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Pup production assessment of the Australian sea lion *Neophoca cinerea* at Dangerous Reef and English Island, South Australia

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SD Goldsworthy[1], B Page[1], A Lowther[1,2], P Rogers[1], K Peters[1,2], PD Shaughnessy[3]

[1] SARDI, 2 Hamra Avenue, West Beach SA 5024
[2] School of Earth and Environmental Sciences, University of Adelaide, Adelaide, South Australia 5005
[3] South Australian Museum, North Terrace, Adelaide, SA 5000

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SD Goldsworthy, B Page, A Lowther, P Rogers, K Peters, PD Shaughnessy

South Australian Research and Development Institute
SARDI Aquatic Sciences
2 Hamra Avenue
West Beach SA 5024
Telephone: (08) 8207 5400
Facsimile: (08) 8207 5481
www.sardi.sa.gov.au

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Authors:	SD Goldsworthy, B Page, A Lowther, P Rogers, K Peters, PD Shaughnessy
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1 EXECUTIVE SUMMARY

Australian sea lion pup abundance was determined for the Dangerous Reef and English Island populations during the 2008 breeding season. A combination of mark-recapture methods and counts of live and dead pups (including cumulative mortality), were used to estimate pup abundance at Dangerous Reef. A cumulative mark and count method was used at English Island. The breeding season at Dangerous Reef commenced in early February and continued through to November 2008, a period of about nine months. Four surveys were made roughly corresponding to the first, third, fourth and fifth month of breeding. Given the protracted breeding season, mark-recapture and count estimates of pup abundance will underestimate pup production for this breeding season. Mark-recapture estimates in conjunction with cumulative dead pups indicated that 520 pups (95% CL 509-535) had been born by the fifth month of breeding (August 2008). This is comparable to the maximum pup count of 537 pups (based on 201 tagged pups, 135 untagged pups and 201 cumulative dead pups), also in the fifth month of breeding. This represents a decline of around 24% since the 2006-07 breeding season and a departure following at least four consecutive breeding seasons of sustained increases in pup production. This decline most likely reflects a drop in fecundity rates between seasons, rather than a reduction in female population size, although surveying of subsequent breeding seasons will be needed to assess this. Even with a drop in pup production for the 2008 breeding season, the overall trend in pup abundance is still increasing at around 6.9% per season, or 4.5% per year, since 1996.

Surveys undertaken at English Island were compromised by influxes of dispersing pups into the colony, with four tagged pups and an unknown number of untagged pups present by the second survey when pup production to June 2008 was estimated to be 23. We recommend a modification of the cumulative mark and count method be used at sites where dispersal from other populations may compromise surveys. This would involve marking and counting of unmarked pups being directed at pups <1 month of age, and an increase in survey frequency.

Estimated mortality rates of pups to the fifth month of breeding at Dangerous Reef were high (43%). A very clear pattern of alternating high (mean ~38%) and low (mean ~18%) pup mortality between winter and summer breeding seasons, has now been established at Dangerous Reef. Although the causes of this pattern are uncertain, it is unlikely that sources of mortality identified in other studies (including con-specific trauma), would elicit such a strong seasonal pattern. Based on studies from other sea lion and fur seal populations, we propose that climate and season induced fluctuations in hookworm (*Uncinaria* spp.) infection in combination with enteritis-bacteraemia complexes explain the observed marked inter-seasonal fluctuation in pup mortality. Given these fluctuation could induce a large variance in recruitment and age-structure, there is a critical need to understand the role of disease and parasites on pup survival and on the broader population structure and demography of Australian sea lion populations.

2 INTRODUCTION

The Australian sea lion (*Neophoca cinerea*) is Australia's only endemic and least numerous seal species. It is unique among pinnipeds because it is the only species that has a non-annual breeding cycle, which is temporally asynchronous across its range. They have the longest gestation period of any pinniped and a protracted breeding and lactation period. The evolutionary determinants of this unusual reproductive strategy remain enigmatic. These factors, including the species small population size (~14,700 individuals), which is distributed over numerous, small colonies, make the Australian sea lion vulnerable to extinction (Goldsworthy & Page 2007). Recent population genetic studies have indicated little or no interchange of females among breeding colonies, even those separated by short distances (Campbell et al. 2008). The important conservation implication is that each breeding colony is a closed population. As such, the Australian sea lion poses significant conservation and management challenges. The species is listed as vulnerable under the threatened species category of the Commonwealth EPBC Act, vulnerable under the South Australian National Parks and Wildlife Act (1972) and recently upgraded to endangered by the IUCN Redlist.

The population of Australian sea lions at Dangerous Reef is the largest for the species. Pup abundances have been estimated at Dangerous Reef since 1994. Between 1994 and 1999 pup abundances have been monitored by counting pups. Since 1999, monthly counts through the breeding season in conjunction with mark-recapture methods have been used to estimate pup production. Mark-recapture provides a more robust means to obtain estimates of pup production because the method enables the calculation of confidence intervals around each estimate. This method has now been used for four breeding seasons at Dangerous Reef. During the 2006-07 breeding season, pup production was estimated to be 708 (95% CL 632-779) (Goldsworthy et al. 2007a). The exponential rate of increase in pup production from 1999 until 2006-07, based on maximum live-pup counts plus cumulative mortality and mark-recapture methods, ranged between 6.7-9.9% per breeding season, or 4.4-6.4% per year (Goldsworthy et al. 2007a). This provides further evidence of strong positive growth in the population, which has been occurring since 2000.

Australian sea lion pup abundances have been surveyed at English Island over six breeding seasons. Between 1998 and 2002, between four and 15 pups were recorded (Shaughnessy et al. 2005) and 18 pups were seen in February 1991 (Gales et al. 1994). In the 2005 breeding season pup production was estimated to be 27 (Goldsworthy et al. in press).

Aims and Objectives

The aims of this study were to: 1) monitor the pup numbers at Dangerous Reef and English Island by conducting four pup production surveys during the 2008 breeding season; 2) monitor dead pup numbers during each survey; 3) estimate pup numbers using mark-recapture methods; and 4) compare the 2008 pup production data with data from previous breeding seasons at Dangerous Reef and English Island.

