

Appendix I:
PROTOCOL FOR FUTURE DRUG EXPERIMENTATION DURING
IMMOBILISATION OF SOUTHERN ELEPHANT SEALS AT MARION
ISLAND

PJN de Bruyn & MN Bester

Introduction

Many aspects of wildlife research require intensive work that necessitates physical contact with the study animals. These animals are thus often restrained through the use of certain immobilizing chemical compounds. Chemical restraint of any wild animal should be dependent on a drug or drug combination that adheres to the following principles, namely, potency (drug volume needs to be kept low), rapid induction, safety for the animal, minimum of side effects and is reversible (Meltzer et al. 2004). The difficulties with finding such a drug for any one species are numerous in that the drugs themselves vary in efficacy and effect, the species that are targeted may respond differently to each drug, and each species differs in their response to each drug. It is therefore necessary to investigate/test different drugs and drug combinations for each species, in order to identify the best form of chemical immobilization for the wellbeing of the study animal.

Pinnipeds are one such group of wild animals where researchers require the use of chemical compounds to immobilize their study animals for either superficial, non-painful work (such as attachment of tags) or more intrusive, often painful work (such as surgery) (Lynch et al. 1999). Some research has been done to test the adequacy of various drugs for use with numerous pinniped species (Gales 1989). One species that has enjoyed a large amount of interdisciplinary scientific treatment is the southern elephant seal (*Mirounga leonina*). A great deal of research has in recent years been aimed at demographic aspects of the southern elephant seal populations throughout the Southern Ocean. At Marion Island (as with many other locations), the focus has in particular been on changes in population sizes (Bester and Wilkinson 1994; Pistorius et al. 1999a), and causal factors

contributing to these changes, both proximate and ultimate (Bester and Wilkinson 1994; Pistorius et al. 1999b). The Marion Island elephant seal population has declined by 83% since 1951 (Laws 1994) and by 37.2% between 1986 and 1994 at an annual rate of change of 5.8%, which was linear over the period (Pistorius et al. 1999a). Pistorius et al. (1999b) suggested low adult female survival due to food limitation, to be the proximate cause of the decline in the Marion Island population. In order to investigate these and other questions, seals often need to be weighed (see Chapter 2 – de Bruyn et al. 2009) at various ages, satellite or other tracking devices deployed on the animals, or dietary or physiological studies need to be done. Since these animals are large and potentially dangerous research subjects, such studies require the seals to be immobilized and/or anaesthetised temporarily. McMahon et al. (2005, 2008) showed that such handling and intensive research does not affect either short or long-term survival of elephant seals.

Elephant seals of all ages have been routinely immobilized in the past (Gales 1989). Several experimental procedures have been conducted to assess the usefulness of various drugs for southern elephant seal immobilization, sedation, anaesthesia, and mitigation and reversal of side effects while the animal is drugged (e.g. Ling and Nicholls 1963; Gales and Burton 1987; Bester 1988; Erickson and Bester 1993; Woods et al. 1994, 1995, 1996a, 1996b; Ramdohr et al. 2001; Field et al. 2002). Ketamine-hydrochloride appears to have been among the more successful drugs for the immobilization of southern elephant seals for a number of decades (Ryding 1982; Gales 1989; Woods et al. 1996a). The primary difficulty with the use of ketamine is that this drug is irreversible. This can be potentially problematic for two main reasons where seals are concerned, 1) the animal may return to the sea before induction takes place and can then become immobile in the water, 2) seals are adapted physiologically to live in extreme environments (Kooyman et al. 1981) and when sedated/immobilized often suffer side effects such as apnoea or hypothermia (Gales 1989; Woods et al. 1994) which, if any particular case is severe under the influence of ketamine, cannot be treated by reversal. Most workers have attempted to circumvent this problem by

combining the “knockdown” properties of ketamine with reversible sedatives such as diazepam, midazolam and xylazine (Gales and Burton 1987; Woods et al. 1994, 1995). The α -2 adrenergic receptor agonist, medetomidine, has only been tested once to our knowledge on 12 adult female southern elephant seals (Woods et al. 1996) although the ketamine-medetomidine cocktail has been extensively and successfully used on terrestrial herbivores (e.g. reindeer; Ryeng et al. 2001), carnivores (e.g. mink, Arnemo and Sølvi 1992) and primates (e.g. red howler monkeys, Vié et al. 1998). Other drugs such as tiletamine and zolazepam have been used with success and are preferred over ketamine by some, particularly with intravenous administering (Woods et al. 1994; McMahon et al. 2000). Opioid drugs have been tested for elephant seal immobilization and have rendered erratic results (Ramdohr et al. 2001), however, a key benefit (when the results are not erratic) in using these drugs is their fast induction and complete and quick reversal, both properties treasured by wildlife biologists in the field. Neuromuscular blockers such as succinylcholine chloride proved to have fatal consequences when used to immobilise some southern elephant seals (Ling et al. 1967).

Aims and Objectives

At present we use only ketamine on Marion Island with no added sedatives and while results have been consistent, our aims in this study are particularly focussed on finding at least partially reversible alternatives:

- 1) Combine the α -2 agonist medetomidine and the opioid antagonist butorphanol with ketamine at various ratios, increasing the medetomidine/butorphanol to appropriate levels in order to minimise reversal time using atipamezol while still attaining adequate immobilization for non-painful work. The medetomidine/ketamine cocktail has been used on adult female southern elephant seals but some problems of vomiting and respiratory depression were seen (Woods et al. 1995). The addition of butorphanol to this mixture has shown a decreased tendency for vomiting and respiratory depression in other species (e.g. red wolves, Larsen et al. 2002).
- 2) Assess the usefulness of the opioid drug, thiafentanil (A3080), for the immobilization of elephant seals. The small volume and fast induction

of this drug, and complete reversal with naltrexone may provide a suitable method of immobilization. This relatively new drug has rendered good results in the few tests done on terrestrial species (e.g. nyala antelope, Cooper et al. 2005). This drug has not been tested on any pinnipeds to our knowledge although related morphine derivatives such as etorphine have delivered variable results (Ramdohr et al. 2001).

- 3) Determine the efficacy of intramuscular administering of nalorphine or naltrexone after ketamine immobilization to test for any reversal effect, since recent work with terrestrial animals have given indications of limited reversal of cyclohexylamines by these opioid antagonists (pers. comm. D. Meltzer). Concurrently to test if these drugs assist in alleviation of respiratory depression if administered intramuscularly, intravenously or sublingually. The more commonly used respiratory stimulant doxapram has been shown to have limited, or no effect in alleviating apnoea in southern elephant seals although it did speed recovery times after ketamine immobilisation (Woods et al. 1996b).

Materials and Methods

The study area

Southern elephant seals will be primarily immobilized for purposes of weighing and deployment of satellite and other tracking devices, mostly along the eastern coast of sub-Antarctic Marion Island (46°54'S, 37°45'E) (Fig. 1.2 in Chapter 1). It is primarily during these sessions that the drug effects will be tested.

Field techniques and Data collection

The desired dose will be administered intramuscularly in the dorsal hip area using a handheld syringe or a modified version of the remote injection method (tubing between needle and syringe [Bester 1988 as described by Ryding 1982]). A 100-150 mm, 13–18 gauge needle with sealed tip and lateral hole will be used for injection. Where seals are logistically impossible to weigh, masses will be estimated using the photogrammetric technique initially developed in this thesis (see Chapter 2 – de Bruyn et al. 2009).

Accurate morphological measurements of standard length and maximum girth length will be taken.

An attempt will be made to sample at least 10 different animals per aimed experiment (see Aims and Objectives section - this Appendix) (i.e. total $n \geq 30$). An effort will be made to obtain an even representation of sex – and age classes of seals for the experiments. Each experiment will require certain monitoring procedures to ascertain the response to each drug dose/mixture. The responses monitored will include heart rate, respiratory rate, head response, palpebral response, fore-flipper withdrawal response, hind-flipper response and if possible rectal temperature. If possible a pulse-oximeter will be attached to the immobilized animals' tongue to detect changes in blood oxygen saturation and pressure/pulse. The level of anaesthesia/sedation will be scored according to the eight-point scale developed by McMahon et al. (2000) or the six-point scale developed by Woods et al. (1996) at constant time intervals. The dosages utilized will depend upon initial visual pre-anaesthetic assessment, but will follow the guidelines documented by other authors as far as possible. Where tests on variable doses of the same drug/drug mixtures are to be performed, an effort will be made to keep age – and sex variables as constant as possible. Animals that are visibly excited, injured or in poor condition will not be included in the experiments.

For objective 1:

A small sample of animals will initially be immobilised with only ketamine (2-4 mg/kg) as a control procedure. Thereafter initial drug doses will follow Woods et al. (1996) for ketamine (1.5-2.0 mg/kg) in combination with medetomidine (0.01-0.027 mg/kg). Butorphanol will be added to this mixture at similar doses to medetomidine, i.e. 1:1. Atipamezole will be administered at approximately 0.4 mg/kg to reverse the medetomidine. The total dose of medetomidine will not exceed 10 mg/animal (possibly excluding very large bulls) as this has been shown to cause severe heart rate depression and apnoea in adult females (Woods et al. 1996). Further experiments will follow whence the dose of medetomidine and butorphanol will increase while the dose of ketamine decreases, in order to evaluate whether higher doses of the

reversible drug medetomidine could attain adequate immobilisation for non-painful work.

For objective 2:

The dosages of etorphine used by Ramdohr et al. (2001) will allow a guideline of initial doses of thiafentanil since the two drugs have similar potency (1 : 1.5 – etorphine : thiafentanil) although the induction time of the latter is shorter (Meltzer et al. 2004). Therefore our initial dose of thiafentanil will be at approximately 0.0009 mg/kg and reversal with naltrexone at 25-40 times the total thiafentanil dose.

For objective 3:

Initial ketamine doses will vary between 2-5 mg/kg in order to attain heavy immobilization (score = 5, Woods et al. 1996). Thereafter the naltrexone/naloxone will be administered at doses ranging from 0.01 to 0.02 mg/kg (naloxone) and 0.05 to 0.15 mg/kg (naltrexone), but keeping the dose and frequency of administration constant per individual to assess recovery time and level accurately (Higgins et al. 2002).

In so doing, we intend to provide data that could alleviate the constraints imposed by the non-reversible use of ketamine-hydrochloride in isolation.

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Appendix II:
**COHORT AND TAG-SITE SPECIFIC TAG-LOSS RATES IN MARK-
RECAPTURE STUDIES: A SOUTHERN ELEPHANT SEAL CAUTIONARY
CASE**

WC Oosthuizen, PJN de Bruyn, MN Bester & M Girondot

Abstract

Marker-loss is a common feature of mark-recapture studies and important as it may bias parameter estimation. A slight alteration in tag-site of double tagged southern elephant seals (*Mirounga leonina*) marked at Marion Island from 1983 – 2005 in an ongoing mark-recapture program, had important consequences for tag-loss. We calculated age-specific tag-retention rates and cumulative tag-retention probabilities using a maximum likelihood model selection approach in the software application TAG_LOSS 3.2.0. Under the tag-loss independence assumption, double tag-loss of inner interdigital webbing tags (IIT; 17 cohorts) remained below 1% in the first 5 years and increased monotonically as seals aged, with higher tag-loss in males. Lifetime cumulative IIT tag-loss was 11.9 % for females and 18.4 % for males, and equivalent for all cohorts. Changing the tag-site to the outer interdigital webbing (OIT; 6 cohorts) resulted in increased and cohort dependent tag-loss, although the variation (mean \pm 95% CI) in cumulative tag-loss probabilities never exceeded 5.3% between cohorts at similar age. Although different studies may homogenize techniques, we advocate the importance of dataset-specific assessment of tag-loss rates to ensure greatest confidence in population parameters obtained from mark-recapture experiments. Permanent marking should be implemented where feasible.

