Foraging ecology and diet analysis of Australian sea lions

Final Report to the Department of the Environment and Water Resources

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EXECUTIVE SUMMARY

Recent Commonwealth Department of the Environment and the Heritage (DEH) Ecological Sustainable Development (ESD) assessments of the South Australian (SA) rock lobster (SARLF) and southern and eastern scalefish and shark fishery (SESSF) identified interactions with protected species (particularly seals), as one of the key bycatch issues. The issues are most relevant to SA waters where *threatened* Australian sea lion (ASL) populations are located, and where un-quantified interactions between seals and the SARLF and gillnet sector of the SESSF fisheries are known to occur. Recommendations from fishery ESD assessments, fishery Bycatch Action Plans, and a recently drafted Recovery Plan for the ASL, have all identified the importance of assessing and mitigating interactions between seals and commercial fisheries. This study provides a desk-top risk-assessment of seal fisheries interactions in the SARLF and gillnet sector SESSF in SA and adjacent waters, and makes recommendations on future research and management responses.

A review of the PIRSA and AFMA fishery logbooks identified the major constraint to the assessment of bycatch risk to seal subpopulations was the absence of quantitative data on bycatch rates in both the gillnet sector SESSF and SARLF. Anecdotal evidence and entanglement data suggest there has been significant underreporting of seal interactions in these fisheries.

In SA there are 38 ASL subpopulations that produce around 2,674 pups, with the total population size estimated at about 10,900. However, most pup production (67%) occurs at 6 sites, hence the median pup production is very low (25.5 pups), with the majority of sites producing small numbers of pups (60% produce <30 pups per season). Population viability analysis (PVA) on ASL subpopulations reinforced the recent listing of the ASL as a *threatened* species, by confirming that large numbers of subpopulations with low pup production are vulnerable to extinction. PVA simulations suggested that in absence of anthropogenic mortality, a number of ASL subpopulations will go *quasi-extinct* (ie the number of adult females is too low to ensure population persistence; <10 females), but in the face of small (1-2 additional females/year) but sustained anthropogenic mortality (eg. from fishery bycatch), most other small subpopulations will become *quasi-extinct* and negative growth will become a feature of even the largest subpopulations. There is apparent depletion (ie. very low pup production) of a large number of subpopulations that may be indicative of widespread subpopulation declines in the species. That such declines may be ongoing and attributable to anthropogenic mortality (ie. fishery bycatch) is a hypothesis that requires urgent attention.

The risk of bycatch in the gillnet SESSF and SARLF were assessed based on estimates of interaction probabilities. These were a function of the extent to which historic fishing effort and seal foraging effort (based on foraging distribution and population models) overlap in space and time. ASL demonstrated a high risk of significant depletion and quasi-extinction as a result of fishery bycatch. By combining PVA outcomes with bycatch scenarios based on interaction probabilities, this study identified the subpopulations, regions and marine fishing areas (MFAs) most at-risk from seal bycatch.

Bycatch from the gillnet SESSF is most likely to provide the greatest risk to ASL, because of almost complete spatial overlap in fishing effort with ASL foraging effort, it is a year-round fishery with relatively high fishing effort that can potentially interact with all ASL age-classes. The impact from SARLF is likely to be less because there is less overlap in fishing effort with ASL foraging effort, fishing is restricted to seven months of the year (November-May) and bycatch is likely to be restricted to pups and juvenile seals. However, the potential additive and interactive impacts posed by combined bycatch in these fisheries could be significant.

Results from this study suggest the two fisheries investigated lend themselves to different mitigation approaches to addressing seal bycatch issues. In the gillnet SESSF, gear modification options are limited, but spatial management of fishing effort may provide a range of risk-reduction options, but would need to be coupled with independent observer bycatch data to demonstrate and justify the benefits from different closure options. In contrast, there are significant options for gear modification in the SARLF, with pot-protection devices already used in some parts of the fishery. Quantitative testing of these and alternate protection measures (as is taking place in the WA WRLF), and industry wide adoption of best-mitigation practices may eliminate seal bycatch in this fishery, without the need for an expansive and costly independent observer program. Recommendations for future research are made, that should result in the successful mitigation of seal bycatch issues and as a consequence address the recommendations of the fishery ESD, Bycatch Action Plan, ASL Recovery Plan and assist in the recovery of the *threatened* ASL.

Enhanced spatial tools for risk assessment will be required if spatial management of fishing effort is to become a management strategy for mitigating ASL bycatch in the demersal gillnet fishery. Such tools would provide a simple mechanism for policy makers and managers to evaluate the benefits and costs of different spatial allocations of fishing effort, in terms of increasing or decreasing: 1) risk to sea lion subpopulations and 2) fishery catches. However, further development of such tools are required, because current models are limited by the

absence of data on the foraging movements of sea lions in some high-risk regions, as well as the absence of accurate fishing effort data.

Further satellite tracking of ASLs at subpopulations identified as high-risk was undertaken as part of this study, to improve the accuracy of spatial foraging models. This pilot study demonstrated an approach to refine assessments of sea lion interactions with commercial fisheries, where quantitative data on interactions are not available. This approach may enhance the spatial information on which mitigation options and decisions about spatial management of fisheries are based. Importantly, the pilot study determined that colony-specific information on sea lion foraging effort could be used to refine the spatial management of fisheries when using fishing effort data that was summarised into 1 x 1 degree boxes (Marine Fishing Areas). In 2006, demersal gillnet fishers were required to record the latitude/longitude positions of each net-set, and from July 2007, all vessels will be fitted with satellite-linked vessel monitoring systems that will significantly improve the resolution of fishing effort. Following these improvements to fishing effort data sets, it is recommended that bycatch probabilities be re-estimated with colony-specific information on seal lion foraging effort and used to model the benefits of different spatial-management scenarios that could include area-closures, and reductions or redistributions of fishing effort.

The diet of the ASL is currently poorly understood, which hampers our understanding of their key prey species, habitats and trophic interactions with fisheries. Traditional faecal analysis techniques have proven ineffective in ASL because most prey remains are completely digested. We conducted a feeding trial on captive ASL to determine whether faecal DNA analysis could be used to quantify sea lion diet. This study demonstrated that analysis of faecal DNA can detect the presence of sea lion prey DNA, despite no identifiable prey hard parts being recovered from the same scats. The results from our experiments also demonstrate that DNA extracted from ASL scats is highly degraded, because we could not detect prey DNA using molecular primers more than 100 base pairs in length. Quantitative PCR indicated that the amount of mtDNA amplified from scats was related to the amount of novel prey ingested. Quantitative estimates determined the difference between periods of high consumption of novel prey and low consumption of a particular prey type, but did not pick up smaller differences in consumption rates. Interestingly, mtDNA quantitative estimates were significantly higher for squid than shark from periods when the seal was fed in equal proportions. These differences may be related to the concentration of DNA in the different tissue types of each species.

KEYWORDS: SA rock lobster fishery (SARLF), gillnet sector of the South Eastern Scalefish and Shark fishery (SESSF), Australian sea lion (ASL), bycatch

INTRODUCTION

The Australian sea lion (ASL) *Neophoca cinerea* is one of five sea lion species in the world. Sea lions form around one-third of species in the Otariidae family of seals that includes all of the fur seals and sea lions. Over recent decades there has been growing concern over the status of all five sea lion species. In the North Pacific Ocean, the Steller sea lion, *Eumetopias jubatus*, has been declared endangered in parts of its range and is considered threatened with extinction in other parts (Trites et al. 2007). Although the total population of California sea lions in California and Mexico is increasing (Caretta et al. 2004), the Mexican stock is in decline (Szteren et al. 2006). There have also been reductions in numbers of the Galapagos subspecies of the Californian sea lion, *Zalophus californianus wollebaeki* (Alava and Salazar 2006) and the Japanese subspecies, *Z. c. japonicus,* is possibly extinct (Mate 1982). Numbers of South American sea lions, *Otaria flavescens*, have reduced considerably in recent years (Crespo and Pedraza 1991, Reyes et al. 1999, Shiavini et al. 2004), especially in the Falkland Islands (Thompson et al. 2005). Numbers of New Zealand sea lions, *Phocarctos hookeri* (Lalas and Bradshaw 2003) and ASL (McKenzie et al. 2005) have not recovered from historic sealing and form the smallest populations of all sea lion species.

The ASL is Australia's only endemic and least-abundant seal species. It is unique among pinnipeds in being the only species that has a non-annual breeding cycle (Gales et al. 1994). Furthermore, breeding is temporally asynchronous across its range (Gales et al. 1994, Gales and Costa 1997). It has the longest gestation period of any pinniped, and a protracted breeding and lactation period (Higgins and Gass 1993, Gales and Costa 1997). The evolutionary determinates of this atypical life-history remain enigmatic. Recent population genetic studies have indicated little or no interchange of females among breeding colonies, even those separated by short (20 km) distances (Campbell 2003). The important management implication of extreme levels of female natal site-fidelity (philopatry) is that each colony effectively represents a closed population.