3 METHODS

Field sites

Dangerous Reef (34.870 S, 136.2170 E) is 35 km south-east of Port Lincoln and forms part of the Sir Joseph Banks Group Conservation Park (Figure 1). It comprises Main Reef with nearby East Reef and West Reef. They cover about 12 ha in area (Robinson et al. 1996). Sea lion pups are born on Main Reef, and some of them move to the West Reef several weeks after birth. Dangerous Reef was accessed by vessel from Port Lincoln, between 2 February 2008 and 28 August 2008. Four trips to the island were made over this period. During each visit to the island, sea lion pup numbers were surveyed by direct counting of live pups, surveying of dead pups and for three of the visits to the island by mark-recapture. Each survey is defined as a session. Methodology for these approaches is detailed below.

English Island (34.638 S, 136.196 E) is a small rocky island that forms part of the Sir Joseph Banks Group and is 1.2 km east-north-east of Sibsey Island. Australian sea lion pups were surveyed on two occasions, 21 May and 23 June 2008.

Live and dead pup counts

The number of live pups was counted while slowing walking around the island, taking care not to disturb animals on the top of the island, to reduce the chance of double counting. After counting around the periphery of the island, the counters walked through the centre of the island to count the pups.

We recorded the number of pups that had died since the previous visit. To avoid double counting, dead pups were sprayed with paint or covered with rocks when they were counted. The number of dead pups was added to give the number of ‘accumulated dead pups’. When that number was added to the number of live pups, it gave the best available estimate of pup production to that date.

Mark-recapture

The method of directly counting pups is known to underestimate total pup production, because pups that are hidden from view (sightability bias) or absent from the colony (availability bias) at the time of the survey are not included. The influence of these factors on estimates of pup numbers can be reduced to some degree by undertaking a mark-recapture procedure. Petersen estimate mark-recapture methods have been used to estimate pup production at fur seal colonies in Australia since 1988 (Shaughnessy et al. 1995a, Shaughnessy et al. 1995b, Shaughnessy & McKeown 2002, Kirkwood et al. 2005), but have only been recently applied to estimating pup production in the Australian sea lion population at Seal Bay, Dangerous Reef, Olive and North Page and South Page Islands (McIntosh et al. 2006, Shaughnessy et al. 2006, Goldsworthy et al. 2007c).

A mark-recapture procedure was used to estimate the number of live pups present during each visit to Dangerous Reef in May, June and August 2008 (session 1, 2 and 3, respectively). During each colony visit a sample of pups was tagged in the trailing edge of each fore-flipper with individually numbered plastic tags (Dalton® Size 1 Supertags). During each field trip, individual re-sight records were collected for marked individuals with the aid of binoculars. All dead pups sighted were recorded and rocks were placed on top of carcases to avoid repeat counting. Records of the total number of tagged, untagged and newly recorded dead pups were noted on each field trip. The numbers of re-sights of individually marked pups on the days prior to recapture surveys were used as the number of 'marked' individuals in subsequent recapture events using the Petersen estimate procedure (see below).

During each session, individual re-sight records were collected for tagged individuals with the aid of binoculars. Records of the total number of tagged, untagged and newly recorded dead pups were noted on each field trip. Individual re-sights of tagged pups (usually undertaken over 1-2 days prior to recapture surveys), provided the number of 'marked' individuals in the population available for recapture. Pups sighted in subsequent sessions were assumed to be available for sighting in all preceding sessions. During recapture surveys, the individual identity of tagged pups was determined by reading tag numbers with binoculars. The number of untagged pups seen was also recorded as were newly dead pups that had not been marked.

Mark-recapture estimates of pup numbers ($\hat{N}$) were calculated using a variation of the Petersen method (formula attributed to D.G. Chapman by Seber (1982) with the formula

$$\hat{N} = \frac{(M+1)(n+1)}{(m+1)} - 1,$$

where *M* is the number of marked pups at risk of being sampled during recapture operations, *n* is the number of pups examined in the recapture sample, and *m* is the number of marked pups in the recapture sample.

The variance of this estimate is calculated as

$$\operatorname{var}(\hat{N}) = \frac{(M+1)(n+1)(M-m)(n-m)}{(m+1)^2(m+2)}.$$

Where several mark-recapture estimates ($\hat{N}_j$) are undertaken (one from each recapture session), they are combined by taking the mean (*N*) using formulae from White and Garrott (1990)(pp. 257 & 268):

$$N = \sum_{j=1}^{q} \frac{\hat{N}_j}{q}$$

where q is the number of estimates for the colony (i.e., the number of recapture sessions). The variance of this estimate is calculated from

$$\mathrm{var}(N) = \frac{1}{q^2}\sum_{j=1}^{q}\mathrm{var}(\hat{N}_j).$$

Following Kuno (1977) the square root of var(N) gives the standard error (SE) for the estimation, and the 95 % confidence limits calculated as

$$N \pm (1.96 * SE).$$

The Petersen estimates yields an accurate result as long as a number of conditions are met (Caughley 1977). These include:

- the probability of capturing an individual is the same for all individuals in the population,
- no animal is born or immigrates into the study area between marking and recapturing,
- marked and un-marked individuals die or leave the area at the same rate, and
- no marks are lost.

Test for equal catchability

The key assumption of mark-recapture studies is that the probability of capture is the same for all individuals in the population. This was tested within the tagged population by examining the number of times that individual marked pups were resighted within each capture session. We used Leslie's test for equal catchability, following methods detailed in Caughley (1977), and for each of the six capture sessions, examined the number of times known-to-be-alive individuals were resighted. We used Leslie's test in favour of the zero truncated Poisson test because it enabled us to use data on zero recaptures (animals known to be alive from subsequent recapture session, but not sighted) (Caughley 1977). This could be achieved for all but the final recapture session. The assumption in Leslie's test is that if catchability is constant across all individuals, the recapture frequencies will form a binomial distribution. This assumption can be tested as a Chi-square with $(\sum f)-1$ degrees of freedom, by comparing the observed variance to the expected binomial variance, where

$$\chi^2 = \frac{\sum fi^2 - \frac{(\sum fi)^2}{\sum f}}{\frac{\sum fi}{\sum f} - \frac{\sum n^2}{(\sum f)^2}},$$

and n is the number of individually tagged pups resighted during each recapture, i is the number of times individual pups were resighted during recapture sessions and f is the number of individuals resighted i times (Caughley 1977).