Introduction

Mark-recapture studies are frequently used by ecologists and wildlife managers to estimate demographic parameters of wildlife populations. These parameters provide insight into population processes and allow implementation of appropriate management policies. Mark-recapture data analyses have largely been based on Cormack-Jolly-Seber (CJS) parameterizations (Lebreton et al. 1992). However, violations of the

assumptions of the CJS model can severely bias parameter estimates (Arnason and Mills 1981; McDonald et al. 2003). Fundamental assumptions are that marks do not affect future survival, are not lost or missed during resights (Seber 1982), and are correctly identified (Stevick et al. 2001). However, these assumptions are potentially violated by many marking techniques and may be inherent to all long term tagging regimes. Marker-loss and marker-induced mortality result in population size overestimates through negatively biased survival rates, since individuals that have lost their marks cannot be identified anymore and appear 'dead' to the observer (Arnason and Mills 1981; Pollock et al. 1990; McDonald et al. 2003). Additionally, if recaptured, such individuals appear to be immigrants, biasing recruitment rates upward (Cowen and Schwarz 2006). Time or group dependent marker-loss may additionally result in non-uniform biases in survival estimates through heterogeneous recapture probabilities among groups or over time (Pollock et al. 2001). Therefore, knowledge of violations of fundamental assumptions such as marker-loss is crucial to obtain robust information of life-history data that allows informed decision making (McMahon and White 2009).

The extensive use of non-permanent markers to identify individuals in mark–recapture studies for investigations of life-history, demographics, dispersal, growth and behavior makes estimation of marker-loss rates widely applicable to numerous ecological research disciplines and wildlife authorities across a wide range of species. An increasing number of studies incorporate quantitative estimates of marker-loss rates in conjunction with analytical advances in this field (Barrowman and Meyers 1996; Conn et al. 2004; Cowen and Schwarz 2006). These include tag-loss in invertebrates (Kneib and Huggler 2001), terrestrial and marine vertebrates, (Stobo and Horne 1994; Diefenbach and Alt 1998; Adam and Kirkwood 2001; Casale et al. 2007) and neckband failure (Johnson et al. 1995) and band loss in birds (Spendelov et al. 1994). Marker-loss is usually estimated by double marking individuals and approximating marker-loss by following subjects through time and noting whether one or two marks are retained. In the absence of a permanent mark (in addition to the two temporary markers), marker-loss independence is

assumed, where the probability of losing the second marker is independent of the probability of losing the first marker. This assumption is difficult to test because permanent marking is not easily accomplished. However, in cases where permanent marks facilitated assessment of the assumption, assuming independence has been shown to underestimate tag-loss (e.g., Diefenbach and Alt 1998; Bradshaw et al. 2000; McMahon and White 2009). However, aside from the potential errors associated with the independence assumption, researchers should attempt to quantify inconsistent marker-loss over time or variation therein between groups of marked animals (Spendelow et al. 1994). Failure to account for marker-loss differences between juveniles and adults for example, may lead to erroneous conclusions about the importance of juvenile and adult survival as population growth determinants. Similarly, a false indication of processes such as density dependent survival may be indicated when marker-loss differs according to the population density, physical environment or tagger proficiency at specific colonies or study sites.

Many pinniped species are ideal mark-recapture study subjects due to their ubiquitous terrestrial phases, generally high site fidelity and thus their temporal and spatial accessibility to researchers. Individuals are usually marked by double tagging in the connective tissue of the interdigital webbing of each hind flipper in true seals (Phocidae) or in the trailing edge of both front flippers in fur seals and sea lions (Otariidae) (Erickson et al. 1993). However, in contrast to permanent markings such as branding or tattoos, tag-loss can be substantial (e.g., Stobo and Horne 1994; McMahon and White 2009) and if left uncorrected may severely bias survival estimates. On sub-Antarctic Marion Island (46°54'S, 37°45'E), southern elephant seals *Mirounga leonina* L. have been subject to a 25-year (1983 – 2008) ongoing mark-recapture study. The mark-recapture program forms the foundation of investigations into life-history, demography, dispersal and philopatry of southern elephant seals at Marion Island (e.g., Bester 1989; Pistorius et al. 1999, 2004; Kirkman et al. 2003, 2004; McMahon and Burton 2005; McMahon et al. 2005). Two previous studies have estimated tag-loss for this population, to incorporate tag-loss adjustments into demographic data. Wilkinson and Bester (1997) used a ratio method to calculate tag-loss over the period 1983 to 1990 and Pistorius et al.

(2000) improved on this method, estimating linear tag-loss (1983 – 1993) based on the time at liberty of tagged seals in a maximum likelihood framework. Age and sex specific tag-loss rates from Pistorius et al. (2000) were subsequently used to correct mark-recapture survival estimates of southern elephant seals at Marion Island.

The physical placement of markers can be central to the accuracy of estimates gained from mark-recapture experiments. Incorrect tag placement can result in increased mortality (Kneib and Huggler 2001) or reduced apparent survival as a consequence of increased tag-loss. Tag placement has nonetheless received limited attention in studies beyond fisheries management (e.g., Brennan et al. 2007). Limpus (1992), however, evaluated tag placement in turtles, where tag-loss was higher in the more distal tagging positions on the front flipper. The hind flipper tag-site used to mark elephant seals at Marion Island changed in 2000 from the inner interdigital webbing (between digits two and three or three and four; 1983 – 1999) to the upper, outer interdigital webbing of the hind flippers (between digits one and two; 2000 – 2008). The tag-site adjustment aimed at improving tag visibility for resighting, because the tags placed in the inner interdigital webbing are often obscured by the flipper digits when animals are hauled out on land (WCO, PJNdB, MNB personal obs.). The effect of tag-site on tag-loss has not been quantified here or directly for other pinnipeds and such an adjustment may have important consequences for demographic analyses.

We estimate and compare tag-loss rates for 17 cohorts of southern elephant seals double tagged in the inner interdigital webbing (inner interdigital tags: IIT), and six cohorts double tagged in the upper, outer interdigital webbing (outer interdigital tags: OIT). Variation in tag-loss due to seal age and sex, tagging protocol (tag-site, i.e., IIT and OIT) and tagging proficiency by different personnel (resulting in cohort specific tag-loss rates) are considered.

Methods

Mark-resight framework

Data from cohorts of southern elephant seals born on Marion Island from 1983 - 2005 and resighted up to April 2008 were used. During each breeding season, virtually all recently weaned southern elephant seal pups born on Marion Island were sexed and double tagged in the interdigital webbing of the hind flippers with identical, uniquely numbered, color-coded plastic Dal 008 Jumbotags[®] (Dalton Supplies Ltd., Henley-on-Thames, United Kingdom). These two-piece self-piercing tags are applied with an applicator, and have favorable retention rates as compared to other tag types (Testa and Rothery 1992). The male component of all tags was positioned on the outer surface of the flipper (i.e., the point of this component protrudes from the inner surface). From 1983 to 1999 tags were applied to the center of either of the two inner sections of interdigital webbing of the hind flipper (webbing between digits two and three or three and four; IIT). From 2000 – 2005, tags were applied to the center of the upper interdigital webbing of the hind flipper (between the first and second digit; OIT). At tagging, one third of the tag extended past the trailing edge of the interdigital webbing. During all years tags were applied by two dedicated field personnel, all of whom were trained by MNB (except from 1986 to 1988 when up to six pairs of trained field personnel tagged pups). Further details regarding the tagging procedure appear in Wilkinson and Bester (1997) and Chapter 3 (de Bruyn et al. 2008).

During the entire study period (1983 - 2008), the resighting effort remained constant and included all beaches along a 51.9km coastline where southern elephant seals can haul out, except during the early period (1983 - 1988) when no resights were made during winter (mid-April – mid-August). During the molting and winter periods (mid-November - mid-August), all beaches were searched for tagged seals every ten days, but in the breeding season (mid-August – mid-November) this was done on a seven-day cycle to allow for increased seal numbers and harem density. For every tagged seal that was resighted, the date and locality of the sighting, tag color combination and three-digit number, number of tags remaining (one or two), and the sex of the seal (if identified) were recorded. We assume similar and accurate resight

rates of single and double tags owing to the high and constant resight effort by trained personnel on Marion Island, where both flippers of each animal was always inspected for the presence of tags. This was done to prevent different reporting rates for single or double tags which may bias estimates (Adam and Kirkwood 2001). The haulout pattern of elephant seals (Kirkman et al. 2003, 2004) allowed for confirmation of recorded tag data with subsequent resights, often several times over a season. Shed tags were not replaced.

Estimation of tag-loss

We estimated tag-loss using a maximum likelihood method for individually identifiable mark-resight study subjects in the software application TAG_LOSS (Version 3.2.0; http://www.esu.u-psud.fr/epc/conservation/Tag_Loss/Tag_Loss.html) as presented by Rivalan et al. (2005). This program provides quasi-continuous tag-loss probabilities and incorporates assessment of different trends in tag-loss rates over time. Quasi-continuous tag-loss probabilities based on exact time at liberty remove bias associated with pooled observations (Xiao 1996; Diefenbach and Alt 1998). Model functions described the time-dependent daily probability of tag-loss $p(t)$ (see Rivalan et al. 2005). Model selection was based on Akaike's Information Criteria (AIC), where the model with the smallest AIC value provided the most parsimonious fit (Burnham and Anderson 1998). AIC weights (w_i) provided the relative support for each model. Pups initially tagged with only one tag, seals of unknown sex and pups double tagged, but never resighted, were excluded from analyses. Tagged seals that were not resighted most likely died during their first pelagic foraging trip (60 – 65% first year mortality; McMahon et al. 1999; Pistorius and Bester 2002) or emigrated from the study area (Bester 1989; MNB unpublished data). Double tag-loss within the first year is suggested to be minimal (see Results and Discussion).

The annual haulout cycle of elephant seals, combined with the continuous high resight effort in the current study permits calculation of reliable estimates of tag time at liberty, in contrast to studies where few resights are possible (e.g., leatherback sea turtles *Dermodochelys coriacea*, Rivalan et al. [2005] and loggerhead sea turtles *Caretta caretta*, Casale et al.

[2007]). We constructed individual capture histories for all individuals tagged on day zero (at weaning), and subsequently resighted ($n = 7849$, cohort range $n = 228 - 479$). Three sets of capture histories were considered, following Rivalan et al. (2005): (1) The individual was resighted with two tags intact for the duration of its presence in the study, to estimate the minimum number of days without tag-loss (N_{22} ; $n = 6786$). (2) We initially observed the individual with two remaining tags (N_{22}), but subsequently with only one tag intact (N_{21}). This capture history calculates both the minimum number of days without tag-loss (N_{22}) and the interval length (mean = 212 days) during which one tag was lost (N_{21} ; $n = 952$). (3) Subsequent to double tagging, we only observed the individual with one tag intact for the duration of its presence in the study (N_{21} ; $n = 111$). Because permanent marks were absent, we could not reliably identify animals that had lost both tags (N_{20} or N_{10} ; known $n = 10$), and therefore did not consider such cases. This necessitated the assumption of tag-loss independence, where the probability of losing one tag did not affect the probability of losing the second tag.