There are 73 known breeding locations for ASLs, 47 of which occur in South Australia where the species is most numerous (80% of pups counted), with the remainder (26 colonies) occurring in Western Australia (McKenzie et al. 2005). The species was subject to sealing in the late 18th, the 19th and early 20th centuries, resulting in a reduction in overall population size and extirpation of populations in Bass Strait and other localities within its current range. Total pup production for the entire species during each breeding cycle has been estimated at about 2,500 with an estimated overall population size based on a demographic model

developed by Goldsworthy et al (2003), of around 9,800 (McKenzie et al. 2005). A re-analysis of this demographic model, in conjunction with improved estimates of pup production for some sites, has increased estimates of the SA pup production to about 2,700 per breeding cycle and the size of the ASL population in SA to about 10,900 individuals (Goldsworthy and Page 2007). Based on pup production estimates of 709 for WA sites (Goldsworthy et al. 2003), the total pup production for the species is currently estimated at about 3400 per breeding cycle, with an estimated overall population estimate of around 14,000 (Goldsworthy, unpublished data). The life tables associated with the population model produced population estimates that were 4.08 times that of pup production (Goldsworthy and Page 2007), which is about mid-point of the range expected for pinniped populations (Harwood and Prime 1978).

There are 39 ASL breeding sites in SA, when the criterion for classification as a breeding colony is set at ≥ 5 pups present per breeding cycle (McKenzie et al. 2005, Fig. 1). Of these, only six (16%) produce more than 100 pups, and these account for 67 % of the State's pup production. The largest population is Dangerous Reef in Southern Spencer Gulf (585 pups), followed by The Pages (577 pups) in Backstairs Passage between Kangaroo Island and mainland Australia. The next largest populations are Seal Bay (214 pups) on Kangaroo Island, West Waldegrave (157 pups) and Olive Islands (131 pups) off the west coast of the Eyre Peninsula, and Purdie Island (132 pups) in the Nuyts Archipelago (summarised in Goldsworthy and Page 2007). The median pup production for SA colonies is 25.5 per colony, with 60% of breeding sites producing fewer than 30 pups per season, 42 % fewer than 20 pups, and 13% fewer than 10 pups (Goldsworthy and Page 2007). These analyses do not take into account at least another 11 breeding sites (termed 'haul-out sites' with occasional pupping), where fewer than 5 pups have been recorded at some time (McKenzie et al. 2005).

The ASL is Australia's only endemic pinniped, and was recently listed under the Environment Protection and Biodiversity Conservation Act as *Threatened*, *'Vulnerable'* category (gazetted 14 Feb 2005), and a recovery plan is has been drafted by Commonwealth DEH.

Although the pre-harvested population size of the ASL is unknown, the overall population is still believed to be in recovery. Unlike Australian fur seal, *Arctocephalus pusillus doriferus* and New Zealand fur seal, *Arctocephalus forsteri* populations, which have been recovering rapidly throughout southern Australia, there is a general view that the overall population recovery of the ASL appears to be limited, and it is unclear why.

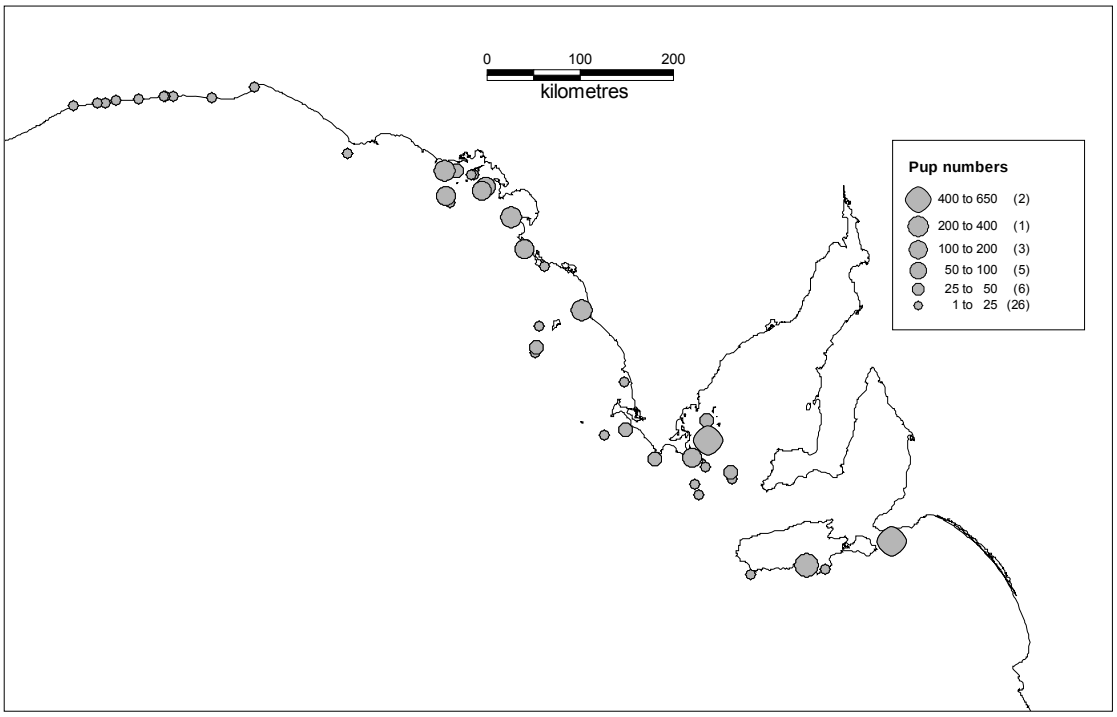

Figure 1. Location and relative size of ASL breeding colonies (grey circles are scaled based on pup production per breeding season) in South Australia.

NEED

Provisions of the Commonwealth Environment Protection and Biodiversity Conservation Act (*EPBC Act*), require strategic assessment of fisheries against the principles of ESD including the need to monitor, assess and, if necessary, mitigate the interactions of fisheries with protected species (Fletcher et al. 2002).

In both the SARLF and gillnet sector of the SESSF there are considerable policy and research requirements relating to fishery interactions with sea lions that need to be undertaken in order to fulfil recommendations detailed in recent Bycatch Action Plans and ESD Assessments (Goldsworthy and Page 2007).

The Australian Governments' National Seal Action Plan requires the estimation of sea lion bycatch in gillnet, trawl, trap, dropline and longline fisheries and quantification of interactions with fishing equipment.

ASL are listed as protected species under the Commonwealth *EPBC Act*, and are known to interact with lobster and gillnet fisheries.

Methods for assessing, monitoring and mitigating the interactions of ASL with lobster and gillnet fisheries are needed urgently. This need is greatest in South Australia, where:
1. The majority of subpopulations of the ASL occur, and where declining populations have been identified;
2. A valuable ($70 M) fishery for southern rock lobster (*Jasus edwardsii*) is located;
3. Un-quantified interactions between ASL and the SARLF and gillnet sector of the SESSF fisheries are known to occur.

The need to assess the interactions between these fisheries and ASL is particularly pressing because it:

1. Is Australia's only endemic pinniped;
2. May be more vulnerable to fishery-induced mortality than other species;
3. Is mainly confined to South Australia, with ~80% of pup production occurring in the State;
4. Has recently been listed as *Threatened* (*Vulnerable* Category) under Commonwealth *EPBC Act*.

AIMS AND OBJECTIVES OF THE REPORT

The aims of this report are to:
1. Use satellite tracking methods to assess the spatial distribution of foraging effort in ASL populations in close proximity to areas of high catch and effort in the SARLF and shark gill-net fisheries;
2. Assess the degree of spatial overlap in ASL foraging space and catch and effort in the SA rock lobster and shark gill-net fisheries;
3. Provide advice to DEH on the risks posed by commercial fisheries that overlap with the foraging space of sea lions and provide advice on the spatial management of the fisheries;
4. To develop novel, quantitative PCR-based faecal DNA assessment methods that can be used to identify key prey taxa and quantify their contribution in the diet;
5. To calibrate the accuracy of the method by undertaking a feeding trial on captive seals.

This report addresses objective 1 as separate chapter and the following two chapters address objectives 2-3 and 4-5, respectively.

THE SPATIAL DISTRIBUTION OF FORAGING EFFORT IN AUSTRALIAN SEA LION POPULATIONS IN CLOSE PROXIMITY TO AREAS OF HIGH CATCH AND EFFORT IN THE SA ROCK LOBSTER AND SHARK GILL-NET FISHERIES

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Introduction

A recovery plan for the Australian sea lion (ASL) was recently drafted by NHT (NHT, 2005), after the species was listed as *Vulnerable* pursuant to the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act). The decision to upgrade the species from *Conservation Dependent* was based primarily on reports to DEWR (formerly DEH and administrator of the *EPBC Act*), which detailed the possible impediments to growth in ASL populations (McKenzie et al. 2005).