English Island

The methodology to survey English Island followed that described by Goldsworthy et al. (2007c) for small colonies, termed the cumulative mark and count (CMC) method. During each visit, attempts were made to mark a number of pups, by clipping a small patch of fur on the rump using scissors. The number of marked, unmarked and dead pups sighted was recorded on each visit to the colony, and where possible, additional pups were marked. Dead pups were covered with rocks to avoid repeat counting on subsequent surveys. Pup numbers were estimated for each visit from the numbers of marked pups and accumulated dead pups, plus the number of live unmarked pups. The last item was estimated in several ways, and the maximum number was used to estimate the number of pups born to date. For the first visit, it was simply the number of unmarked live pups seen. For the latter surveys it was the maximum number of unmarked pups seen in one of the previous surveys, less pups marked since then.

Trends in abundance

The rate of change in pup numbers was calculated using linear regression of the natural logarithm of the mean estimate of pup numbers against year or breeding season (~1.5 years). The exponential rate of increase (*r*) is the slope of the regression line. An exponential rate of increase has been demonstrated for other seal species, for example the New Zealand fur seal on Kangaroo Island (Shaughnessy et al. 1995a). It can be expressed as a percentage increase using the following formula $(e^r-1) * 100$.

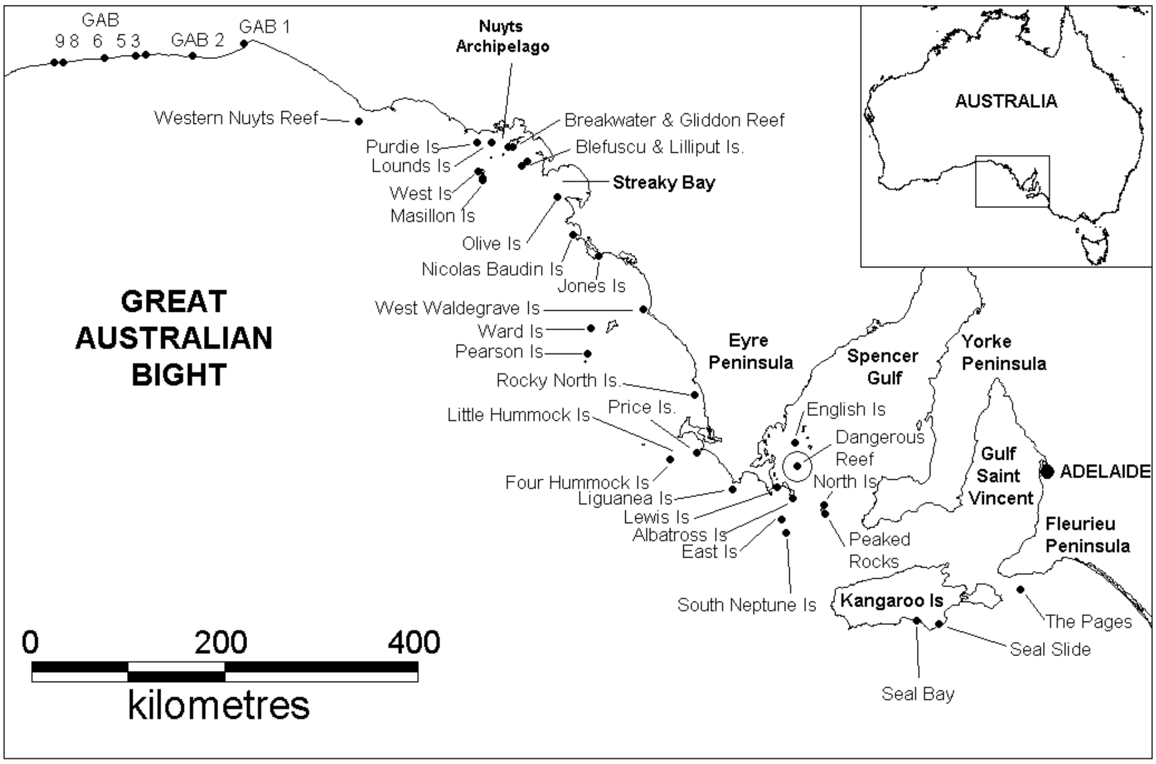

Figure 1. Location of Dangerous Reef and English Island in southern Spencer Gulf, relative to other Australian colonies in South Australia.

4 RESULTS

Dangerous Reef

Pup counts

On the first visit to Dangerous Reef on 2 February 2008, 3 live pups were recorded, indicating that the breeding season had commenced by the beginning of February. On 21 November 2008, approximately 6 young brown pups were sighted, although no systematic counts were undertaken. These observations suggest that the duration of the breeding season for 2008 was at least 9 months. Counts of live and dead pups surveyed at Dangerous Reef during the 2008 pupping season are presented in Table 1 and Figure 2. The largest estimate of pups based on counts of cumulative tagged (201), cumulative dead untagged pups (201) and maximum untagged pups (135) was 537 on 26 August 2008 (Figure 2). This does not include an unknown number of pups that would have been born after 26 August 2008.

Mark-recapture estimates of pup numbers

The mark-recapture estimate procedure utilised 201 tagged pups. Re-sights of these tagged pups over several days prior to recapture surveys were used to provide a pool of tagged pups for each recapture session. Pups sighted in future surveys (known to be alive) were included as being available for re-sighting in previous recapture sessions. The number of tagged pups available to be re-sighted varied considerably between surveys (109-170, Table 2). Mark-recapture estimates of the number of live pups were the greatest during the first session (mean 334, 95% CL 322-346), and then progressively declined in the second (mean 331, 95% CL 315-347) and third recapture sessions (mean 289, 95%Cl 275-304, Table 2). Adding accumulative dead pups to these values provided the reverse pattern of increasing estimates of 399 (95% CL 387-411), 493 (95% CL 447-509) and 520 pups (95% CL 509-535), respectively, highlight the significant pup mortality during this breeding season (Table 1).