We tested three different trends in daily tag-loss over time: constant rate, monotonic increase/decrease and a two-step function (e.g., rapid initial tag-loss, followed by a decreased continuous loss pattern and then a further increase or decrease with varying slope; this function can adjust to many different daily tag-loss rate modalities over time). Tag-loss patterns were assessed separately for each sex and cohort and also for cohorts and sexes pooled over time. We used the best fitting trend to test for a sex, cohort and tag-site effect on tag-loss. For the sex and cohort variables, we compared the AIC model fit for separate sex/cohort models, compared to a single model grouping sexes/cohorts. Constructing models that separated IIT and OIT and subsequently evaluating model fit assessed tag-site variability. TAG_LOSS 3.2.0 converted parameter estimates from the best model to daily tag-loss probabilities, age-specific tag-loss probabilities and cumulative tag-loss rates (Rivalan et al. 2005). Age-specific tag-loss is the conditional probability that a tag is lost during one year among the tags that were still present at the beginning of that year. Standard errors of parameters were calculated by the square-root of the inverse of the Fisher information matrix (Abt and Welch

1998). The proportion of animals retaining at least one tag is presented as identification probabilities (1 - cumulative tag-loss probability). Identification probabilities therefore represent the proportion of individuals still identifiable (retaining either one or two tag/s) for each age group.

Results

Tag-loss was best described by a monotonic increase of tag-loss rate with time for both male and female groups (AIC w_i (monotonic) = 1) (Table 1). Although several cohorts showed support for the constant model or two-step function, few of these cohorts showed substantial support against the monotonic increasing model. Males from cohort 17 deviated most from the monotonic trend ($\Delta\text{AIC} = 17.01$), while only one other cohort (cohort 10 females) with a ΔAIC value > 4 ($\Delta\text{AIC} = 7.95$) indicated some support for an alternate model. Both these groups received support for the two-step function model, indicating initial high tag-loss soon after tagging, but leveling off over time.

Tag-site had a significant effect on tag-loss rates, with cohort and sex differences in tag-loss also dependent on the tag-site. Tag-loss did not differ between cohorts with IIT, and the model with a general estimate calculated over all 17 cohorts received the most AIC support (Table 2). In contrast, strong support for variable tag-loss rate between cohorts was found for OIT (cohorts grouped, $\Delta\text{AIC}_{18-23} = 190.03$) (Fig. 1). Cohort dependent OIT tag-loss was not unexpected, as field observations indicated that cohort 23 showed uncharacteristically high initial tag-loss associated with suboptimal (shallow - tag extends more than one third past the trailing edge of the flipper webbing) tagging. In addition, we suspected that cohorts 19 and 20 might show different tag-loss rates that could lead to inter-cohort variation as these tags were sometimes tagged too deep (tag not extending by one third past the trailing edge of the flipper webbing). We subsequently removed cohorts 19, 20 and 23 from the OIT model, to test whether cohort variability existed among the remaining three cohorts. Cohort specific variability persisted (cohorts grouped $\Delta\text{AIC}_{18, 21, 22} = 59.34$, cohorts separate $\Delta\text{AIC} = 0$), and we continued analyses considering all OIT cohorts separately. Separate sex models, with

increased tag-loss in males, improved model fit for IIT (Table 3). Conversely, a single model for males and females combined was sufficient to describe tag-loss for OIT cohorts.

Table 1. Model selection results for tests of southern elephant seal tag-loss trend over time, at Marion Island.

Cohort	Tag-site	Males				Females			
		Model of change of tag-loss rate				Model of change of tag-loss rate			
		Constant	Monotonic	direction	2 steps	Constant	Monotonic	direction	2 steps
1	IIT	119.52	112.51	+	116.50	171.85	169.18	+	173.17
2	IIT	120.48	117.53	+	121.41	200.60	201.12	+	205.12
3	IIT	278.71	273.20	+	277.20	300.49	291.10	+	295.10
4	IIT	170.28	173.42	-	177.44	257.17	254.50	+	258.50
5	IIT	227.20	224.72	+	228.70	221.33	216.38	+	219.98
6	IIT	245.02	237.28	+	240.87	258.25	256.04	+	259.28
7	IIT	258.11	257.76	+	261.74	247.33	251.28	+	255.26
8	IIT	239.07	234.04	+	238.04	229.30	230.17	+	234.16
9	IIT	214.48	213.38	+	217.38	327.08	322.64	+	326.64
10	IIT	222.12	220.40	-	219.05^a	400.89	404.89	=	396.93^b
11	IIT	248.59	248.45	+	252.38	288.61	285.10	+	284.12
12	IIT	134.55	134.05	+	138.05	278.11	277.29	+	280.93
13	IIT	153.06	155.56	+	156.82	177.04	175.69	+	179.17
14	IIT	162.68	158.19	+	156.42^b	378.33	367.55	+	371.54
15	IIT	219.67	199.89	+	203.88	215.89	205.05	+	209.05
16	IIT	175.11	178.12	+	181.36	318.64	319.25	+	323.20
17	IIT	167.68	171.69	=	154.68^a	210.59	205.46	-	202.98^c
18	OIT	224.71	199.69	+	203.69	243.94	218.45	+	221.40
19	OIT	189.52	180.91	+	184.91	305.19	289.70	+	293.70
20	OIT	268.39	265.37	+	269.37	324.18	325.70	+	328.43
21	OIT	345.78	339.70	+	343.70	483.32	486.05	+	490.04
22	OIT	89.64	90.72	+	93.55	167.01	169.35	+	171.97
23	OIT	378.02	378.56	-	381.46	393.78	397.78	-	401.28
Total AIC		4852.39	4765.10		4818.59	6398.92	6319.70		6381.97
Δ AIC		87.25	0.00		53.45	79.22	0.00		62.27
AIC w_i		0.00	1.00		0.00	0.00	1.00		0.00

^a High rate just after tagging and it becomes null after 1500 days

^b High rate just after tagging

^c Rate becomes null after 2000 days

Age-specific tag-loss rates (Fig. 1) were derived for cohorts 1 – 17 (IIT; grouped), and cohorts 18 – 23 (OIT; separately). IIT showed low initial tag-loss rates that increased monotonically over time. Age-specific tag-loss of adult females followed a slight convex curve, with tag-loss increasing at a low rate for adult females above age five. Cumulative IIT tag-loss rates (double tag-loss; Fig. 2) were less than 1% up to age five for both sexes. Tag-loss in males increased more as they aged when compared with females, although older age classes are represented by fewer males than females (e.g., 13 males and 106 females above age 12), leading to greater confidence in female retention rates to this age. Near the maximum life expectancy, close to 81% of males, and 88% of females were expected to remain identifiable under the tag-loss independence assumption. Age-specific tag-loss rates for OIT were generally higher than those of IIT and predominantly increased over time, apart from cohort 23 in which tag-loss declined after high initial loss. Cumulative tag-loss rates increased from cohort 18 to 23 (Fig. 2), with the exception of cohort 22 (cumulative tag-loss cohort 18 < 19 < 22 < 20 < 21 < 23), *i.e.*, apart from cohort 22, tag-loss increased as resight time decreased. The variation in cumulative tag-loss rates between individual OIT cohorts and between grouped OIT and grouped IIT cohorts was relatively small, despite model-supported separation. The maximum difference observed between IIT and OIT cohorts was for cohort 23, indicating a 5.2% lower identification probability at age 2 as compared to cohorts with IIT. When cohort 23 was not considered, OIT inter-cohort variation did not differ by more than 0.16% \pm 0.16 (mean \pm 95%CI) for ages 0-2. Cohort variation for OIT increased as animals aged (age 3-7), but 95% confidence intervals never spanned more than 5.3% for any age (Fig. 3).

Table 2. Model performance based on AIC for cohort dependent and independent tag-loss rates in southern elephant seals at Marion Island.

Cohort grouping	Males	Δ AIC	AIC w_i
1,...,23	4765.14	25.59	0.00
1-17, 18,...,23	4739.55	0.00	1.00
1,...,17, 18-23	4860.33	120.78	0.00
1-17, 18-23	4834.75	95.19	0.00
1-23	4910.24	170.68	0.00

Cohort grouping	Females	Δ AIC	AIC w_i
1,...,23	6319.70	0.00	0.93
1-17, 18,...,23	6324.90	5.19	0.07
1,...,17, 18-23	6421.61	101.91	0.00
1-17, 18-23	6426.81	107.10	0.00
1-23	6511.57	191.87	0.00

Cohort grouping	M+F	Δ AIC	AIC w_i
1,...,23	11084.85	12.06	0.00
1-17, 18,...,23	11072.78	0.00	1.00
1,...,17, 18-23	11274.87	202.09	0.00
1-17, 18-23	11262.81	190.03	0.00
1-23	11431.83	359.05	0.00

Table 3. Model selection for a sex-effect, dependent on tag-site, for southern elephant seal tag-loss from Marion Island. Males in cohorts 1-17 showed higher tag-loss rates than females.

Cohorts	Sexes separated	Sexes grouped	Δ AIC	AIC w_i
1-17	7722.49	7730.82	8.333	0.98
18-23	3539.07	3531.99	7.073	0.97

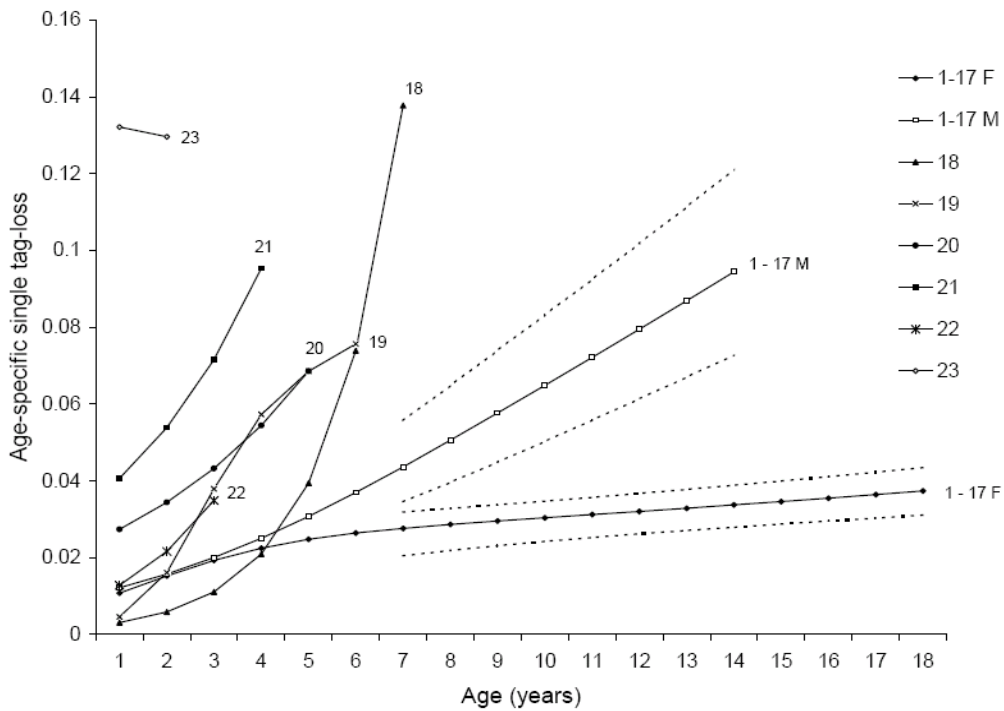


Fig. 1. Maximum likelihood functions for age-specific single tag-loss rates over time. Inner interdigital tags (IIT; cohorts 1-17) are represented by two general functions for males and females. Standard errors (tag-loss probability \pm 2SE) are presented for IIT tags above age 7.