The recent report to DEWR identified a number of factors that might result in population declines, with anthropogenic factors of top-down (mortality driven) origin being considered the most likely and relevant (McKenzie et al. 2005). Direct killing, pollutants and toxins, plus fishery by-catch and entanglement were considered as possible contributors, although there is currently no evidence that direct killing or pollution and toxins are contributing factors. However, a number of sources indicated that fishery by-catch and entanglement might be a contributing mortality factor in some parts of the range of ASL (Page et al. 2004, Goldsworthy and Page 2007). As a consequence, the report ranked fishery by-catch and entanglement as the most important contributor to limited growth in some populations and declines in others (McKenzie et al. 2005).

A substantial body of information exists regarding operational interactions between ASL and southern rock lobster (*Jasus edwardsii*) pots and demersal gummy shark (*Mustelus antarcticus*) and school shark (*Galeorhinus galeus*) gill-nets. The drowning of significant numbers of ASL pups in Western Australia and juvenile Australian fur seals in Victoria in rock lobster pots when attempting to remove either baits or rock lobster has been

speculated over the past 30 years (Warneke 1975; Gales et al. 1994). However, recent reports now confirm that these interactions may occur in large enough numbers to potentially impact on the conservation of some breeding populations in Western Australia (Campbell 2004). Anecdotal reports that operational interactions between seals and shark fishing operations are more likely to occur in coastal waters and the admission by one fisher who claimed to have caught about 20 ASL yearly both suggest that sea lions also drown in shark gill-nets (Shaughnessy et al. 2003). The high incidence of ASL entanglements in monofilament shark gill-net material on Kangaroo Island suggests that the level of interaction with shark gill-nets may be high (Page et al. 2004).

The recent interest in the impact of commercial fisheries on ASL populations has now confirmed that operational interactions occur with rock lobster pots and shark gill-nets. In spite of this, their occurrence was completely absent from logbooks submitted by the South Australian Rock Lobster Fishery (SARLF) during a recent review (Hamer 2007). The same review found that rates of operational interactions with shark gill-net fishing operations in the Southern and Eastern Scalefish and Shark Fishery (SESSF) were very low, with only seven mortalities reported between 1999 and 2004. Low interaction rates with shark gill-net fishing activities in Victorian waters were also reported in logbook records (Walker et al. 2005). The disparity between these records and the apparent broad scale occurrence of operational interactions between ASL and these two fisheries suggest that there has been and is currently a high degree of underreporting in both of these fisheries.

Commercial fisheries have been required to undergo strategic assessment pursuant to the EPBC Act since its enactment in 2000. In particular, their activities are assessed under Part 10, against the *Guidelines for the Ecologically Sustainable Management of Fisheries* , reflecting internationally recognised principles of ecologically sustainable development (ESD). Monitoring, assessing and mitigating operational interactions with protected species subject to a recovery plan is the manifestation of these principals. The requirement for this course of action was also reflected in the original listing advice for the species, which also confirmed the need to establish a unified approach under the recovery plan for the species with complementary threat abatement through changes in fishing practices.

The SARLF and the gill-net sector of the SESSF have recently undergone strategic assessment, in April 2003 and November 2002, respectively (Department of the Environment and Heritage 2003a & b). DEWR responded by handing down a number of

recommendations for improvement to the management of each fishery, some of which specifically focused on operational interactions with fur seals and sea lions. In September 2003, DEWR recommended that the SESSF gill-net sector mitigate interactions with seals by establishing a reporting system within 12 months (18i) and appropriate mitigation tools, such as spatial closures, within 3 years (18ii). Similarly, DEWR responded in October 2003 to the SARLF ecological assessment by recommending that a mandatory reporting system be established within 18 months (10) and appropriate mitigation measures be implemented within 2 years (11).

All of the time limits associated with each DEWR recommendation for both fisheries have now expired without action. However, the existence of each of these fisheries is contingent on receiving an exemption to trade in a native species, pursuant to Parts 13 and 13A of the EPBC Act. Compliance to this aspect of the EPBC Act is of particular relevance, because all declarations, agreements and decisions made in relation to such activities pursuant to the EPBC Act are in recognition of the *Convention on International Trade in Endangered Species of Wild Fauna and Flora 1973* (CITES).

The need to address these DEH recommendations is greatest in South Australian waters, where the majority of subpopulations of ASL occur and where declining populations have been identified (Goldsworthy et al. 2003; Shaughnessy et al. in press). This is particularly important while operational interactions between ASL and southern rock lobster pot and shark gill-net fishing activities remain unquantified.

In the absence of data and quantified information regarding interaction rates, management agencies have struggled to determine the appropriate course of action for management of ASL. This includes justification of observer programs, which are typically costly to industry, especially if observer effort is to be representative of fishing effort across the fleet and its geographic range. In addition, a relatively low level of operational interactions may have a relatively large impact on small breeding colonies, but the impact of relatively high interaction rates adjacent to large breeding colonies may be relatively small. How best to estimate the potential impacts of by-catch on protected species and how to recognise the most appropriate mitigation options is a perennial issue faced by the managers of fisheries and protected species.

A key aspect of assessing the potential threat of fishing activities on ASL populations is to examine the extent to which they spatially utilise the marine environment and the degree to which their efforts overlap (Goldsworthy et al. 2003). Data obtained through

satellite telemetry will be used to determine the spatial utilisation of the marine environment by foraging adult female sea lions from breeding colonies in close proximity to regions of high rock lobster pot and shark gill-net fishing effort. These results are then compared with the results of a spatial model developed by (Goldsworthy and Page 2007), to assess the level of agreement between the two sources and how a combination of the two might be used to refine the degree of seal-fisheries overlap in these regions. The results will assist in the implementation of the draft recovery plan, by providing information on the degree and distribution of spatial overlap between the two and the subsequent distribution of risk to individual populations.

Methods

Capture, restraint and anaesthesia

To determine the spatial distribution of ASL foraging effort, satellite transmitters (KiwiSat 101, Sirtrack, Havelock North, New Zealand) were deployed on sexually mature females. This demographic was chosen, as they are most important for providing recruits to the next generation of the population through the contribution of pups. A hoop-net was used to initially restrain individual animals. Anaesthesia was induced and maintained using Isoflurane® (Veterinary Companies of Australia, Artarmon, Australia), administered via a purpose-built gas anaesthetic machine with a Cyprane Tec III vaporiser (Advanced Anaesthetic Specialists, Melbourne, Australia). Once anaesthetised, a satellite transmitter was then attached to the guard hairs along the mid-dorsal line, between the fore-flippers, using a thin layer of Araldite® 2107 (Vantico, Basel, Switzerland), a flexible, two-pack epoxy adhesive. The welfare and health of the animal was monitored throughout the procedure, in accordance with the procedures approved separately by Primary Industries and Resources SA (PIRSA) and University of Adelaide Animal Ethics Committees.

Data collection

Once anaesthetised, the length (nose to tail) and mid-planar girth (from behind the fore-flippers) of each sea lion was measured (± 1 cm). Unique numbered plastic tags (Supertags®, Dalton, Woolgoolga, Australia) were applied to the trailing edge of each fore-flipper of animals at breeding colonies currently included in a broader, long term population monitoring program.

Most satellite transmitters were recovered by recapturing animals with the hoop net. However, animals that consistently remained very alert or close to the water, thus reducing the likelihood of being successfully restrained in this manner, were administered Zoletil® (~1.1 to 1.2 mg per kg) via a 1.0 cc barbless dart (Pneu-Dart®, Pennsylvania, USA), delivered by a CO_2 powered dart gun (Taipan 2000, Tranquil Arms Company, Melbourne, Australia). The satellite transmitter was then removed by cutting through the guard hairs immediately beneath the unit.

Data analyses

Satellite location data were obtained through Service Argos Inc. Due to the variability in spatial quality of the information obtained, the least accurate (class *Z*) positions were omitted from the analysis (Sterling and Ream 2004). Spatial analysis was conducted using R statistical software (version 2.3.0, R Development Core Team, R Foundation for Statistical Computing, Vienna) and the *timeTrack* package (version 1.1-5, M. D. Sumner, University of Tasmania, Hobart) and were used to redistribute at-sea locations during foraging trips (McConnell et al., 1992), based on a maximum possible horizontal speed of 11.93 km/h (Goldsworthy et al., unpublished data).