Comparisons of mark-recapture estimates with direct counts at Dangerous Reef have now been made over five breeding seasons (Table 3). Mark-recapture estimates were between 1.19 and 1.38 times the direct count of pups (95% confidence limits of comparisons range from 1.12 to 1.45). This indicates that estimates derived from mark-recaptures were similar to direct counts of pups in the five pupping seasons. The discrepancy between the direct counts and the mark-recapture estimates on each occasion results from the difficulty of sighting all pups in the colony. Some pups may not be viewed during counting because they are away from the island, swimming in the shallows or obscured by rocks.

Equal catchability

Results from Leslie's test of equal catchability are presented in Table 4. Results from the second and third recapture sessions were non-significant, indicating that the assumption that the distribution of recaptures was binomial and that catchability of tagged pups was equal is supported.

Trends in abundance at Dangerous Reef

Live and dead pup surveys

For the Dangerous Reef Australian sea lion population, estimates of pup numbers by direct counting are available for 13 pupping seasons from 1975 to 2008, and range from 248 to 585 with an average of 403 (sd = 116) (Table 5, Figure 2).

Because dead pups were not counted in the 1994-95 season, the number of live pups in that season has been adjusted to estimate the number of births (Table 4, see Shaughnessy (2005b). Using the maximum live-pup counts and numbers of cumulative dead pups over these 13 breeding seasons (1975 to 2008) as an index of pup production, the number of pups born at Dangerous Reef has increased at an exponential rate of r = 0.029 or 3.0% per breeding season (~ 1.5 years) or r= 0.019 or 2.0% per year. The trend is significant over time (linear regression, $F_{1,12}$ =10.073, P=0.009, R^2=0.478).

Data from three pupping seasons are considerably smaller than the others: 262 pups in 1976-77, 260 in 1990 and 248 in 1997-98 (Figure 2). Each of these counts was made in the fourth month after pupping began, whereas maximum counts for all but one of the other seasons were made in the fifth month or later (Table 5). Counting that ended in the fourth month of a pupping season is likely to underestimate pup production considerably. Data for the1994-95 season were incomplete, did not include counts of dead pups and were adjusted for mortality based on the averages from the preceding three seasons (Table 5). The most accurate pup count data have been collected since 1996. Analyses of eight pupping seasons from 1996 (excluding 1997-98), indicate that pup counts have increased at r = 0.066 or 6.9% per breeding season, equivalent to *r* = 0.044 or 4.5% per year. This is the best interpretation of these data and the increasing trends are significant over time (linear regression, $F_{1,7}$ =33.475, P=0.001, R^2=0.848) (Figure 2).

Mark-recapture surveys

Mark-recapture estimates for live pups plus cumulative dead pups to the time of survey, have been undertaken over five breeding seasons (1995 to 2008, Table 3). Trend data for the five seasons show an increase between seasons of r = 0.057 or 5.9% per season, which is equivalent to r = 0.038 or 3.9% increase per year (Figure 2). With the drop in pup numbers during the 2008 season, and with the limited time series of mark-recapture data, these trends are not significant over time (linear regression, $F_{1,4}$ =2.943, P=0.185, R^2=0.495).

Pup mortality

For the 2008 pupping season at Dangerous Reef, 231 dead pups were recorded by 26 August when the estimated number of births reached a maximum of 537, giving an incidence of pup mortality of 43.0% for the 2008 breeding season (Table 5).

For the last nine pupping seasons at Dangerous Reef (since 1996), the incidence of pup mortality has ranged from 15.3% to 44.6% (Table 3.5). It was high for pupping seasons that occurred predominantly in winter (30.3% in 1996, 42.0% in 1999, 44.6% in 2002 and 31.1% in 2005, 43.0% in 2008, with unweighted average 38.2%) and lower for pupping seasons that occurred predominantly in summer (15.3% in 1997-98, 22.9% in 2000-01, 18.6% in 2003-04, and 13.9% in 2006-07 with unweighted average 17.7%). For this analysis, data for pupping seasons before 1996 have been omitted because insufficient attention had been directed at dead pups. A one-way ANOVA comparing the mortality rate between summer and winter breeding seasons, indicated that mortality rates (proportion of dead pups) were significantly higher in winter breeding seasons ($F_{1,7}$ =26.442, P=0.0013, arcsine transformed data).

Despite variable and often high rates of pup mortality between season, the minimum number of live pups (maximum pup count – maximum cumulative dead pups), still showed a significant increase between seasons (r = 0.063, 6.5%) and year (r = 0.044, 4.3%) using data from 1996 (both linear regressions, $F_{1,8}$ =6.592, P=0.037, R^2=0.485) (Figure 2).

Table 1. Summary of details of Australian sea lion pup counts, tagging, cumulative mortalities and various direct count and mark-recapture abundance estimates during 9 visits (sessions) to Dangerous Reef between February and August 2008.

Session		**1**	**2**	**3**
Date	**2-Feb**	**20-May**	**25-Jun**	**26-Aug**
Cumulative tagged		170	201	201
Maximum untagged counted	3	109	99	135
Maximum count (live)	3	270	242	210
Cumulative dead (un-tagged)	0	65	146	201
Cumulative dead (tagged)	0	0	16	30
Total accumulative dead	0	65	162	231
Maximum count (live) + cumulative dead	3	335	404	441
Cumulative tagged + dead (unmarked) + max un-tagged	3	344	446	537
Petersen Estimate (live)		334	331	289
Petersen Estimate Lower – Upper CL		322-346	315-347	275-304
(No. recapture estimates)				
Petersen Estimate (live) + cumulative dead		399	493	520
Lower – Upper CL		387-411	447-509	506-535

Table 2. Details of Petersen mark-recapture procedures undertaken at Dangerous Reef between May and August 2008. M = number of marked pups in the population, n = the total number of pups sampled and m = the number of marked pups in each recapture sample. N = the estimated pup population size, sd = standard deviation and s = variance. % = the percentage of marked pups in each sample, CV = the coefficient of variation. The lower and upper 95% confidence limits (CL)of each estimate, respectively.