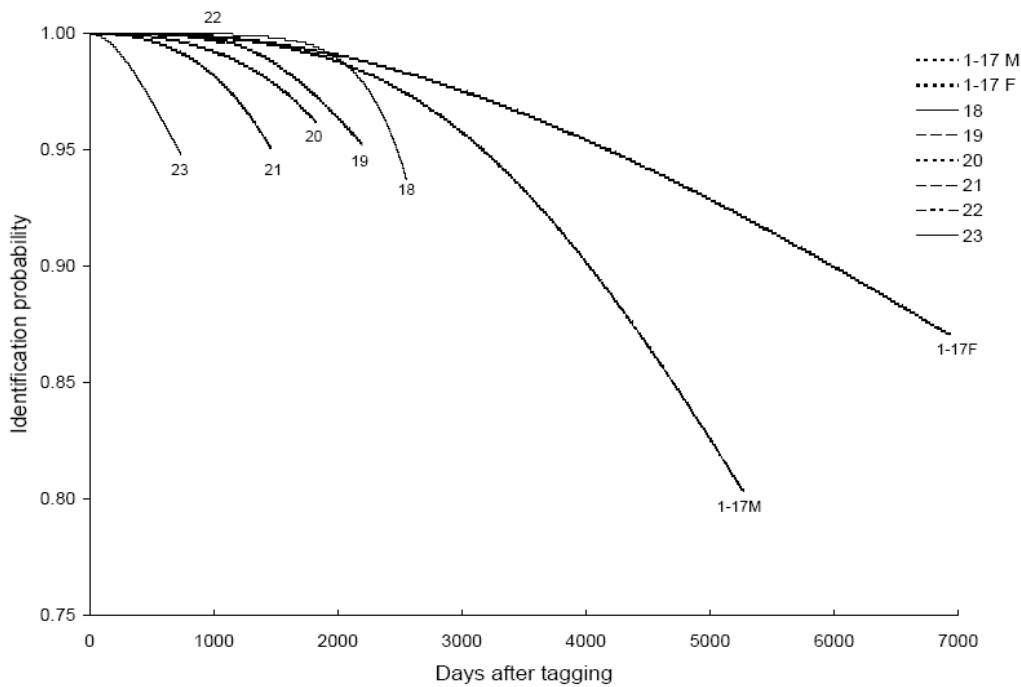


Fig. 2. Identification probabilities of double tagged southern elephant seal cohorts at Marion Island. An individual is rendered unidentifiable when both tags are lost.

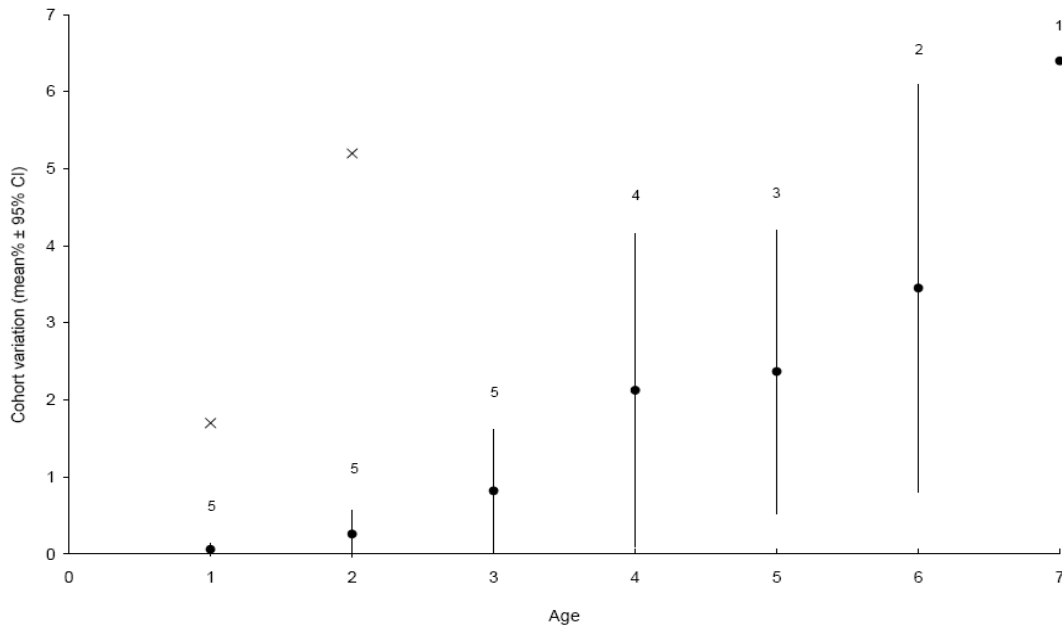


Fig. 3. Variation in southern elephant seal tag-loss rates between cohorts 18 – 23, double tagged in the outer interdigital webbing of the hind flipper (OIT). Points represent the mean tag-loss rate over cohorts 18 - 22, with numerical values indicating the available sample size (number of cohorts). Cohort 23 (x at ages 1 and 2) is not included in the calculation of mean cohort differences.

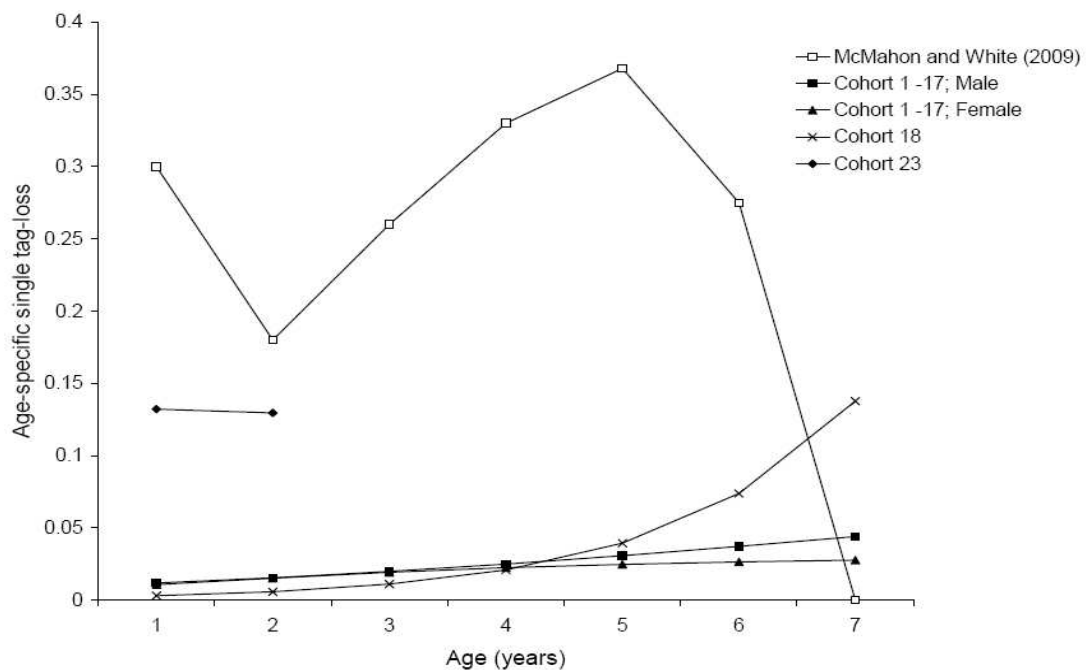


Fig. 4. Age-specific single tag-loss (N_{21}) in southern elephant seals from Macquarie Island (McMahon and White 2009) and Marion Island (data shown for IIT [cohort 1-17 males and females] and OIT cohorts 18 [longest OIT time-series] and 23 [cohort with greatest tag-loss measured]). Tag-transition from two to one tag is accurately measured at both locations, and not influenced by the independence of tag-loss assumption

Discussion

Tag-loss for both IIT and OIT cohorts of southern elephant seals is best described by a monotonic increase over time, although the pattern of tag-loss did deviate in some cohorts. Because all tags were applied to recently weaned individuals (~23 - 30 days old; see Chapter 3 – de Bruyn et al. 2008), tag-time and seal age cannot be differentiated. The increase in tag-loss rates over time is assumed to be generally related to an increase in seal- and flipper size and webbing thickness, rather than tag failure due to breakage (Pistorius et al. 2000). Extreme sexual dimorphism is present in adult elephant seals and the higher tag-loss rates in males tagged with IIT reflect this. Age-specific IIT tag-loss probabilities of males increased relative to those of females from age 3 to 4 onwards. Sexual body size differences become evident at age 3, whereas extreme sexual dimorphism manifests after male elephant seals undergo a secondary growth spurt between ages 4 and 6 (Laws 1984). In contrast, model selection favored a combined male and female model for OIT cohorts. The lack of a sex effect for the OIT cohorts may possibly be explained by a lack of statistical power, due to fewer years post-tagging (maximum = 7 years) to detect such effects. For IIT, model selected support for different sex models was only present when all cohorts were grouped, and not for individual cohorts (results not shown). Therefore, there may be insufficient statistical power to detect sex differences when using individual cohorts, or only a few combined cohorts (OIT, $n = 6$). However, seals tagged in the outer webbing of the hind flipper at Macquarie Island, similarly did not show sex differences in tag-loss (McMahon and White 2009). This may indicate that the influence of flipper size may be important for IIT-loss, but less so for tags applied to the outer webbing (OIT).

Cumulative tag-loss rates for both IIT and OIT are low in comparison with other phocid studies (e.g., Stobo and Horne 1994, McMahon and White 2009) and with previous assessments for a shorter time-series of this same mark-recapture program (Pistorius et al. 2000). Cumulative tag-loss rates from birth to age 15 computed by Pistorius et al. (2000) were 35% and 17% for males and females respectively, which is higher than those reported here. Pistorius et al. (2000) included 11 cohorts (1983 – 1993), with resighting data

up to 1998. Our data included an additional 6 cohorts with IIT, and a further 10 years of resighting data. The added cohorts, and more importantly the longer resighting time period would modify the tag-loss estimation. This may be especially important for the adult age categories (e.g., 13 males over age 12 in this study vs. only 2 males in Pistorius et al. 2000). The different analytical approach between this study and Pistorius et al. (2000) furthermore resulted in these differences. The Pistorius et al. (2000) function constrained tag-loss to be a straight line, with the younger age categories (the region in the graph that has the more weight in the likelihood output, because more individuals are included) guiding the initial slope of the linear trend line. Age-specific IIT tag-loss (this study) did not fit the linear function exactly, but were rather slightly concave up (males) or convex down (females), leading to lower cumulative loss.

Outer interdigital tags were shed at a higher rate than IIT. In aquatic mammals, body and fin/flipper undulations pass water posterior along the body with increasing force, creating body-bound vorticity. This vorticity is transmitted along the body to the trailing edge of the fin, or flipper, where it is shed in a thrust jet (Fish et al. 2008). In swimming phocids, the center of the flipper is in addition more rigid than the flipper extremities (Fish et al. 1988). While swimming, OIT may thus be subjected to increased drag and movement, leading to increased tag-loss. On land, IIT are usually protected from the substrate and environmental variables (e.g., ultraviolet light) because the tag is typically enclosed in the folds of the resting flipper (between adjacent flipper digits). OIT visibly protrude more, improving tag visibility for resighting and reducing disturbance to seals during tag resighting (the objective of the change in tag-placement in this study program). However, OIT are therefore also more likely to make contact with the substrate, plausibly leading to more abrasion and snagging, and potential loss from the flipper. Additionally, OIT exposed to more UV radiation than the enclosed IIT may become weakened over time and result in increased tag breakage, although we rarely observed such breakage.