It was assumed that all the sea lions carrying satellite transmitters would consistently return to their colony to suckle pups, so all foraging trips were separated by haulout periods. Precise coordinates were entered for the central location of each breeding colony, so the beginning (when the animal departed) and end (when the animal hauled out) of foraging trips could be estimated. Foraging trips typically started after a few days of non-transmission and ended after the transmitter sent several high-class hits, prior to the timer (activated by a salt water switch) temporarily turned the unit off. If a satellite transmitter failed while an animal was at sea, that entire foraging trip was excluded from the analyses. However, in many cases the satellite transmitter did not give any locations after the sea lion appeared to have hauled out on land. In these cases, the following criteria were used to estimate when the sea lion hauled out: 1) no locations were received by satellites for > 8 hr, possibly indicating that the haulout timer had switched off the satellite transmitter because the animal was not in the water, 2) the distance to the colony was calculated from the last satellite location to determine if it was close to the colony and 3) the direction of travel indicated that it was travelling toward the colony,

Due to the inherent inaccuracy associated with the location data, determining when foraging trips began and ended was difficult at best. We calculated the distance between the first/last location at sea and the colony where the seal hauled out and interpolated the start/end times based on average travel speeds between these locations. The average travel speed of sexually mature female ASL was used in this calculation, based on outbound (2.63 $\pm$ 0.62 km/h) and inbound (6.43 $\pm$ 1.29 km/h) estimates derived from previous studies at Dangerous Reef, South Australia (Goldsworthy et al., unpublished data).

The R statistical software and *timeTrack* were used to determine the number of different 1 km^2 grid cells entered by each seal and the proportion of time they spent in each. We assumed a constant horizontal speed between each filtered location and interpolated a new position for each 15 minute block of time along the path between them. The number of original and interpolated positions within each cell were then summed and assigned to a central node within it. To ensure the different deployment durations recorded for different seals did not bias comparisons, the amount of time spent in each cell was converted to a proportion of the total time spent at sea for each individual. The proportional values of the time spent in each 1 km^2 grid cell were then plotted, using the triangulation with a smoothing function in VerticalMapper® and MapInfo® (version 2.5 and 8.0 respectively, MapInfo Corporation, New York, USA).

Fishing effort data for the gill-net sector of the SESSF, within and adjacent to South Australian waters, were derived from fishery logbooks obtained from the Australian Fisheries Management Authority submitted over a 32 year period, between 1973 and 2004. Fishing effort data for the SARLF were obtained from SARDI Aquatic Sciences for the 35 year period between 1970 and 2004. The spatial resolution of logbook data has traditionally been poor in both of these fisheries, with management areas for both typically following degree lines of longitude and latitude and referred to as marine fishery areas (MFAs).

The same processing techniques used to determine the proportion of time that a seal spent in each 1 km^2 grid cell were used to determine the proportion of time spent within each MFA, by pooling the values for all cells that fell within the boundary of each MFA. The proportion of fishing effort (rocklobster: pot-lifts.year-1, shark: km net-lifts.year-1), were also calculated across all MFAs adjacent to the South Australian coast. The degree of spatial overlap between sea lions and each fishery was then calculated by multiplying

the proportional seal foraging effort (time) in each MFA by the proportional fishing effort in the MFAs visited by sea lions from each site.

Using the distributions of foraging effort data from satellite tracking data and the models developed by Goldsworthy and Page (2007), the total amount of foraging effort within each fishery MFA was calculated by summing the values for each grid cell within each fishery MFA. The proportion of the total sea lion foraging effort (*FE*), which occurred within each fishery MFA, was then derived from satellite tracking data and the models. Based on the historical fishing effort records, the product of the proportion of (modelled and actual) seal *FE* and fishing effort (*F*) were calculated within each MFA. The extent of overlap between fishing effort and seal foraging (overlap index, *OI*, adapted from Schoener (1968)) within each MFA was then calculated as a probability (*f*) by dividing the product of the proportion of fishing effort (F_{FMA}) and the proportion of seal foraging effort (FE_{FMA}) within each MFA, by the sum of all the products from all MFAs, as follows:

$$f(OI)_{FMA} = \frac{f(FE_{FMA}).f(F_{FMA})}{\sum_{FMA=1}^{FMA=N_{FMA}} f(FE_{FMA}).f(F_{FMA})}$$

where N_{FMA} is the total number of MFAs

We summarised the foraging behaviour of each seal to calculate differences in the habitats they utilised (tracking data) and the habitats that a distance-based foraging model (Goldsworthy and Page 2007) predicted that sea lions from Olive and South Page Island would use. The models developed by Goldsworthy and Page (2007) assumed that seals from Olive and South Page Islands foraged within a set range from their colony, according to the normal probability density function. We calculated the differences in the location of actual and modelled foraging habitats based on the proportional values of time-spent in area. We also calculated differences in the depth of habitats between the actual and modelled distributions of sea lion foraging locations. Mean depth values for both the actual and modelled data were weighted by the amount of time spent over each depth. Depth data were obtained from the GeoScience Australia 1 x 1 km grid. The depth values for each location were interpolated as functions of their distance from the nearest nodes and assigned to each 15 min interval.

Results

Satellite transmitters (KiwiSat 101, Sirtrack, Havelock North, New Zealand) were deployed on 13 (Olive) and 10 (South Page) adult females. Summary maps showing the SESSF and SARLF MFAs and the spatial distribution of foraging effort of the adult females from Olive Island and South Page Island are presented in Figures 2-5. Maps for each individual seal are presented in the Appendix. Details on the morphology of individual seals and the duration of the satellite tracker deployments are summarised in Table 1.

Adult females tracked form Olive Island averaged 154.2 ± 6.6 cm in length (n = 12, range: 145-167.5 cm) and averaged 89.7 ± 5.7 cm in girth (n = 12, range: 84-100) (Table 1). Adult females tracked from South Page Island averaged 152.5 ± 6.5 in length (n= 9, range: 143-161 cm) and averaged 85.3 ± 6.6 cm in girth (n = 9, range: 77.5-95 cm) (Table 1). On average, sea lions were tracked for 30 ± 8 d (range: 6-35) at Olive Island and 80 ± 39 d (range: 3-105) at South Page Island (Table 1). The mean maximum distance reached by individuals from Olive Island was: 58 ± 20 km (n = 12, range: 32-107) and from South Page Island was 92 ± 32 km (n = 10, range: 32-147) (Table 1, Fig 2, 4).

The average direction in which 4 individuals (seal no. 1, 923, 951 and 952) from Olive Island travelled was west to southwest, indicating they typically used offshore, shelf habitats while foraging (Table 1, Fig 4,5). The remaining 8 individuals typically foraged between north and east of the colony, predominantly within Streaky Bay (Table 1, Fig 4,5). The direction in which 3 females (# 956, 957 and 959) from South Page Island travelled indicated they typically foraged to the south and southeast of the colony, concentrating their efforts in a region parallel to the 100 m depth contour, likely containing a bathometric feature (Table 1, Fig 2). The other 7 females typically foraged northwest of the colony, along the north coast and to the north of Kangaroo Island, within the southern part of the Gulf of St Vincent (Table 1, Fig 2).

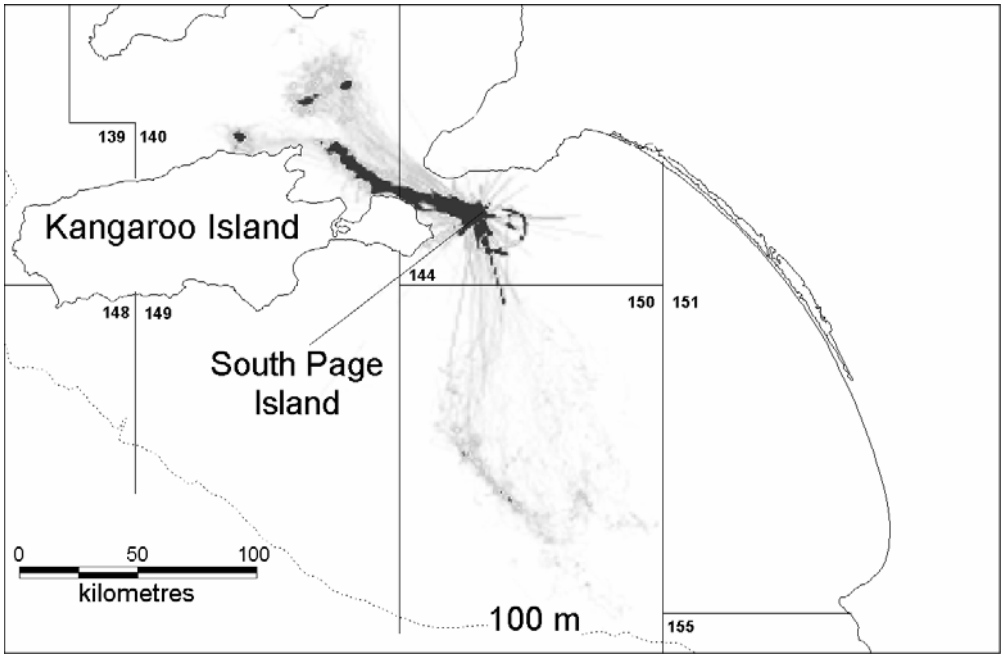

Figure 2. Geographic distribution of the amount of time spent in 1 km^2 cells by lactating female ASL that were satellite-tracked from South Page Island (n = 10). Dark grey/black areas indicate regions of relatively high foraging effort. SESSF MFAs are shown and numbered.