Date	**Recapture No.**	**Marked M**	**Examined n**	**M-R m**	**N**	**sd**	**s**	**%**	**CV**	**Nlo**	**Nup**
Session 1											
20-May	1	170	207	111	317	12	142	54%			
20-May	2	170	156	87	304	15	223	56%			
20-May	3	170	203	110	313	12	141	54%			
20-May	4	170	220	111	336	13	171	50%			
20-May	5	170	184	79	394	24	583	43%			
20-May	6	170	218	109	339	14	185	50%			
				Mean	**334**	**6**		**51%**	**1.9%**	**322**	**346**
Session 2											
26-Jun	1	151	175	79	333	19	357	45%			
26-Jun	2	151	184	89	311	15	225	48%			
26-Jun	3	151	131	56	351	28	759	43%			
26-Jun	4	151	160	72	334	21	431	45%			
26-Jun	5	151	180	87	312	15	238	48%			
26-Jun	6	151	175	76	346	21	430	43%			
				Mean	**331**	**8**		46%	**2.5%**	**315**	**347**
Session 3											
26-Aug	1	110	216	81	293	13	169	38%			
26-Aug	2	110	191	70	299	17	284	37%			
26-Aug	3	110	174	70	273	15	223	40%			
27-Aug	4	109	183	74	269	13	181	40%			
27-Aug	5	109	210	77	297	14	206	37%			
27-Aug	6	109	181	62	317	21	441	34%			
27-Aug	7	109	197	86	249	9	83	44%			
27-Aug	8	109	191	71	292	16	254	37%			
27-Aug	9	109	170	56	329	25	603	33%			
28-Aug	10	108	188	73	277	14	202	39%			
				Mean	**289**	**5**		**38%**	**2.5%**	**275**	**304**

Table 3. Summary of mark-recapture estimates of the abundance of Australian sea lion pups at Dangerous Reef over four breeding seasons, highlighting comparison between mark-recapture estimates and live pup counts. For the 2006/07 season comparisons between methods can be made for two of the three mark-recapture estimates.

Date	Max. direct count (inc. dead)	Direct count of pups	Mark-recapture estimate of pups	Comparison	95% confidence interval	No. month since pupping commence to		Source
						Max count	Mark-recapture estimate	
July 1999	383	240	285	1.19	1.12 to 1.25	4	4	(Shaughnessy & Dennis 1999)
Jan 2004	499	333	423	1.27	1.21 to 1.31	5.5	5	(Shaughnessy 2004)
July 2005	585	272	326	1.20	1.15 to 1.25	6	6	(Shaughnessy 2005a)
Nov 2006	397	330	436	1.32	1.26 to 1.38	4	4	(Goldsworthy et al. 2007b))
Jan 2007	575	495	629	1.27	1.12 to 1.42	6	6	(Goldsworthy et al. 2007b)
Aug 2008	537	210	289	1.38	1.31 to 1.45	6-7	6-7	This report

Table 4. Leslie's test for equal catchability across each recapture session at Dangerous Reef. *n* is the number of individually tagged pups resighted during each recapture, *i* is the number of times individual pups were resighted during recapture session and *f* is the number of individuals resighted *i* times. Chi-squared (X^2) and degrees of freedom (df) values are also given. Non-significant (NS), probability (*P*) values indicate equal catchability.

Session No.	Recapture No.	*n*	n^2	*i*	*f*	*f. i*	$f.i^2$	X^2	df	*P*
2	1	48	2304	0	66	0	0			
	2	22	484	1	39	39	39			
	3	30	900	2	21	42	84			
	4	32	1024	3	18	54	162			
	5	27	729	4	8	32	128			
	6	18	324	5	2	10	50			
				6	0	0	0			
	$\sum$	177	5765		154	177	463	0.045	153	>0.05
3	1	58	3364	0	17	0	0			
	2	74	5476	1	12	12	12			
	3	53	2809	2	25	50	100			
	4	60	3600	3	18	54	162			
	5	60	3600	4	21	84	336			
	6	44	1936	5	18	90	450			
	7	65	4225	6	11	66	396			
				7	0	0	0			
	$\sum$	414	25010		122	356	1456	0.017	121	>0.05

Table 5. Estimated number of births of Australian sea lions at Dangerous Reef, South Australia for 13 pupping seasons between 1975 and 2008. Data are collated from Dennis (2005), Shaughnessy and Dennis (2001) and (2003), Shaughnessy (2004) and (2005a), Goldsworthy et al. (2007a) and this report. The data for 1994-95 includes an adjustment to account for pup mortality because only live pups (295) were counted in that season (following Shaughnessy (2005).

Pupping season	Cumulative dead pups at max. live count [a]	Max. pup count [b]	Pup mortality (%)	Month of max. live count since pupping began	Max. cumulative dead pup
1975	73	356	20.5	5	73
1976-77	26	262	9.9	4	26
1990	55	260	21.2	4	55
1994-95	-	354 [c]	not estimated	6.5	
1996	110	363	30.3	-	110
1997-98	38	248	15.3	4	43
1999	161	383 [d]	42.0	4	165
2000-01	90	393	22.9	7	90
2002	190	426 [e]	44.6	6	190
2003-04	93	499 [f]	18.6	5	100
2005	182	585 [g]	31.1	5	274
2006-07	80	575 [h]	13.9	6	88
2008	231	537	43.0	6-7	231

[a] ‘Cumulative dead pups’ refers to the number of dead pups counted through to the maximum live pup count.
[b] ‘Max. pup count’ refers to the maximum live pup count plus cumulative dead pups up until the date of the maximum live pup count.
[c] Adjusted for pup mortality using: “Maximum pup count” x 1.19954, where 0.19954 is the un-weighted average proportion of dead pups in three summer pupping seasons, 1997-98, 2000-01 and 2003-04.
[d] In addition, 23 newly-born pups were recorded on the last two visits; that number plus the previous estimate (of 383) leads to an estimate of pup numbers for the season of 406.
[e] In addition, 29 newly-born pups were recorded on the last visit; that number plus the previous estimate (of 426) leads to an estimate of pup numbers for the season of 453.
[f] In addition, 27 newly-born pups were recorded on the last visit; that number plus the previous estimate (of 499) leads to an estimate of pup numbers for the season of 526.
[g] In addition, 32 newly-born pups were recorded on the last three visits; that number plus the previous estimate (of 585) leads to an estimate of pup numbers for the season of 617.
[h] In addition, 4 newly-born pups were recorded on the last visit; that number plus the previous estimate (of 575) leads to pup count for the season of 579.