McMahon and White (2009) compared tag-loss at Macquarie Island to Marion Island, and suggested that tag placement may affect tag-loss as tag-loss was much greater at Macquarie Island (OIT) than at Marion Island (IIT, from Pistorius et al. 2000). Our results support their interpretation. However, the large difference in tag-loss between the OIT from Macquarie Island (McMahon and White 2009) and Marion Island (this study) indicate that other factors are also important. We suggest that the timing of tagging is a critical determinant in life-time retention rates. At Marion Island, pups are always tagged post-weaning (age ~ 23 – 30 days), while pups on Macquarie Island were tagged at birth. McMahon and White (2009) rejected this hypothesis, as they calculated tag-loss from weaning (when pups were branded) only; and not birth. However, it is probable that flipper damage from tagging at birth may lead to increased tag-loss post-weaning and not necessarily only during the pre-weaning period as assumed by McMahon and White (2009). For example, newborn pups may be more immuno-compromised than weaned pups. Tag-loss due to immune reaction may, however, only occur in a few months time, rather than within the 3 – 4 week pre-weaning age. Weaned pups, in contrast, may be less susceptible to infection of the tag-site than newborn pups, leading to lower tag-loss. We recommend that pups are tagged at weaning only and suggest an alternative method to identify pre-weaned pups (see Chapter 3 – de Bruyn et al. 2008). On Marion Island, pre-weaned pups are marked with temporary Supersmall[®] tags (Dalton Supplies Ltd., Henley-on-Thames, U.K.) that are designed to minimize injury to the tag-site on the pre-weaned pup's delicate hind flipper. These tags are applied to the inner interdigital webbing of the hind flipper. At weaning (when hind flippers are sturdier), pups are tagged in the outer webbing of the hind flipper with the more robust Jumbotags[®]. This tagging protocol allows identification of pre-weaned pups (see Chapter 3 – de Bruyn et al. 2008), while postponing marking with long-lasting tags to a period when; a) the pup flipper is stronger; b) the pup is generally in better condition and has greater immunity than at birth; and c) tagging of weaned pups occurs well outside the harem (without the need to return the pup to the harem) that enable the precise placing of tags, which is required for low loss rates. The correlation between tag-loss, tag-site and time of tagging between Marion Island and Macquarie Island may

further be influenced by different tag types used (Dal 008 Jumbotags[®] [Marion] vs. Supertag[®] Size 1 [Macquarie]; Dalton Supplies Ltd., Henley-on-Thames, United Kingdom) and possibly the practice of cutting and filing the tag pin on Macquarie Island. If this procedure puts any strain on the tag itself (e.g., holding the tag to file it down, while the pup tries to move the flipper), tag-loss may be increased due to damage/enlargement of the tag-site during this procedure.

Differences in tagging proficiency of personnel may lead to heterogeneous tag-loss. Tag-loss in South African fur seals *Arctocephalus pusillus pusillus*, for example, varied between 6.8 - 33.8% for different tagging personnel (Shaughnessy 1994). Stobo and Horne (1993) reported cohort variation in tag-loss among year-old grey seals *Halichoerus grypus* which varied between 7.2 – 18.8%. In the present study, interannual variation in tagging proficiency may result in cohort specific tag-loss rates, despite stringent efforts to maintain constant tagging technique. Wilkinson and Bester (1997) compared tag-loss of one-year old elephant seals at Marion Island, and found no significant variation amongst 8 cohorts. This trend continued for all age groups in the 17 IIT cohorts, and no important variation in tag-loss between cohorts was evident. Conversely, OIT tag-loss varied by cohort. Field observation indicated that tag placement in three of the OIT cohorts were marginally suboptimal. It thus appears as if tag placement for OIT needs to be even more exact (~ 5mm) as compared with IIT, as only a slight misplacement of the tags (OIT) at tagging may render tags attached too deep (cohorts 19 and 20) or too shallow (cohort 23). Tags applied too deep in the webbing may increase infection and tissue necrosis of the tag-site as the flippers grow while tags applied too shallow are more likely to tear out of the trailing edge of the flipper. The outer webbing of the hind flipper also provides a smaller surface area in which to place a tag as compared to the neighboring inner webbings between digits two and three and three and four. This may therefore explain the cohort dependence observed (and the suggested need for more precise tag placement) for OIT, but not IIT.

Although tag location on the outer interdigital webbing may lead to a lower retention rate, it is assumed that loss of tags should occur randomly among cohorts when they are similarly tagged. Yet, even when we removed cohorts that we a priori believed might have been responsible for the observed bias, the remaining three cohorts still could not be grouped. The estimates of OIT are hampered by a lower sample size (6 cohorts) for a maximum time at liberty of 7 years, compared to 17 cohorts at a maximum time at liberty of over 24 years for IIT cohorts. As such, OIT resight data exists for only five cohorts of adult females, and two cohorts of adult males (based on the age at maturity for this species at Marion Island; Kirkman et al. 2003). Tag-loss probabilities are based on the time at liberty of tags: the time from application of the tag, to the last occasion that the individual was seen with two tags (N_{22}) or first seen with one tag (N_{21}). However, cohorts have different maximum times at liberty (maximum resight time decreases by one year for successive cohorts) which could influence parameter estimates, especially when the time at liberty is relatively short. This potential bias is apparent in the current study, significantly more so for OIT with shorter time at liberty and few sexually mature cohorts. For example, cohort 23 tags have a maximum time at liberty of around 900 days, in comparison with the first OIT cohort (18) at liberty for more than 2700 days. Inter-cohort variation should be more pronounced when the data are sparse, and a few random cases of tag-loss could potentially help to drive cohort specific differences. For five of the six OIT cohorts (cohort 22 being the exception), tag-loss estimates increased as absolute cohort age was younger. Therefore, we attribute at least part of the cohort specific tag-loss rates observed for OIT as an effect of fewer days to maximum time at liberty (specifically the time-span of N_{22}). We suspect that as more data becomes available, tag-loss of OIT should become more homogenous among cohorts, although at higher rates than the IIT cohorts.

The cohort effect observed for OIT necessitates caution when deriving life-history parameter estimates (such as age-specific survival rates) from these cohorts. Because animals from separate OIT cohorts lose tags at different rates, the proportion of marked animals in the population at any period will differ between cohorts, and not represent a homogenous group

with respect to tag-loss. An increase in tag-loss rate between IIT and OIT per se does not present considerable analytical drawbacks as survival rate corrections may simply be structured to represent the two different tag-sites. Extreme cohort variability, however, would negate the implementation of survival models structured at a population level (rather than a cohort level) as population level survival trends might be influenced by cohort specific tag-loss rates.

To demonstrate the differences in tag-loss rates between OIT cohorts, we considered an arbitrary cohort of 500 double tagged pups, a good approximation of the number of pups born annually on Marion Island in recent years (MNB unpublished data). On average, at age 2, cumulative tag-loss will render all individuals identifiable for three of the OIT cohorts (and all of the IIT cohorts). Tag-loss in cohort 20 and 21 will result in double tag-loss in 2 and 4 pups respectively, while tag-loss in cohort 23 rendered 26 pups unidentifiable. Therefore, in a mark-recapture framework, within the first two years of life, only cohort 23 had biologically meaningful variation in tag-loss rates – which may lead to a decrease of 5% in apparent survival rate [$1 - (26/500) = 0.948$]. The maximum variance in OIT loss was present at age 4, where one (cohort 18) or 25 (cohort 21) pups out of 500 are expected to lose both tags, leading to a 4.8% decrease in apparent survival rate of cohort 21 at this age. Survivorship is chiefly responsible for population regulation at Marion Island (see Pistorius et al. 1999, 2004), and indeed in many mammal populations where immigration and emigration is limited; accurate estimates of these rates are therefore invaluable. In this case, apparent survival rates for juveniles should not be biased by tag-loss even if IIT and OIT cohorts were combined in survival analysis (excluding cohort 23). However, variation in tag-loss between cohorts can negatively bias estimates for sub-adult male and adult age classes if such cohorts are pooled.

Assumption of independent tag-loss

The results presented assume tag-loss independence. Violations of the independence assumption will result in a greater proportion of animals retaining two or losing two tags, with few animals retaining only one tag. This

would result in an overestimate in tag retention rates, and negative bias in survival rates. Testing for dependence in tag-loss requires the permanent marking of study subjects. Permanent marking of southern elephant seals at Marion Island is not possible to facilitate testing of this assumption. Tattoos for example (Diefenbach and Alt 1998), although useful in a mark-recapture framework, are not realistic in our mark-resight design. Southern elephant seals have been successfully branded on Macquarie Island with no long-term influence on survival or condition (McMahon et al. 2006a). This protocol has allowed testing of the independent assumption for the period where flipper tagging and branding overlapped (McMahon and White 2009). However, branding is logistically impractical at Marion Island and this technique incorporates animal welfare concerns (Jabour Green and Bradshaw 2004) that resulted in the termination of the Macquarie Island southern elephant seal monitoring program (McMahon et al. 2006b, 2007). Passive Integrated Transponder (PIT tags) have been successfully used to mark numerous species (Gibbons and Andrews 2004) including southern elephant seals (Galimberti et al. 2000). PIT tags facilitate reliable long-term identification of elephant seals up to adult age-classes for both sexes (F. Galimberti personal communication). However, in contrast to external hind flipper tags which can be sighted from a distance, PIT tags require scanning each seal from a close distance (<20cm) with an electronic reader. This is often impossible to achieve, in particular for breeding females within harems, and aggregations of seals during the molt haulout (F. Galimberti personal communication). PIT tags are additionally more invasive than external tags, may itself incur tag-loss (Gibbons and Andrews 2004) and are expensive. Therefore, by comparison, double tagging with plastic tags remains the preferred choice of marking in elephant seals. Still, where PIT tags can be used in combination with double-tagging to provide an additional mark this method will be useful to improve tag-loss estimates. Previously tagged animals can in some cases be identified through scarring, and Bradshaw et al. (2000) used flipper scarring in New Zealand fur seal *Arctocephalus forsteri* pups to address dependence in tag-loss estimates. Such scarring (tag punctures in the flipper) is sometimes visible in elephant seals, but it is virtually impossible to regularly and

accurately distinguish seals that have lost both tags from untagged seals (e.g., transients/immigrants).

Several studies have provided evidence of dependent tag-loss, and the assumption of independence appears to be biologically unrealistic. Double ear tag-loss in sea otters *Enhydra lutris* (Siniff and Ralls 1991) and black bears *Ursus americanus* (Diefenbach and Alt 1998), and flipper tag-loss in fur seal pups (Bradshaw et al. 2000) and leatherback sea turtles (Rivalan et al. 2005) were all greater than expected under the independence assumption. Similar results for elephant seals from Macquarie Island have been shown (McMahon and White 2009), and the expectation is therefore that tag-loss at Marion Island would also be dependent. Dependent tag-loss varies according to individual attributes, leading to heterogeneous tag-loss probabilities (McMahon and White 2009). In black bears, individual behavior such as fighting, mother-pup grooming or playing probably influences tag-loss (Diefenbach and Alt 1998). In fur seal pups, mechanical abrasion is thought to induce tag-loss, which is likely influenced by substrate, pup behavior and condition (Bradshaw et al. 2000). Dependent tag-loss in leatherback sea turtles is probably related to individual immunity, as the majority of tags are lost as a result of tissue necrosis. Individuals prone to infection may therefore be more likely to lose the second tag if the first tag was already lost (Rivalan et al. 2005). Tag-loss in elephant seals at Macquarie Island seems to be more dependent on pup wean mass. Lower wean mass concurrent with lowered immuno-competence is suggested to result in a greater incidence of dependent tag-loss, while larger pups generally exhibit independent tag-loss (McMahon and White 2009). To what extent observations from different species or different marking protocols can be used to infer dependent tag-loss in individual elephant seals at Marion Island is unsure. Even species-specific comparison is intricate due to the large difference in age-specific single tag-loss rates between Marion Island (0.0 - 0.14, this study) and Macquarie Island (0.0 – 0.364, McMahon and White 2009). Ideally, because the degree of tag-loss heterogeneity varies among study species, physical environment and tagging protocol, with the bias associated with assuming tag-loss

independence reliant on the magnitude of tag-loss, dataset-specific assessment of this assumption will be of greatest value.

