravenous anaesthesia still has advantages over this method. The practicalities of weighing and measuring elephant seals would make it impossible to administer the anaesthetic approximately every two minutes (Kusagaya and Sato 2001) to attain the durations of anaesthesia required.

The results of this study have established a relationship between the condition and age of elephant seals and their duration of anaesthesia. Weight-specific doses of tiletamine and zolazepam injected intravenously, combined with information on the age and body condition of a seal, have made it possible to reduce dose rates and to tailor the desired level and duration of immobilisation. By using this method, the risk of apnoea and other side effects associated with anaesthesia in large, wild seals was virtually eliminated.

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