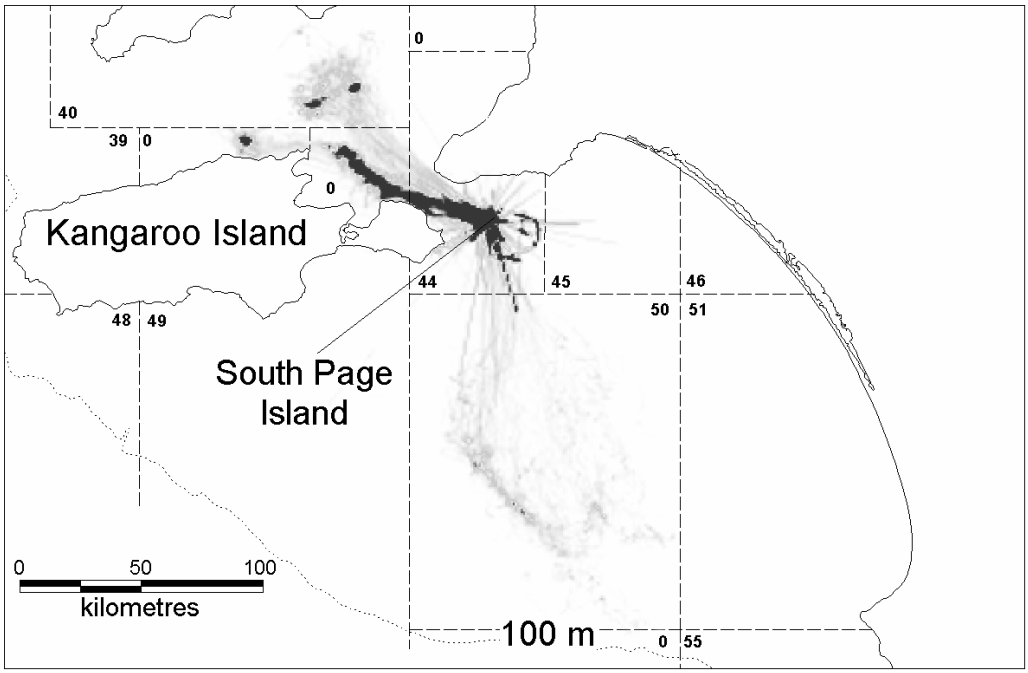

Figure 3. Geographic distribution of the amount of time spent in 1 km^2 cells by lactating female ASL that were satellite-tracked from South Page Island (n = 10). Dark grey/black areas indicate regions of relatively high foraging effort. SARLF MFAs are shown and numbered.

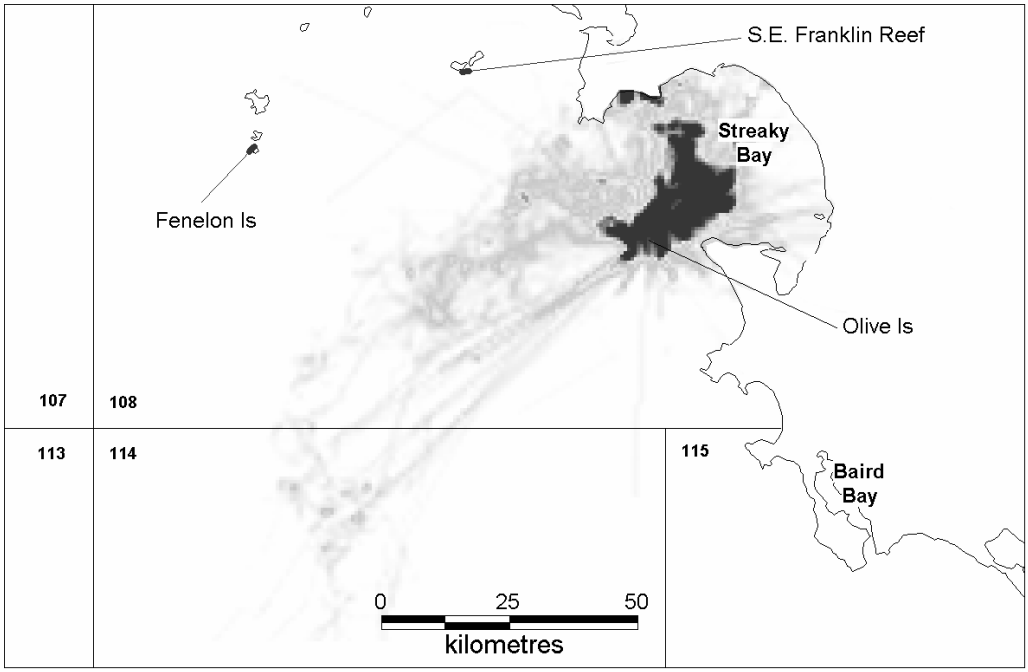

Figure 4. Geographic distribution of the amount of time spent in 1 km^2 cells by lactating female ASL that were satellite-tracked from Olive Island (n = 12). Dark grey/black areas indicate regions of relatively high foraging effort. SESSF MFAs are shown and numbered.

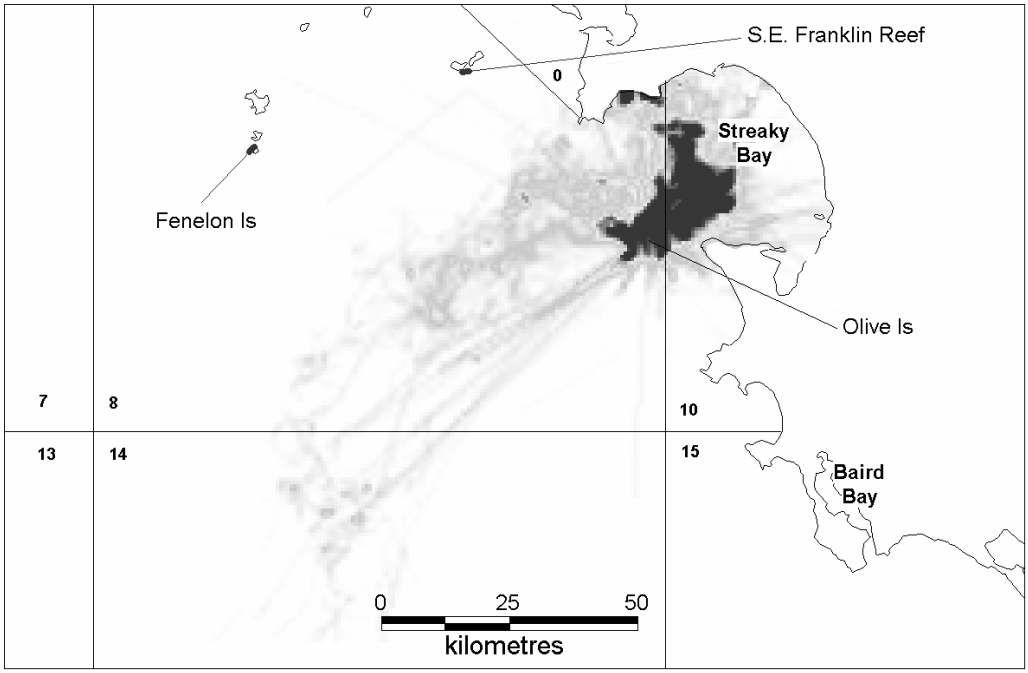

Figure 5. Geographic distribution of the amount of time spent in 1 km^2 cells by lactating female ASL that were satellite-tracked from Olive Island (n = 12). Dark grey/black areas indicate regions of relatively high foraging effort. SARLF MFAs are shown and numbered.

Table 1. Length and girth, duration of satellite transmitter deployments, maximum distance reached and average bearing travelled of individual ASL from Olive Island (n = 12) and South Page Island (n = 10).