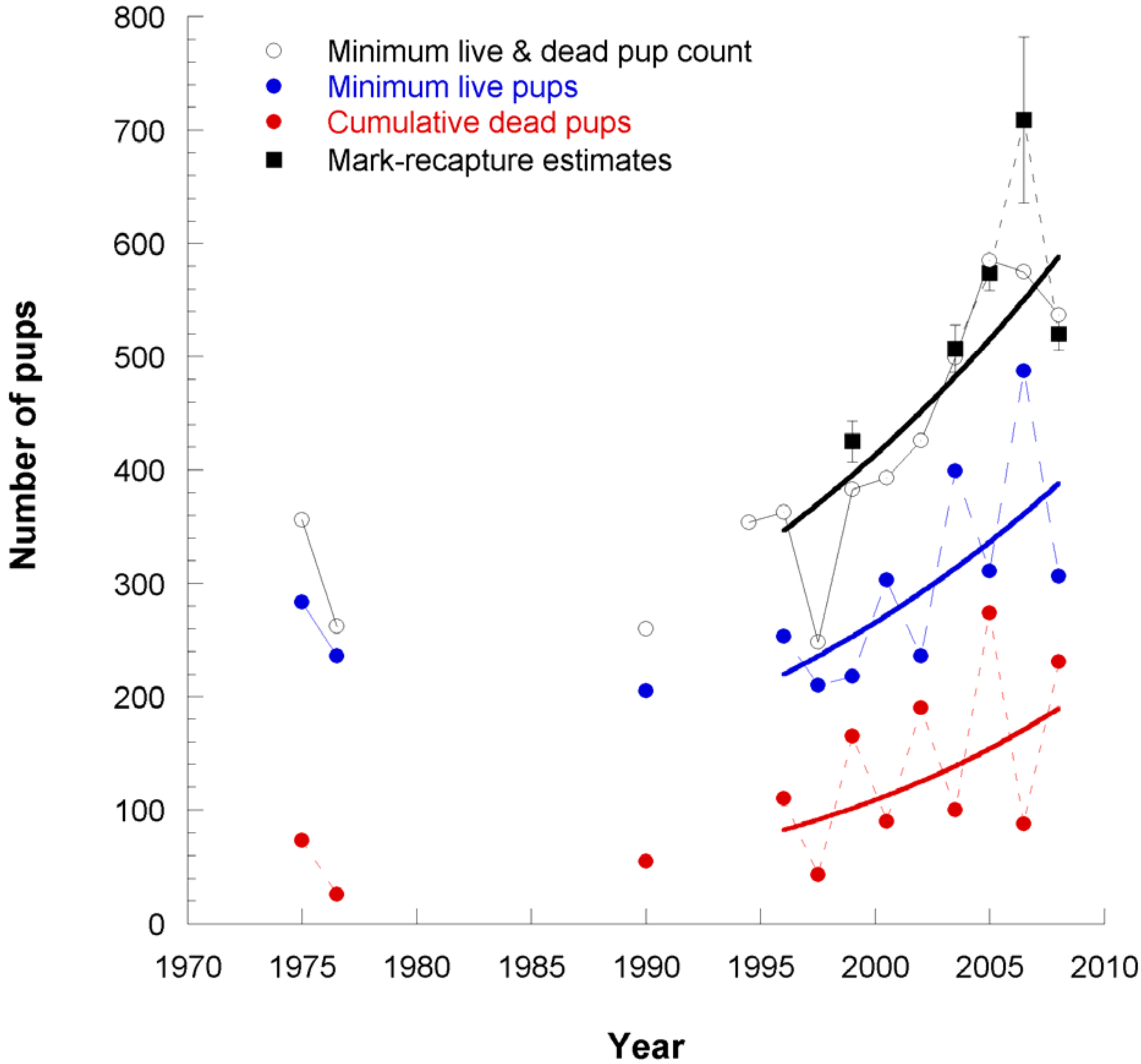

Figure 2. Trends in the abundance of Australian sea lion pups at Dangerous Reef, based upon minimum live and cumulative dead pup counts, minimum live pups count, cumulative dead pups and mark-recapture estimates (inclusive of cumulative dead pups) for 14 breeding season between 1975 and 2008. Error bars around mark-recapture estimates are ± 95% CL. Exponential curves are fitted to subsets of minimum live and cumulative dead pup counts, minimum live pups count and cumulative dead pups.

English Island

Pup counts

English Island was surveyed on two occasions, 21 May and 23 June 2008. Details on the number of unmarked, marked and dead pups sighted on each survey are presented in Table 6. The minimum number of marked, dead and unmarked pups present in the population, based on the re-sight and marking history is also presented. On the first visit, 16 live (6 black, 10 brown) and 3 dead pups were observed, and 11 of the live pups (5 black, 6 brown) were marked. Black pups are typically <4 weeks of age, and brown pups between 4 and 12 weeks of age. On the next survey, 12 live pups were sighted (4 black, 8 brown), and only 2 of these (brown pups) were marked. Based on this survey, the minimum number of pups estimated at English Island subpopulation was 24 (11 marked + 10 clear + 3 dead) (Table 6). However, given that four tagged pups from Dangerous reef were sighted at English Island on the second survey (not included in pup tally), it cannot be certain that all of the unmarked pups sighted were born at English Island. Because of the dispersal of Dangerous Reef pups, and the implications of the presences of these animals on the confidence of pup production estimates, no further surveys were undertaken during the 2008 season (Table 6). At Dangerous Reef during the June surveys, a maximum of 185 live tagged pups (201– 16 dead) and a minimum of 242 live untagged, represents approximately 43% tagged. Given this, the presence of four tagged pups from Dangerous Reef at English may suggest that up to 9 additional pups (including the 4 tagged) from Dangerous Reef (4 x (1/0.43)), were present at English Island during the June survey (ie. another 5 untagged pups). Based on these results, the best estimate of pup production is likely to be derived from adding the 4 black pups (new births since the first survey) to the total estimate of the first survey, as it can be certain that these pups were born at English Island. This gives a total estimate of 23 pups for the 2008 season.