Pistorius et al. (2000) expected a low degree of bias caused by dependent tag-loss for the Marion Island tagging regime owing to; a) an observed increase in tag-loss rate over time instead of an apparent decline (see Xiao et al. 1999); b) low absolute tag-loss rates resulting in modest bias; c) high resight frequency, where most animals are seen multiple times per year, reducing the probability of missing tag transition from N_{22} - N_{20} (i.e., not seeing the seal changing tag status from two to one tag), and d) the relatively high proportion of resightings of seals with one tag remaining for extended periods. Diefenbach and Alt (1998) predicted from observations of permanently marked animals that low tag-loss and frequent resightings should result in little bias from dependent tag-loss. Therefore, as the rate of tag-loss from two tags to one tag (N_{21}) is markedly lower at Marion Island than at Macquarie Island (Fig. 4), dependence of tag-loss should result in a smaller bias of survival rate at Marion Island, and results should be fairly robust in dealing with these violations, especially for IIT. However, we acknowledge the potential bias in our results and agree that the tag-loss estimates provided here will be underestimated due to partial dependence of tag-loss in individuals. In cohorts with relatively high tag-loss (cohort 23 for example), the bias in survival rates will be higher.

Conclusion

Generally, small shifts in demographic rates of large-mammal populations, especially adult female survival, are able to produce a change in population growth (Eberhardt and Siniff 1977; Pistorius et al. 1999; McMahon et al. 2005). This illustrates the importance of accurate estimation of survival rates in mark-recapture studies, as biased estimates of demographic rates may result in erroneous conclusions and implementation of inappropriate management strategies leading to failure in management objectives (Brook et al. 1997; McMahon and White 2009). Tag-loss corrections in mark-recapture studies improve accuracy by adjusting survival estimates upwards. Double tagging with high retention tags, in conjunction with permanent marking

(where possible) should be used, while frequent resight/recapture occasions should improve life-history estimates (McDonald et al. 2003). Fluctuation in tag-loss rates between years, tag-site and other variables (e.g., colony and habitat differences) must be considered. We illustrate that small changes in tagging methodology can have potentially serious consequences for life-history estimates of a population if such changes are not investigated and, ideally, quantified. While homogenizing techniques for extensive use across study locations are appropriate for comparative purposes, we advocate the importance of dataset-specific assessment of tag-loss rates to ensure greatest confidence in population parameters obtained from mark-recapture experiments.

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Supplementary material.

Table S1. Estimated cumulative age specific tag retention probabilities for elephant seals at Marion Island. Cohort independent, sex specific probabilities are given for inner interdigital tags (IIT; cohorts 1 to 17), while outer interdigital tags are separated by cohort (OIT; cohorts 18,..., 23).

Age	1-17 F	1-17 M	18	19	20	21	22	23
1	1.000	1.000	1.000	1.000	0.999	0.998	1.000	0.983
2	0.999	0.999	1.000	1.000	0.996	0.992	0.999	0.948
3	0.998	0.998	1.000	0.997	0.990	0.977	0.995	
4	0.996	0.995	0.998	0.988	0.979	0.950		
5	0.992	0.991	0.994	0.973	0.962			
6	0.988	0.984	0.979	0.952				
7	0.983	0.974	0.936					
8	0.977	0.961						
9	0.970	0.944						
10	0.962	0.924						
11	0.954	0.900						
12	0.945	0.874						
13	0.935	0.846						
14	0.925	0.816						
15	0.915							
16	0.904							
17	0.893							
18	0.881							

Appendix III:

PRELIMINARY ANALYSIS OF THE SOCIAL STRUCTURE OF KILLER WHALES, *Orcinus orca*, AT SUB-ANTARCTIC MARION ISLAND

CA Tosh, PJN de Bruyn & MN Bester

Abstract

Studies of social differentiation between populations of killer whales (*Orcinus orca*) are important due to the cosmopolitan nature of the species, both in terms of distribution and feeding habits. The following research provides preliminary findings describing the social structure of the killer whale population at sub-Antarctic Marion Island. We provide evidence for consistent, observable patterns of social interactions with animals associating and disassociating in non-random patterns. We show that the social structure of this population may follow a new pattern of association, displaying a blend of the traditional resident/transient model displayed in the Northern Hemisphere. However, we emphasize the critical need for further studies related to the sociality, biology and life history of Southern Ocean killer whales.

Introduction

Social structure in cetaceans has been extensively described and analysed (Matkin et al. 1999; Baird and Whitehead 2000; Mann et al. 2000; Gowans et al. 2001; Weinrich et al. 2006) however, it is acknowledged that intra-specific generalizations can not be made in animals of advanced social structure (Di Fiore and Rendall 1994; Costa and Fitzgerald 1996). An ideal example is the differences in diet, behaviour and social organization of “transient” and “resident” killer whales in the North Pacific (Heimlich-Boran 1988; Bigg et al. 1990; Felleman et al. 1991; Baird et al. 1992; Ford et al. 1998; Baird and Whitehead 2000). It is thus particularly valuable to document the variability in such factors for different populations. The degree of social organization and the ability of animals to “learn” from individuals within the same population has implications for long-term survival, especially in marine environments (Boran and Heimlich 1999; Whitehead 2007a). Knowledge of the social differentiation between different populations of killer whales is vital

for conservation since different strategies will apply to distinct populations that may react to similar threats in diverse ways (Whitehead et al. 2004).

Killer whale social behaviour and organization are poorly understood in the Southern Indian and Atlantic Oceans. There is even uncertainty of the number of species and degree of ecological specialization for killer whales around the Antarctic continent (Pitman and Ensor 2003). Killer whales at Marion Island have been studied at a population level and local movements have been preliminarily analysed (Condy et al. 1978, Keith et al. 2001; Pistorius et al. 2002). Killer whale sightings occur regularly throughout the year, peaking between October and December (Keith et al. 2001; Pistorius et al. 2002). This peak presence coincides with the breeding season of southern elephant seals (*Mirounga leonina*) and numerous penguin species (Condy et al. 1978; de Bruyn et al. 2007). This pattern has also been observed at Iles Crozet (Guinet 1991) and Punta Norte, Argentina (Hoelzel 1991). Scant record of the diet of Marion Island killer whales exists (Condy et al. 1978; Keith et al. 2001; Pistorius et al. 2002; Kock et al. 2006). Indications from these publications and the personal observations of numerous field personnel suggest that Marion Island killer whales include a combination of seal, penguin and fish in their diet. Killer whales at Marion Island have been observed to participate in active searching and hunting, feeding and resting activities (Condy et al. 1978). To date, no comprehensive research has been done on the social organization, distribution patterns and ranging behaviour of sub-Antarctic killer whales. Hoelzel (1991) described behavioural and social factors of the southern killer whale population around Punta Norte, Argentina (latitudinally 5° north of Marion Island, not classified as sub-Antarctic).

This study aims to reveal the potential social structures of killer whales at Marion Island and provide a basis for future studies. We suggest that continued and focussed long-term data collection would provide valuable information about killer whale sociality at Marion Island.

Methods

Marion Island (46°54'S, 37°45'E) is the larger (296 km²) of a pair of islands comprising the Prince Edward Islands group. It is situated in the Southern Indian Ocean with the nearest landmass being the Iles Crozet (950km to the east) (Fig. 1). Oceanographically, Marion Island lies in the direct route of turbulent water masses (eddies) originating in the Thomas Bain fracture zone, a component of the South West Indian Ridge (Ansorge and Lutjeharms 2005). The interplay between the Antarctic Circumpolar Current and the prominent bottom topography of the South-West Indian Ridge results in productive turbulent water masses around the Prince Edward Islands (Ansorge and Lutjeharms 2005), sustaining the numerous mammal and bird species that use Marion Island as a breeding ground.

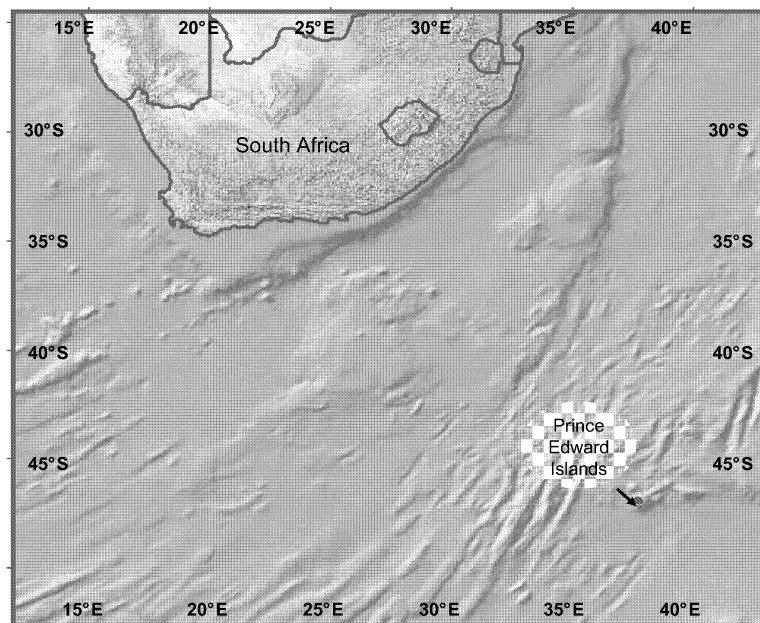


Fig. 1. The position of the Prince Edward Islands in relation to South Africa

Opportunistic land-based observations of killer whales around Marion Island were made from April 2006 - April 2007. As many individuals as possible at each sighting were photographed using digital cameras of various makes and capabilities by 25 different observers. Most observations were made within 0 – 100m of the shore and were 0 – 10 minutes in duration. The height of the observer varied depending on the locality of the sighting and varies between 0 - 15m above the subject. All identified individuals observed

in an encounter were considered to be associated. Individuals were identified based on unique dorsal fin markings, cuts or scratches on the body and any other unique markings. Each identified animal was assigned a unique name code, following the protocol established by the Dolphin Biology Research Institute (DBRI, Urian and Wells 1996), detailing its sex and unique number (e.g. MF001, **M**arion **F**emale number **001**). Subsequent identifications were only considered if there was certainty about the identity of the animal. Matching of photographs was only done by an experienced individual, using restrictive criteria. If any uncertainty existed, a second opinion was sourced and if the uncertainty persisted then the photograph was rejected. Sex and age was assigned to each animal based on dorsal fin size and shape (Leatherwood et al. 1976). For this study, three classes were identified, namely adult female, adult male and sub-adult/juvenile, the last category denoted with a **U** (unknown sex). Date and time of observation, direction of movement, observation site and group structure information (sex and age class of each individual) were also collected.

Quantitative analysis was performed in two steps. Preliminary data analysis described the number of sightings made throughout the year, the number of photographed sightings and the number of identified individuals in those sightings. The average group size, percentage of the group that was identified, the average number of males, females, sub-adults and calves associated with each identified individual are also given. A preliminary social analysis, calculating the degree of social differentiation, was first run with all identified individuals ($n=21$) and then with individuals that were identified four times or more ($n=11$). All identified animals seen three times or less were excluded from further analyses in order to lend strength to the analysis for which substantial data exists. The potential implications of these animals on pod identification are discussed. The analysis was thus limited to 11 individuals that were seen repeatedly throughout the sampling period (13 months). All encounters in which known animals occurred were used in the analyses. Associations were analysed using SOCPROG 2.3 for MATLAB 7.4 (Whitehead 2007b).