Seal no.	Body length at deployment (cm)	Body girth at deployment (cm)	Duration analysed (d)	Maximum distance from colony (km)	Average bearing	SD
Olive Island						
1	167.5	99.5	68	66	245	72
923	149.0	95.0	54	107	229	26
924	153.0	95.5	58	43	85	71
925	161.0	98.0	63	46	71	70
942	159.0	85.0	74	50	80	117
943	151.0	86.0	65	55	94	76
944	161.0	89.0	72	59	81	84
945	155.0	86.0	69	32	114	141
949	148.0	84.0	64	62	88	96
950	145.0	87.0	58	39	92	122
951	150.5	88.0	63	83	253	62
952	150.0	83.5	67	59	265	106
South Page Island						
946	150.0	77.5	103	105	275	34
954	-	-	104	95	293	32
955	158.0	93.5	25	76	264	53
956	147.0	89.0	49	103	185	39
957	158.5	84.0	101	147	184	36
958	146.5	79.0	101	93	295	38
959	150.5	81.5	101	126	171	43
960	161.0	89.0	3	32	219	80
961	158.0	95.0	105	68	268	57
982	143.0	79.0	104	74	265	46
Olive: mean	154.2	89.7	64	58	-	-
S Page: mean	152.5	85.3	80	92	-	-
Overall: mean	153.5	87.8	71	74	-	-

At Olive Island, the main difference between the results of satellite tracking data and the outcome of the spatial model (Goldsworthy and Page 2007) occurred because satellite tracked sea lions typically utilised regions that were closer to the colony than indicated by the model. Tracked animals from Olive Island spent 95.9% of their time at sea within SESSF MFA 108 (inshore, where the island is located) and 4.1% in MFA 114 (offshore), while the model indicated 83% and 10.9% respectively (Table 2). Similarly, tracked animals spent 53.1% of their time in SARLF MFA 10 (inshore) and 42.7% of their time in SARLF MFA 8 (offshore), while the model indicated 18.3% and 61.5% respectively. However, the regions utilised were the same between the tracked data and the model for both management schemes (depicted in Figures 3 and 4). Under both management schemes, the model predicted that individual animals spent more time offshore, rather than around the colony and in inshore waters. Depths utilised by satellite tracked animals were 25.8 $\pm$ 22.2 m in SESSF MFA 108 and 73.7 $\pm$ 7.8 m in SESSF MFA 114, while the

model indicated 38.8 ±23.2 m and 65.2 ±2.2 m respectively. This suggests that waters adjacent to the colony, where tracking data indicated that individuals spent more time when compared to the model, were shallower.

The level of overlap between sea lion foraging effort and fishing effort (for both SARLF and SESSF management schemes) at Olive Island was generally greater when tracking data was used, compared with the distribution of foraging effort predicted by the model. This was the result of the model redirecting a higher proportion of time toward MFAs that had relatively higher fishing effort (Table 2). Foraging effort determined from tracking data was 13% higher in SESSF MFA 108, which encompasses the colony, than predicted by the model. This is particularly important because 49% of the shark fishing effort that occurred in SESSF MFAs visited by the sea lions tracked from Olive Island occurred there. Conversely, seal foraging effort determined from tracking data was lower in SESSF MFAs 114 and 115 (which are some distance to the south of the colony) by 7% and 6% respectively, when compared with the model. These two SESSF MFAs account for 22% and 29% of the shark fishing effort in SESSF MFAs visited by tracked females from Olive Island.

Spatial analysis using MFAs used by the SARLF produced similar results. Foraging effort of sea lions from Olive Island determined from tracking data was 35% higher in SARLF MFA 10 than predicted by the model. Again, this is particularly important because this SARLF MFA accounts for 26% of the shark fishing effort in the SARLF MFAs visited by the tracked sea lions. In contrast, seal foraging effort determined from tracking data was 19% lower in SARLF MFAs 8 than predicted by the model, accounting for 20% of the shark fishing effort in SARLF MFAs visited by the tracked animals. In addition, seal foraging effort reduced by 14% in regions that were not used in the SARLF.

In contrast to the tracking and model comparisons at Olive Island, animals that were satellite tracked from South Page Island generally used regions that were further from the colony than the model predicted. Tracking data indicated that female sea lions from South Page Island spent 43.5% of their time at sea within SESSF MFA 150 and 38.5% in MFA 140 (at distance from the colony), while the model predicted 14.4% and 7.8% respectively (Table 2). Conversely, tracking data indicated that sea lions spent 18% of their time in SESSF MFA 144, which encompasses the colony, while the model predicted 71.8%.

Spatial analysis using MFAs used by the SARLF in the South Page Island region produced similar results to those for the SESSF. Satellite tracked animals in SARLF MFA 44, which encompasses the colony, spent less time there than the model predicted, being 14.6% and 52.6% respectively. In addition, animals tracked in SARLF MFA 45, nearby and to the east of the colony, spent less time there than the model predicted, being 3.3% and 18.7% respectively.

Discussion

Spatial management of fisheries can be effective at reducing interactions with protected species that are not randomly distributed in the region used by fishers (Hall 1996). This study demonstrates a suitable approach to assessing spatial overlaps between pinnipeds and commercial fisheries, where quantitative spatial data on interactions are not available. By comparing actual spatial data with spatial models, such as those developed by Goldsworthy and Page (2007), the accuracy of the models can be determined on a case by case basis, at a regional or sub-population scale. Importantly and specifically, this approach provides a means to assess the spatial models as a tool for managing the potential effects of the SARLF and SESSF gill-net sector on ASL populations across the range in which the two spatially overlap.

In this study, the level of agreement between the two approaches – satellite tracking data and the model – is limited, with discrepancies in the amount of time that the tracked animals spent in particular management zones, compared with the amount of time predicted by the model. However, while data pertaining to the spatial distribution of foraging effort from many populations remains either limited or absent altogether, the model provides the most realistic and readily accessible approach. Furthermore, continuing to develop this approach, by refining models with data collected from satellite tracked animals, is particularly important considering the practical limitations and cost prohibitive nature associated with tracking a sufficiently representative number of animals. Moreover, comparing the model with satellite data allows the user to determine whether a sufficient quantity of animals have been tracked to ensure that the model has become representative of the sub-population or breeding colony and whether a generic model is suitable for application at a colony specific scale.

Table 2. Comparison of the: 1) distributions of foraging effort, 2) mean depths used, 3) proportional overlap with fishing effort (rocklobster: pot-lifts.year^{-1}, shark: km net-lifts.year^{-1}) based on satellite tracking and distance-based models (Goldsworthy and Page 2007) for each MFA. The differences between using satellite tracking data and modelled data are shown.

	Time spent in MFAs			Mean depth used by ASL					Fishing effort		Seal-fishery overlap		
MFA	Model	Tracking	Difference	Model	SD	Tracking	SD	Difference	km & pot	%	Model	Tracking	Difference
Shark													
Olive Island													
108	83	96	13	39	23	26	22	-13	1594	49	40	47	7
114	11	4	-7	65	2	74	8	9	723	22	2	1	-1
115	6	0	-6	45	25	-	-	-	968	29	2	0	-2
Total, mean	**100**	**100**		**50**	**14**	**50**	**34**	**0**	**3285**	**100**			**4**
South Page Island													
140	8	39	31	21	9	23	10	2	627	8	1	3	2
144	72	18	-54	36	10	48	20	12	751	10	7	2	-5
149	6	0	-6	45	13	-	-	-	1498	20	1	0	-1
150	14	43	29	52	6	35	14	-17	2062	28	4	12	8
151	0	0	0	48	8	-	-	-	2458	33	0	0	0
Total, mean	**100**	**100**		**40**	**12**	**35**	**13**	**-5**	**7396**	**100**			**4**
Rocklobster													
Olive Island													
8	62	43	-19	49	16	45	19	-4	22500	20	12	9	-3
10	18	53	35	12	17	11	10	-1	29100	26	5	14	9
14	0	4	4	-	-	74	8	-	0	0	0	0	0
15	6	0	-6	45	25	-	-	-	59300	53	3	0	-3
0	14	0	-14	51	27	-	-	-	0	0	0	0	0
Total, mean	**100**	**100**		**39**	**18**	**43**	**32**	**4**	**110900**	**100**			**3**
South Page Island													
40	1	12	11	26	2	33	2	7	47800	30	0	4	3
44	53	15	-38	34	10	45	21	11	4100	3	1	0	-1
45	19	4	-15	40	11	61	6	21	23900	15	3	1	-2
46	0	0	0	39	11	-	-	-	10300	6	0	0	0
49	5	0	-5	45	13	-	-	-	54400	34	2	0	-2
50	14	43	29	52	6	35	14	-17	19700	12	2	5	4
0	8	26	18	20	8	18	8	-	0	0	0	0	0
Total, mean	**100**	**100**		**37**	**11**	**38**	**16**	**2**	**160200**	**100**			**2**

This study tracked adult female sea lions from two of the subpopulations that are most at risk from fisheries by-catch (Goldsworthy and Page 2007). These two colonies were identified as such in a recent desktop analysis by Goldsworthy and Page (2007), which combined a PVA for the ASL at a colony scale and fishery data to assess their relative degree of risk to decline due to interaction probabilities and subsequent fishery induced mortalities. These analyses identified that subpopulations from two regions were most at-risk. Firstly, the top seven subpopulations at greatest risk were in the Nuyts Archipelago and Streaky Bay regions, off the western Eyre Peninsula (Olive Island, East Franklin Reef, West Island, Purdie Island, West Franklin Reef, Lounds Islands and Breakwater Reef), while the next three subpopulations occurred south and east of Kangaroo Island (Seal Bay, the Seal Slide and The Pages).