Trends in abundance at English Island

Australian sea lion pup abundances have now been surveyed at English Island over seven breeding seasons. Between 1998 and 2002, between 4 and 15 pups were recorded (Shaughnessy et al. 2005) and 18 pups were seen in February 1991 (Gales et al. 1994). In the 2005 breeding season pup production was estimated to be 27 (Goldsworthy et al. in press), and with this most recent breeding season, a minimum of 23 pups have been reported. Clearly previous surveys have also been confounded by the issue of pups dispersing to the island from Dangerous Reef during the breeding season, as well as high variability in survey number and effort across breeding seasons. As such it is not possible at present to discern trends in pup production at English Island.

Table 6 Details of pup surveys undertaken at the Australian sea lion colony at English Island in May and June 2008. The number of clear (unmarked), marked, dead and total pups seen on each survey is indicated, in additional to the number of new marks applied. The number of marked pups available to be re-sighted at each survey is presented, along with the cumulative number of dead pups recorded. The minimum number of pups at each visit is estimated by summing the number of pups marked, maximum number of unmarked pups and cumulative dead pups.

Date	Clear count	Marked count	Dead clear	Dead marked	Total live count	Total live & dead count	New marked	Cum. marked	Min Alive	Cum. dead clear	Min Total
21-May	16	0	3	0	16	19	11	11	16	3	**19**
23-June	10	2	0	0	12	12	1	12	21	3	**24***

* Not including 4 tagged pups from Dangerous Reef sighted (tag numbers 612, 642, 655, 732).

5 DISCUSSION

Based on surveys undertaken at Dangerous Reef during the 2008 breeding season, there has been an apparent decrease in pup production of about 24% (based on mark-recapture estimates) between the 2006-07 and 2008 breeding seasons. However, there are a number of factors about the 2008 Dangerous Reef breeding season and the surveys undertaken that cast some doubt over the accuracy of this survey. The most important of these were the extended breeding season (up to nine months in duration), and the limited number of surveys (funding only permitted four). The highly protracted breeding season meant that an unknown number of pups were born over the three months following the final survey. The proportion of pups born by the final survey is unknown, and hence estimates of pup production for the 2008 season based on these surveys represent an underestimate.

For most Australian sea lion breeding seasons, four surveys are usually enough to derive an accurate estimate of pup production, as long as they are adequately spaced. The first survey is used to determine the commencement or stage of the breeding season, and to estimate when the third month of breeding will occur. For large colonies where mark-recapture methods are appropriate, undertaking these surveys on the third, four and fifth month of breeding covers most of the breeding season. At Seal Bay, the four most recent breeding seasons (2002-03 to 2007) have lasted between 6-9 months, with 90% of births occurring over a 4.0 month period (range 3.4 – 4.6, n = 4) (Goldsworthy et al. 2008a). The 2002-03 breeding season at Seal Bay was of similar duration to the 2008 Dangerous Reef breeding season, lasting about 9 months. The first births were recorded in December 2002, with 90% of births occurring between 2 January and 21 May (4.6 months) (Goldsworthy et al. 2008a).

Given the timing of surveys at Dangerous Reef during 2008, and based on a similar duration breeding season at Seal Bay, more than 90% of births were likely to have occurred by the time of the final survey in late August, almost 7 months after the commencement of the breeding season. As the duration of breeding seasons cannot be reliably anticipated in advance, the number of surveys required to minimise errors in estimates of pup production for any given breeding season can be difficult to determine. Clearly, for the 2008 breeding season at Dangerous Reef, additional surveys at month 5 and 8 would have enabled better estimates to be determined. Future surveys should build in some contingency (if possible) to conduct additional surveys if required.

Despite some uncertainties in the accuracy of the 2008 pup production estimate, there has clearly been a decline in pup production from the 2006-07 breeding season, although the decline of around 24% between seasons is likely to be an over estimate, based on the likelihood that the 2008 pup production has been somewhat underestimated. The decline in pup production between years represents a notable departure following at least four breeding seasons of successive sustained increases in pup production of around 10% per breeding season (Goldsworthy et al. 2007b). Given this, the decline most likely reflects a drop in fecundity rates between seasons, rather than a reduction in female population size, although surveying of subsequent breeding season will be needed to assess this. Marked variance in pup production between successive seasons has also been noted at Seal Bay and The Pages (Shaughnessy & Goldsworthy 2007, Goldsworthy et al. 2008a). At Seal Bay over four consecutive breeding seasons between 2002-03 and 2007, between season pup production increased by 27% (2002-03 to 2004), fell by 24% (2004 to 2005-06) and then increased by 19% (2005-06 to 2007), a mean deviation of 23% between seasons (Goldsworthy et al. 2008a). These results demonstrate the challenges faced in using pup production estimates to determine trends in Australian sea lion populations, and the imperative for long time-series of data to better understand population status and trends in abundance.

Although this study has identified a reduction in pup production for the 2008 breeding season, the overall trends from mark-recapture and pup counts support the general trend that the population has increased markedly in size since the 1990s (Figure 2). Goldsworthy et al. (2007a), identified that this recent pronounced increase in pup abundance at Dangerous Reef has occurred coincidentally with the cessation of the demersal gillnet shark fishery in Spencer Gulf in 2001. Bycatch of sea lions in this fishery has been identified as a key threat to the species (Goldsworthy & Page 2007, Goldsworthy et al. in press), and the recent recovery of the Dangerous Reef population provides circumstantial evidence that positive growth has followed a reduction in anthropogenic mortality for this population (Goldsworthy et al. 2007a).

Difficulties were also met in undertaking a survey of pup production at English Island. Due to dispersal of Dangerous Reef pups to English Island during the breeding season, surveys can be significantly compromised because it is not possible to know the origin of all pups sighted. We estimated that up to 9 pups from Dangerous Reef were present on the final survey undertaken at English Island on 23 June 2008. Our best estimate of pup production up to 23 June was 23 pups. The most effective way to eliminate inclusion of dispersed pups in surveys is to make more regular surveys and only count and marking new black coat pups (<1 month old). This approach has been used to overcome the issue of dispersed pups (from Seal Bay) being included in surveys at The Seal Slide on Kangaroo Island (Goldsworthy et al. 2008a). This alternate method for small colonies where dispersal from nearby colonies cannot be ruled out, is essentially a modification of the cumulative mark and count method (Goldsworthy et al. 2007c, Goldsworthy et al. 2008b), where marking and counting of unmarked pups is directed only at pups <1 month of age.