The degree of societal differentiation is displayed as the coefficient of variation (CV) of the true association indices, which is equated to the proportion of time dyads spend together. The coefficient of variation also depicts how varied the population is in terms of social structure (<0.3 representing homogenous societies; >0.5 representing well differentiated societies and >2.0 extremely differentiated societies). The strength of the analysis was displayed as an estimate of the correlation coefficient (CC) between the true association indices and the calculated association indices [0 (poor analysis) – 1 (strong analysis)]. Both the aforementioned values were calculated using likelihood methods with a resolution of integration = 0.001 (Whitehead 2007b). Standard errors were calculated using bootstrap with 100 replicates.

The basic procedure outlined in Baird and Whitehead (2000) was followed with a simple-ratio index being calculated to estimate the proportion of time each pair spent associating for all animals identified four times or more. The simple-ratio index does not overestimate associations between pairs as is the case between half-weight and twice-weight indices (Ginsberg and Young 1992). This resulted in the creation of an association matrix that is displayed as a cluster diagram (individuals are presented on the y-axis and strength of association on the x-axis) and a sociogram (individuals are arranged around a circle and associations are represented by lines of varying thickness/strength of association). Pods were assigned if animals spent 50% or more of their time associating (Bigg et al. 1990). A test for preferred and avoided associations was also run, based on the method suggested by Bedjer et al. (1998) and modified according to constraints based on testing associations within samples (Whitehead 1999, Whitehead 2007b). This test can only detect long-term preferred/avoided associations and tests the null hypothesis that there are no preferred companions between sampling periods.

Results

From start-April 2006 to end-April 2007, a total of 243 killer whale observations were made at various locations around Marion Island. Of these, 110 observations were photographed, with 56 encounters producing

identifiable individuals. The proportion of individuals identified within groups varied between sightings (Fig. 2), with 100% of individuals observed in 43% of the observations. There was a slight bias towards more individuals being observed in smaller groups (Fig. 3). Within this period 21 individuals (13 females, 5 males and 3 sub-adults) were identified according to unique characters and markings. The killer whale society at Marion Island is well differentiated ($CV_{21}=1.36\pm 0.23$; $CV_{11}= 0.80\pm 0.1$) based on a strong analysis ($CC_{21}=0.63\pm 0.05$; $CC_{11}=0.63\pm 0.11$), there is thus a strong relationship between calculated and true association indices in the present study (Whitehead 2007b), irrespective of the sample used.

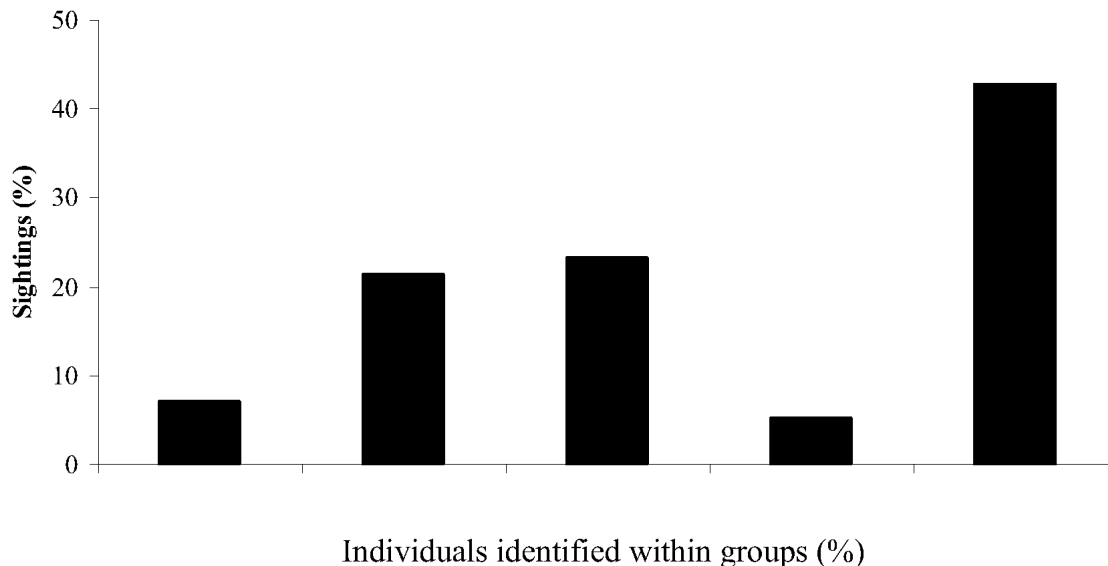


Fig. 2. The proportion of individuals identified within groups at each sighting

The average (standard deviation) group size for all identified animals was 3.43(0.87) whales, with group sizes ranging from 7 to 1 individual (Table 1). Within the groups, $74.97\% \pm 16.25\%$ of individuals were identified (Table 1). The average number animals sighted with each individual varied between 1 and 2 males, 1 and 3 females, 1 and 2 sub-adults and 1 calf (Table 1).

Table 1. Group information for all identified individuals (n=21).

ID	Pod	Number of times observed	Average group size (range)	Percentage of group identified (average \pm SD)	Number of adult σ 's observed within groups (average)	Number of adult ρ 's observed within groups (average)	Number of sub-adults observed within groups (average)	Number of calves observed within groups (average)
MF001	M1	8	4.25 (7-2)	61.79 \pm 37.71	1.00	2.38	1.00	1.00
MF014	M1	11	3.82 (7-2)	75.11 \pm 31.93		2.00	1.00	1.00
MF020	M1	6	3.83 (6-3)	96.67 \pm 8.16		2.33	1.00	1.00
MU021	M1	6	3.83 (6-3)	96.67 \pm 8.16	2.33	1.00	1.00	
MF002	M2	9	3.67 (5-2)	59.07 \pm 26.31	1.00	1.78	1.50	1.00
MM005	M2	5	4.00 (7-2)	62.38 \pm 35.25	1.00	1.75	1.33	1.00
MF003	M3	14	2.64 (6-1)	89.88 \pm 21.73	1.33	1.80	1.00	1.00
MM007	M3	5	2.80 (4-2)	61.67 \pm 26.09	1.00	1.33	1.00	
MU004	M3	12	3.25 (7-2)	88.99 \pm 21.18	1.00	2.00	1.00	1.00
MF010	-	5	2.60 (5-1)	57.33 \pm 39.33	1.00	1.80		1.50
MF012	-	5	3.80 (5-2)	53.00 \pm 13.04	1.00	2.00	1.40	1.00
MF006	-	2	4.50 (7-2)	46.43 \pm 5.05		1.00		
MF009	-	1	4.00 (4)	50.00	1.00	3.00		
MF013	-	3	2.33 (4-1)	83.33 \pm 28.87	1.00	2.00		
MF015	-	2	5.50 (7-4)	64.29 \pm 50.51	1.00	3.00	1.00	1.00
MF017	-	2	3.00 (3)	100.00		2.00	1.00	
MF018	-	2	3.00 (3)	100.00		2.00	1.00	
MM008	-	2	2.50 (4-1)	75.00 \pm 35.35	1.00	2.00		
MM011	-	3	1.67 (3-1)	77.78 \pm 38.49	1.00		2.00	
MM019	-	1	4.00 (4)	75.00	1.00	2.00	1.00	
MU016	-	2	3.00 (3)	100.00		2.00	1.00	

Table 2. Social characteristics of 11 identified killer whales used in the present study.

Individual (ID)	Sex	Number of observations	Mean level of association	Maximum level of association	Pod ID
MF001	F	8	0.34	1	M1
MF002	F	9	0.16	0.67	M2
MF003	F	14	0.28	1	M3
MF010	F	5	0.03	0.25	--
MF012	F	5	0.07	0.67	--
MF014	F	11	0.34	1	M1
MF020	F	6	0.33	1	M1
MM005	M	5	0.12	0.67	M2
MM007	M	5	0.27	0.5	M3
MU004	U	12	0.28	1	M3
MU021	U	6	0.33	1	M1

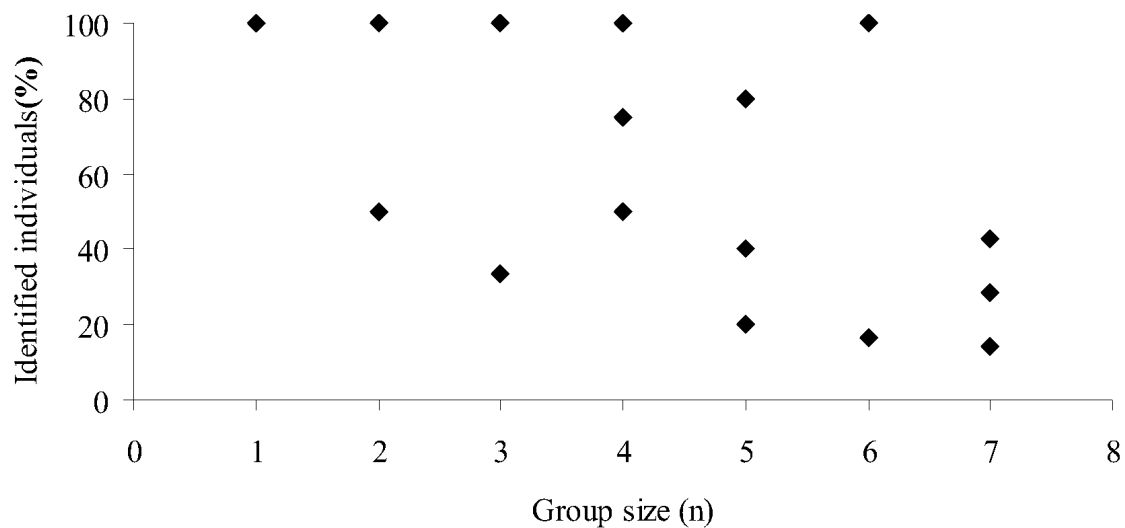


Fig. 3. Relationship between group size and the number of individuals identified within the group.

Eleven individuals (7 females, 2 males and 2 sub-adults) were seen on more than 3 occasions (Mean = 7.64; Range = 4-13), and were thus used for the data analysis (Table 2). There was a total of 84 identifications within the study period, mean number of interactions between dyads (pairs) = 0.75, and mean number of associations per individual = 7.45. Nine of the 11 frequently identified animals could be assigned to pods (Fig. 4). The grouping of animals into pods is also supported by the sociogram (Fig. 5). Strong relationships around the perimeter of the circle and the asymmetry of linkages through the axes of the circle indicate non-random associations. The varying thicknesses of the lines in the sociogram show different levels of association between dyads. The sociogram also supports weak or temporary associations made within the population. The thinner lines crossing the centre of the sociogram show weak or temporary associations. Pod M1 consists of 4 individuals (MF020, MU021, MF014 and MF001) spending 67% of their time together; within this pod MF020 and MU021 are seen to associate 100% of the time possibly due to a parent-offspring relationship. MF014 and MF001 are also closely associated with a strong association index of 1.0. Two individuals (MF002 and MM005) associated 67% of the time, these two animals were only seen alone once and the rest of the time as part of a larger group consisting mainly of females. These two animals are possibly part of a larger group, which could be classified as M2. MF010 and MF012 were not assigned to pods but did display loose relationships with other of animals in the study, namely MF002. Further observations are needed in order to identify if these animals could potentially be the missing members of pod M2. Pod M3 consists of three individuals; MF003 and MU004 (which are associated 100% of the time owing to a possible parent-offspring relationship), and MM007. The addition of MM007 is based on a small sample size; he was only seen 5 times and of these on two occasions with MF003 and MU004 (20/12/2006 and 08/01/2007) (Fig. 6). There was some interaction between pod M1 and M3 (Fig. 6). On 14 December 2006, all the individuals from pod M1 and M3 (except for MM007) were seen together in a group of 6 individuals and there was movement by MF001 from pod M1 to pod M3 on occasion.