The distance-based models used by Goldsworthy and Page (2007) did not incorporate directional information on sea lion foraging effort and the models used maximum distances that were based on data from other sites. The non-random distributions of sea lion foraging effort (Fig 2-5, Table 1) confirmed that satellite tracking data can be used to refine assessments of seal-fisheries overlaps. Differences in the mean maximum distances travelled by sea lions from Olive (58 km) and South Page (92 km) Islands highlighted the importance of acquiring tracking data from other colonies that are at risk from fisheries by-catch, so that the degree to which the model represents the foraging distribution of a particular colony can be determined. If the model and the spatial data sourced from satellite tracking are similar, then the model would be useful for assessing the risk of decline of small populations due to fisheries induced mortality at a finer scale than is currently feasible.

Based on the findings of this study and the premise that spatial management of the non-randomly distributed foraging habits of protected species, the spatial management of shark gill-net fishing activity in areas where ASL forage could markedly enhance the conservation of the conservation of ASL. This is of particular importance when considering that there are limited options for modifying gear or fishing behaviour in demersal gill-nets. Such tools would provide a simple approach for policy makers and managers, enabling them to evaluate the benefits and costs of different spatial allocations of fishing effort, in terms of increasing or decreasing the risk to sea lion subpopulations. However, further development of such tools is required, because current models are limited by the absence of data on the foraging movements of sea lions in some high risk regions, as well as the absence of accurate fishing effort data at

appropriate spatial scales. Spatial management of fishing effort, which could aid in reducing the risks to particular sub-populations is attractive because the outcome is immediate risk-reduction. In many cases, the historic catch in regions of greatest overlap between sea lions and fishing activity suggest that the impact on the productivity of the fisher may be minimal. Spatial management options could be investigated to assess how effort and catch could be reallocated to reduce the impact of by-catch on high-risk ASL subpopulations.

Enhanced spatial tools for risk assessment will be required if spatial management of fishing effort is to become a viable management strategy for mitigating ASL by-catch in this, or any other gillnet fishery. Such tools would provide a simple mechanism for policy makers and managers to evaluate the benefits and costs of different spatial allocations of fishing effort, in terms of increasing or decreasing: 1) risk to sea lion subpopulations and 2) fishery catches. However, further development of such tools are required, because current models are limited by the absence of data on the foraging movements of sea lions in some high-risk regions, as well as the absence of accurate fishing effort data. Further satellite tracking of ASL at subpopulations identified as high-risk should be undertaken to improve the accuracy of spatial foraging models.

In 2006, demersal gillnet fishers were required to record the latitude/longitude positions of each net-set, and from July 2007, all vessels will be fitted with satellite-linked vessel monitoring systems that will significantly improve the resolution of fishing effort. Following the improvements to the spatial information to the spatial resolution fishing activates that is likely to eventuate, it is recommended that by-catch probabilities be re-estimated and used to model the benefits of different spatial-management scenarios that could include area-closures, and reductions or redistributions of fishing effort. If spatial management is implemented it needs to remain flexible, be assessed regularly and be responsive to changes in the fishery. Improved fishery observer coverage will be essential to provide real estimates of by-catch rates and support for spatial-closures in regions of high-risk. They will also be essential for providing ongoing performance measures to managers about the effectiveness of spatial management approaches adopted. Ongoing abundance estimates of ASL subpopulations will also be the fundamental performance measure of spatial management in the fishery.

RISK-ASSESSMENT OF SEAL INTERACTIONS IN THE SOUTH AUSTRALIAN ROCK LOBSTER AND GILL-NET SECTOR OF THE SOUTHERN AND EASTERN SCALEFISH AND SHARK FISHERY

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Introduction

The aim of this chapter is to undertake a desktop risk assessment of seal bycatch in the SA rock lobster fishery (SARLF) and gillnet sector of the Commonwealth Southern and Eastern Scalefish and Shark Fishery (SESSF). The seal species investigated is the Australian sea lion (ASL, *Neophoca cinerea*) and the approach taken is to:

- Develop population and foraging distribution models for seal populations so that the spatial distribution of foraging effort for different sex and age classes can be estimated.
- Undertake a population viability analysis (PVA) of seal subpopulations to identify those most vulnerable to bycatch.
- Collate historic data on the spatial and temporal variation in fishing effort in both the gillnet SESSF and SARLF, and estimate probabilities of seal-fishery interactions by overlaying spatial distribution of seal foraging effort with historical fishing effort.
- Combine interaction probabilities with bycatch scenarios and PVA to identify subpopulations/regions/marine fishing areas (MFAs) with the greatest risk from fishery bycatch.

Methods

Seal distribution, population size and population viability analysis

Location of breeding sites

The location of ASL breeding colonies, and the pup production at each site (subpopulation) within South Australian waters was derived from published and unpublished sources (Tables 3 and 4). Pup production estimates (numbers of pups born per breeding cycle) for each subpopulation, were used as the basis for estimating subpopulation sizes, with the aid of life tables. Breeding colonies were defined as those sites where a minimum of five pups has been recorded at least once during the past 20 years (McKenzie et al. 2005).

Population estimates

The population size of each subpopulation was estimated utilising life tables and pup production estimates. For the ASL, which breeds about every 17.6 months (Higgins 1993), survival was calculated for every 1.5 year interval following the approach used by Goldsworthy et al. (2003). This study set longevity for females to 30.5 years (to provide the same number of reproductive opportunities as available to annually breeding seals). However, recent age-estimates for the species (R. McIntosh, La Trobe University), using annual growth-layer groups identified from sectioned teeth, have identified the oldest female at 25 years (R. McIntosh, unpublished data). Based on these data, the age-specific survival model developed by Goldsworthy et al. (2003) was adjusted and balanced by increasing annual survival levels and scaling to a maximum of 25.5 years (17 x 1.5 year stages) for females; (S = 0.627-0.048a + 0.001a^2-(0.159 x 10^{-4})a^3; and 15 years for males: S = 0.627-0.082a + 0.005a^2-(0.962 x 10^{-4})a^3).

The sex ratio at birth was assumed to be 1:1. The number of live individuals N, in each age-class a and sex s, was calculated as:

$$N_{a,s} = N_{a-1,s} S_{a-1,s} \qquad (1)$$

where S is the age-specific survival rate. The size of a population N was estimated as:

$$N = \sum_{s=1}^{s=2} \sum_{a=1}^{a=A} N_{a,s} \qquad (2)$$

where *A* is the number of age classes (stages) in the population.

All subpopulations were assumed to have the same population parameters as detailed above.

Leslie matrix and population model development

Simple deterministic and density-independent (exponential) Leslie matrices were developed to project the subpopulations through time (Table 5). We used the RAMAS® Metapop software (Version 3.0, Applied Biomathematics, Setauket, New York, Akçakaya and Root 1998) to model female populations. Age-specific survival estimates from life-tables along with estimates of age-specific fecundity were adjusted until a balanced population model was developed (population size remained stable over time, with finite rate of increase (λ) equal to 1, Table 5). Fecundity estimates were based on those determined for closely related species (eg. Boyd et al. 1995; Lima and Paez 1997; Barlow and Boveng 1991) and a minimum age of reproduction of 4.5 years (R McIntosh pers. comm).

Because only the female part of subpopulations was modelled, pup production was halved (assuming 1:1 sex-ratio at birth) and fecundity defined as the proportion of female offspring born to each female per stage. For each subpopulation being modelled, initial population abundances were set so that the estimated numbers in the first stage (pups) equalled half of the estimated pup production for that subpopulation. Final stage survival rates were set to zero, and a standard deviation of 0.1 set for all stage survival and fecundity estimates.

Density-independent models were used for a numbers of reasons. Firstly, populations are believed to be below their carrying capacity, following significant range and population reductions and incomplete recovery from historic sealing (Gales et al. 1994, Goldsworthy et al. 2003, Ling 1999). Secondly, pre-sealing or carrying capacity population estimates are unavailable, hence it is unclear at what population threshold density-dependent factors would become significant. For ASL, most subpopulations are so small that we believe present density levels would not elicit a significant feedback on a subpopulation's vital rates (although there is some

evidence for density dependence in pup mortality at some subpopulations, Ling and Walker 1977, Campbell 2005). Similarly, the importance of Allee effects (where there is a positive relationship between aspects of fitness and population size) in regulating pinniped populations is poorly understood. Although there is growing appreciation for the importance of Allee Effects and the need to incorporate them into population models (Stephens and Sutherland 1999), given the uncertainty in the significance of their role in ASL populations, we have chosen to exclude them from our subpopulation modelling.