Pup mortality

The mortality rate of pups at Dangerous Reef determined for the 2008 season (43.0%) is second only to that reported for the 2002 breeding season (44.6%, Goldsworthy et al. (2007b). There is now a very clear pattern of alternating high and low pup mortality between breeding seasons at Dangerous Reef, with high mortality seasons (mean ~38%) corresponding with breeding seasons that occur mostly over the winter months, and low mortality seasons (mean ~18%) occurring mostly over summer months. A difference in pup mortality between a winter and a summer pupping season has also been observed by Gales et al. (1992)) at islands in the Jurien Bay region on the west coast of Western Australia. They reported high pup mortality in the first five months of a breeding season that included the 1989 winter, averaging 24% over the three islands. Pup mortality rates were considerably lower (7%) in the preceding pupping season, which occurred during the summer. At Seal Bay, there is also evidence over four consecutive breeding seasons of alternate high (33%) and low (22%) mortality seasons, although contrary to the pattern for Dangerous Reef and the Jurien Bay colonies in Western Australia, the correlation with season of breeding appears to be reversed, with high mortality corresponding with summer/autumn breeding, and low mortality corresponding with winter/spring breeding seasons (Goldsworthy et al. 2007b).

The cause of the large variance and apparent seasonality in mortality rates at Dangerous Reef is presently unknown. McIntosh (2007) provides some of the best information on causes of mortality in Australian sea lion pups. During three breeding seasons at Seal Bay (2002-03, 2004, 2005-06), 128 pups were examined to determine the cause of death. In 51% of cases cause of death could be determined and included trauma from con-specific aggression (31.6%), emaciation (10.4%), still-birth (7.6%) and possible shark attack (1.4%). However, in 49% of cases the cause of death could not be assessed, and it is possible that disease and parasites (not assessed in necropsies) were the primary cause of mortality in these cases. It would seem improbable for there to be a strong seasonal pattern in the prevalence of the main causes of mortality identified by McIntosh (2007) (eg. conspecific aggression). It is more likely that the seasonal pattern of mortality observed at Dangerous Reef is related to disease or parasites where seasonality in the environment may influence the prevalence and severity of infection.

Hookworm, *Uncinaria hamiltoni* (Beveridge 1980) and tuberculosis, *Mycobacterium pinnipedii* (Mawson & Coughran 1999, Cousins et al. 2003) have been recorded in Australian sea lions and New Zealand fur seals. Their prevalence in wild populations and their effect on survival and reproduction are unknown. Hookworms are common parasites of fur seals and sea lions, and have recently been recorded in pups at Dangerous Reef (R. Gray, pers. comm.). Hookworm can cause anaemia and enteritis and has been associated with morbidity and mortality of sea lion and fur seal pups (Lyons & Keyes 1978, Sepulveda 1998, Lyons et al. 2001, Castinel et al. 2004, Spraker et al. 2004, Lyons et al. 2005, Castinel et al. 2007a, Castinel et al. 2007b, Spraker et al. 2007). Although the relationship between infection rate and mortality is unclear (Lyons et al. 2001), hookworms (*Uncinaria* spp.) have been identified as the primary cause of death in northern fur seal and Californian sea lion pups (*Zalophus californianus*) in some years (Lyons 1963, Lyons et al. 1997, Lyons et al. 2001, Lyons et al. 2005). A hookworm enteritis-bacteraemia complex was the main cause of California sea lion pup mortality at San Miguel Island in 2002-03, and was thought to be a density-dependent disease (Spraker et al. 2007). Hookworm also appears to play a role in the mortality of pups of the New Zealand sea lion, *Phocartos hookeri* (Castinel et al. 2004, Castinel et al. 2007a, Castinel et al. 2007b), South American sea lion, *Otaria flavescens* (Beron-Vera et al. 2004) and Steller sea lion, *Eumetopias jubatus* (Burek et al. 2004) indicating the importance of this pathogen in sea lion populations.

The main point of infection of hookworm to seal pups is via trans-mammary transmission of third-stage larvae (L_3) through the colostrum (first-milk) within the first few days following birth (Castinel et al. 2007a). Larvae mature into adults in this *intestinal phase*, with hookworm eggs appearing in pup faeces by the time they are 2-3 weeks old (Castinel et al. 2007a). Larvae develop through stages L_1 to L_3 within the eggshell, before hatching around the 23rd day (Castinel et al. 2007a). In this *free-living phase*, L_3 larvae can remain in the soil for some period, before they burrow through the skin or are ingested directly by seals where they migrate to fatty tissue (usually in the ventral abdominal blubber and/or mammary glands) in what is know as the *tissue phase* (Castinel et al. 2007a). The L_3 larvae can then remain in arrested development until migrating to the mammary glands in lactating females, potentially under a hormonal signal (Lyons 1963, Lyons & Keyes 1978).

There is still much uncertainty about the ecology of hookworm, particularly how long larvae can survive in the soil, in other substrates types, and the role of temperature and moisture on larval survival during the free-living phase. Over-wintering larvae have been detected in the soil on the Pribilof Islands which are cold and wet, but not at San Miguel Island in California which is warmer and dryer (Olsen & Lyons 1965, Lyons et al. 2001). In addition, there is uncertainty about the relative contribution of the L_3 larvae surviving in the free-living and tissue phases, as the source of infection of pups born in the next breeding season. This point is particularly pertinent in the case of Australian sea lions, which are the only non-annually breeding pinniped, and where hookworm larvae would need to survive up to 18 months in their *free-living* or *tissue phases* in order to infect the next cohort of pups. Given the marked seasonal temperature and moisture fluctuations experienced at Dangerous Reef, marked differences in the survival of free-living larvae produced during summer and winter breeding season is likely, and the hypothesis that climate and season induce fluctuations in hookworm infection and their consequential enteritis-bacteraemia complexes, appears a plausible explanation for the observed marked inter-seasonal fluctuation in pup mortality.

Such marked fluctuations in pup mortality between seasons, is likely to induce marked variance in recruitment and age-structure within ASL populations, and this may explain why we often observe marked inter-seasonal variance in pup production in this species. As such, there is a critical need to understand the role of disease and parasites on pup survival and on the broader population structure and demography of Australian sea lion populations, as it may exert strong density-dependence, as has been shown for other sea lion species (Lyons et al. 2005).

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