Preferred and avoided associations were detected using the Bedjer et al. (1998) test. Constant results were obtained using 1000 random permutations with 100 flips /permutation. Long term preferred associations were indicated by high standard deviation and coefficient of variation (SD=0.20; CV=1.8) of real association indices when compared to randomly generated association indices (SD=0.11; CV=1.5). Avoided associations were also indicated by a lower proportion of non-zero elements in the real data (proportion = 0.49) when compared to the randomly generated data (proportion = 0.53).

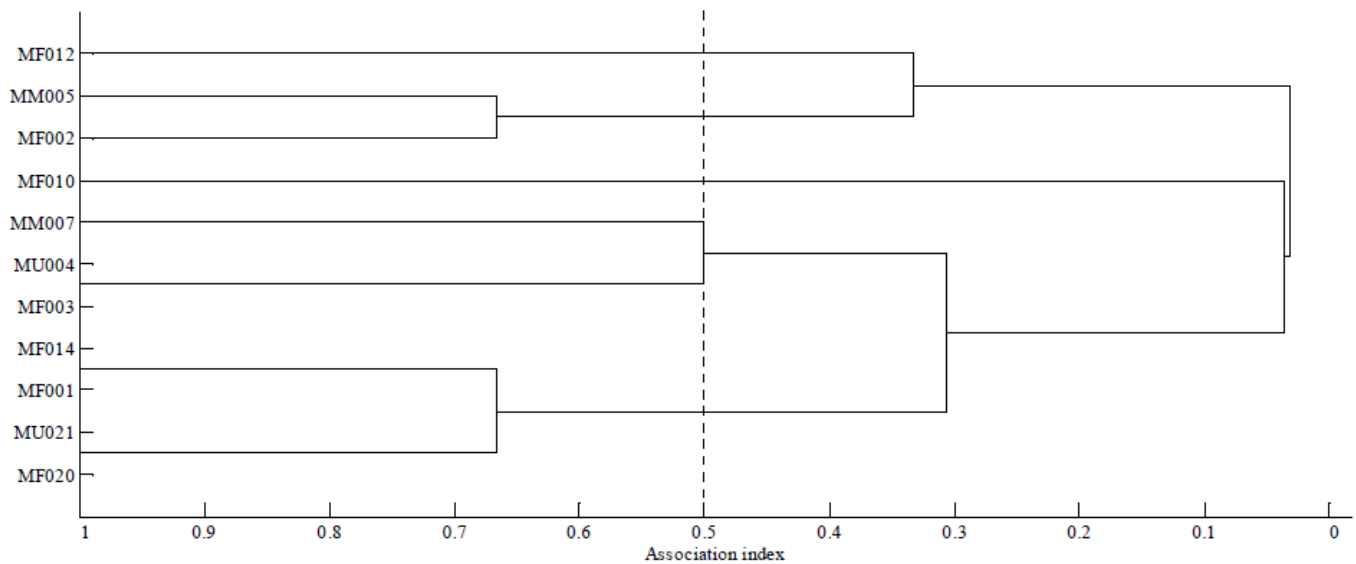


Fig. 4. Cluster diagram showing the association indices between identified individuals. Any association with an index greater than 0.5 (dotted line; Baird and Whitehead 2000) delineates a pod.

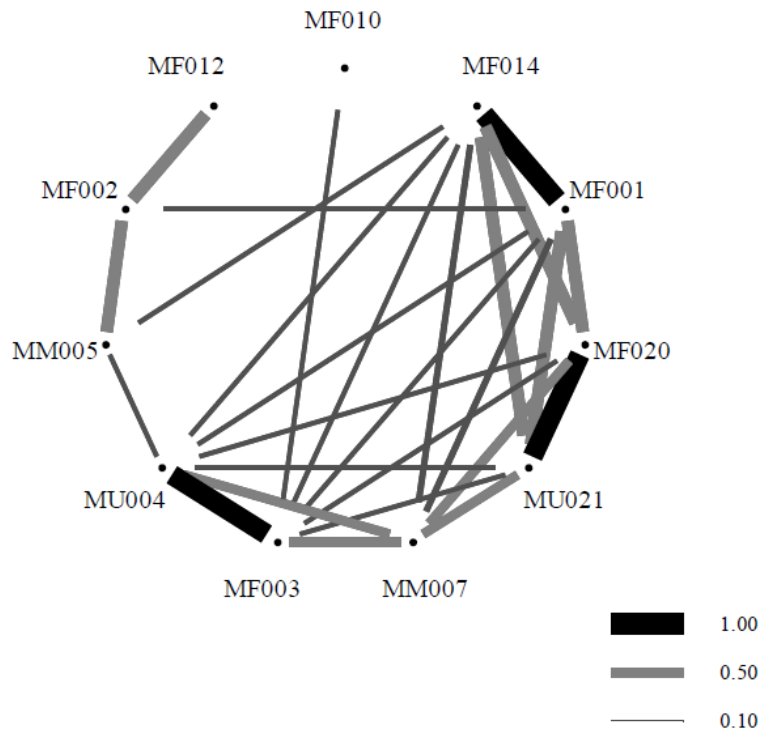


Fig. 5. Sociogram for the 11 individuals in this study. The key shows line thickness for three values, the linkages between individuals vary according to association indices (Association = 1.00 is highest).

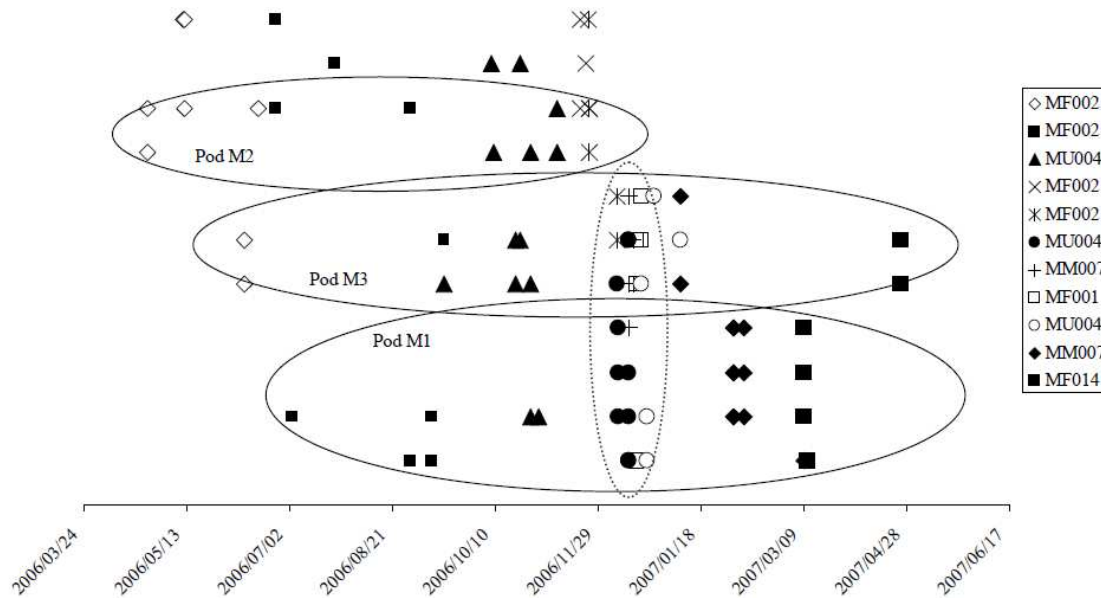


Fig. 6. Timeline of observations within the study period showing pod delineations as defined by association indices ≥ 0.5 (Bigg et al. 1990), The shaded area shows a period when Pod M1 and M2 were observed in the same group.

Discussion

Killer whale research on Marion Island is limited by land based data collection methods. This restricts the ability of the observer to identify all animals within the groups and makes the description of interactions between individuals within groups difficult. The Marion Island population is relatively small; with approximations of between 25 and 30 individuals in 2000 (Pistorius et al. 2002). Small population size limits the number of possible sightings of animals and therefore identifications. Although observations were limited to land based methods of a small population, this study is still based on 53% of all identified individuals. The exclusion of animals identified fewer than 3 times may have implications for the present study, but given that those animals sighted more than 3 times were often seen in smaller groups where all individuals were identified, basic pod composition is not affected. A bias exists against larger groups where not all individuals were identified (Fig. 3). Identification is constrained by poor visibility (weather and distance from shore), lack of identifiable marks and the opportunistic nature of the sightings, factors that affect all studies based on photographic methods (Friday et al. 2000). In the present study, we adopted the criteria as suggested by the IWC (1990) and used only experienced personnel, restrictive criteria and double confirmations if any doubt existed for the matching of individuals in order to reduce false-negative errors where two sightings of the same animal are marked as different (Stevick et al. 2001).

These factors notwithstanding, this study describes a well-differentiated killer whale society with certain individuals clearly associating with specific other individuals. The society seems to be female dominated, supporting findings for killer whales in the northern hemisphere (Brault and Caswell 1993), with females associating with their offspring foremost and then with other females. The role of male killer whales, at this stage, is difficult to define as there is no readily available relatedness information. Condy et al. (1978) describes a male biased composition of the Marion Island adult killer whale population, however, no correction for repeat identifications of the same animals was done. Our results indicate adult female biased (72%) composition among the 18 identified adult killer whales within the population,

which is more aligned with findings by Keith et al. (2001) for the Marion Island population. Difficulty in comparisons between studies arise due to the problems associated firstly with identification of individuals and secondly, distinguishing adult females and sub-adult males.

The relationships between Marion Island - and Antarctic killer whales are as yet unknown, but it seems that they share common characteristics with the type-B killer whales as described by Pitman and Ensor (2003), with a large eye-patch and dorsal cape. Further research is needed to reveal if the killer whale population around Marion Island appear to display similar social organisation to northern hemisphere transient killer whales as described by Baird and Whitehead (2000) or if they display a typically resident social structure (Heimlich-Boran 1986). Small groups (Marion Island: mean = 3.56 individuals (Keith et al. 2001), British Columbia: mean = 2.4 individuals (Baird and Whitehead 2000)), and females that are gregarious (high average association rates) seem to be common between populations. Baird and Whitehead (2000) attributed the social structure of transient killer whales in British Columbia to ecological factors, with energetic constraints imposed by foraging on other marine mammals limiting group size to 3 or less individuals (Baird and Dill 1996). Marion Island killer whales could potentially be displaying a small-group resident model, with animals showing a high degree of philopatry but with group size being energetically constrained. Long term residence in an area, or repeated visits to a site over many years has been shown for killer whales from Marion Island (Condy et al. 1978, Keith et al. 2001) and from Punte Norte (Hoelzel 1991). The presence of killer whales at Punte Norte is limited to a set period of high quality food availability and their behaviour is constrained by the need to maximise prey intake at beaches where hunting success is maximised (Hoelzel et al. 1991). The tendency to patrol beaches of varying topography (Condy et al. 1978, Pistorius et al. 2002), the varied food sources and the temporal variability in food sources may all be factors contributing to observed patterns at Marion Island. Further research is needed to draw conclusions on the link between social structure and diet/foraging at Marion Island, as can be done for killer whales at other localities (e.g. Hoelzel 1991, Baird and Dill 1996).

With the development of a killer whale identikit, further research detailing the temporal changes in associations and the strengths of associations between different classes will be possible. Given the paucity of knowledge about killer whales in the Southern Ocean, where uncertainty exists even at species level (Pitman and Ensor 2003), the current study proves that killer whales can be researched successfully and productive conclusions drawn over a relatively short time using shore-based observation data. Marion Island may serve as a focal point for observations of killer whales in the Southern Ocean, providing research opportunities difficult elsewhere.

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**“I don’t see much sense in that,” said Rabbit
“No,” said Pooh humbly, “there isn’t. But there was going to
be when I began it. It’s just that something happened to it
along the way.”**

Winnie the Pooh by *A. A. Milne*