Individual subpopulations were modelled separately, and assumed to be closed (ie. no immigration or emigration). For ASL, there is good evidence to support this assumption, with population genetic data indicating that the species demonstrates one of the highest levels of population subdivision among pinnpeds, with very high levels of mtDNA haplotype fixation among subpopulations (Campbell 2003). These findings suggest that ASL females display extreme levels of philopatry, with little or no interchange of females among breeding colonies.

Table 3. Summary of estimates of pup production per breeding cycle for ASL breeding sites (subpopulations) in South Australia, including the census date, source of information and location. Only colonies where 5 or more pups have been reported are listed. Data were current in April 2006.

Breeding site	Pups	Census	Sources	Lat	Long
The Pages[1]	577	Oct-05	Shaughnessy (2005a)	-35.767	138.300
Seal Slide (Kangaroo Is.)	11	Sep-04	Shaughnessy et al. (2006)	-36.028	137.539
Seal Bay (Kangaroo Is.)	214	Jun-03	McIntosh et al (2006a)	-36.000	137.333
Peaked Rock	24	Mar-90	Gales et al. 1994	-35.183	136.483
North Is.	28	Jul-05	Goldsworthy et al. 2005	-35.117	136.467
English Is.	27	Jun-05	Goldsworthy et al. 2005	-34.633	136.200
North Neptune, East	14	May-05	Goldsworthy et al. 2005	-35.226	136.077
South Neptune, Main	6	1993	Shaughnessy et al. 2005	-35.333	136.117
Dangerous Reef	585	Jun-05	Shaughnessy (2005b)	-34.817	136.217
Lewis Is.	73	Nov-05	Goldsworthy et al. 2005	-34.983	136.033
Albatross Is.	15	Jul-05	Goldsworthy et al. 2005	-35.067	136.183
Liguanea Is.	43	Jan-05	Shaughnessy (2005a)	-35.000	135.617
Four Hummocks Is. (North)	12	Jan-96	Shaughnessy et al. 2005	-34.767	135.033
Price Is.	25	Jan-96	Shaughnessy et al. 2005	-34.717	135.283
Rocky (North) Is.	16	Jan-96	Shaughnessy et al. 2005	-34.267	135.267
Pearson Is.	27	Sep-03	B Page pers. comm	-33.950	134.267
Ward Is.	8	Nov-95	Shaughnessy et al. 2005	-33.750	134.300
West Waldegrave Is.	157	Jul-03	Shaughnessy et al. 2005	-33.600	134.783
Jones Is.	15	Jan-05	Shaughnessy et al. 2005	-33.183	134.367
Nicolas Baudin Is.	72	Feb-02	Shaughnessy et al. 2005	-33.010	134.126
Olive Is.	131	Jan-05	Shaughnessy (2005b)	-32.717	133.983
Lilliput Is. (E Franklin Reef)	67	Mar-05	Goldsworthy et al. 2005	-32.433	133.700
Blefuscu Is. (W Franklin Reef)	84	Mar-05	Goldsworthy et al. 2005	-32.467	133.650
Gliddon Reef	7	Jun-05	Goldsworthy et al. 2005	-32.323	133.564
Breakwater Is.	17	Jun-05	Goldsworthy et al. 2005	-32.322	133.529
Fenelon Is.	21	Sep-90	Gales et al. 1994	-32.583	133.283
Masillon Is.	9	Sep-02	Robinson et al. 2003	-32.562	133.286
West Is.	56	May-05	Goldsworthy et al. 2005	-32.517	133.250
Lounds Is.	26	Nov-90	Gales et al. 1994	-32.283	133.367
Purdie Is.	132	May-05	Goldsworthy et al. 2005	-32.283	133.233
Western Nuyts Reef	14	Apr-04	Shaughnessy et al. 2005	-32.117	132.133
GAB B1[2]	15	1995	Dennis & Shaughnessy 1996, Goldsworthy et al. 2003	-31.492	131.067
GAB B2[2]	5	1995	Dennis & Shaughnessy 1996, Goldsworthy et al. 2003	-31.594	130.583
GAB B3[2]	31	1995	Dennis & Shaughnessy 1996, Goldsworthy et al. 2003	-31.580	130.150
GAB B5[2]	43	1995	Dennis & Shaughnessy 1996, Goldsworthy et al. 2003	-31.589	130.050
GAB B6[2]	12	1995	Dennis & Shaughnessy 1996, Goldsworthy et al. 2003	-31.609	129.767
GAB B8[2]	38	1995	Dennis & Shaughnessy 1996, Goldsworthy et al. 2003	-31.643	129.383
GAB B9[2]	17	1995	Dennis & Shaughnessy 1996, Goldsworthy et al. 2003	-31.648	129.300
Total	2,674				

[1]The Pages comprise two islands (North and South Page) and ASL breed on both. For the purposes of this study, they have been considered a single subpopulation.

[2]Apportioning pups among the Bunda Cliffs subpopulations in the Great Australian Bight (GAB) follows the approach used by Goldsworthy et al. 2003.

Table 4. Simplified hypothetical life-tables for ASL, including age-specific survival (*S*), and numbers (N) per stage. Numbers are based on pup production estimates from Tables 3 and 4, assuming a 1:1 sex-ratio at birth.

	Age (y)	S	N
Females	0	1.000	1,337
	1.5	0.558	746
	3	0.495	662
	4.5	0.438	585
	6	0.386	516
	7.5	0.339	453
	9	0.296	396
	10.5	0.258	344
	12	0.223	298
	13.5	0.193	257
	15	0.165	221
	16.5	0.141	188
	18	0.119	159
	19.5	0.100	133
	21	0.082	110
	22.5	0.067	89
	24	0.052	70
	25.5	0.039	52
Female total			6,617
Males	0	1.000	1,337
	1.5	0.514	687
	3	0.416	557
	4.5	0.334	447
	6	0.265	354
	7.5	0.207	277
	9	0.160	214
	10.5	0.121	162
	12	0.089	119
	13.5	0.062	83
	15	0.039	52
Male total			4,288
Total Population Estimate			10,905

Population viability analysis (PVA)

PVA provides a means to predict future population abundances, the time to extinction (or a prescribed level of reduced abundance) and the probability of extinction or reaching an abundance threshold within a specified period. These are usually undertaken using stochastic simulation models (Shaffer 1981, Gilpin and Soulé 1986, Reed et al. 2002). We used the Leslie matrices to undertake PVAs on their subpopulations. Two measures of risk were calculated, *terminal extinction risk* (the probability that a population will go extinct during a specified time period) and *quasi-extinction time* (Q_t, the time for the median of the simulated population trajectory replicates to go *quasi-extinct*) (Akçakaya 1998). We defined quasi-extinction (Q) as occurring when the numbers of females in a subpopulation fell to, or below a threshold of 10 individuals. Demographic stochasticity was simulated within RAMAS® Metapop, by sampling the number of survivors from a binomial distribution and young from a Poisson distribution (Akçakaya 1998).

PVA was undertaken to investigate the potential implication of additional (anthropogenic) mortality on the conservation status of each subpopulation. This was achieved by applying virtual harvests of female seals to each subpopulation, and determining the level of additional mortality required to increase the risk of extinction. RAMAS® Metapop allows the user to define the number animals from each stage to be harvested from each year. For consistency, we removed pre-recruit seals from the first stage (<1.5 years old in ASL) when undertaking simulations. The potential implications of additional mortality were investigated under three scenarios of population trajectory: increasing, stable and decreasing. The increasing trajectory was set at 5%/year based on current growth in the Dangerous Reef subpopulation (Shaughnessy unpublished data). Although the Dangerous Reef subpopulation appears to be increasing at a higher rate, part of this is likely to be an artefact of improved census methodology in recent years (Shaughnessy unpublished data). The base model (above) was used as the stable trajectory. The decreasing trajectory was based in part on the current rate of decline observed in the Seal Bay subpopulation (-0.77%/year, Shaughnessy et al. 2006). We rounded this to the nearest integer (-1%). Different population growth models were simulated by adjusting relative survival levels and then calculating the resultant population trajectory (500 replicates of 100 stages). The exponential rate of increase (r), calculated from the slope of exponential regressions of population size over time was expressed as a percentage increase as follows, $(e^r - 1) \times 100$. Relative survival multipliers of 1.0985 and 0.9801 were used

to simulate increasing (5%/year) and decreasing (-1%/year) population trajectories, respectively.

PVA outputs for each subpopulation scenario were based on 1,000 replicates for 100 stages (ie. 150 years for ASL), and categorised against four risk criteria, adapted from Mace and Lande (1991):
1) Quasi-extinct – defined here as <10 females
2) Critical – 50% probability of extinction within 5 years or 2 generations, whichever is longer.
3) Endangered – 20% probability of extinction within 20 years or 10 generations, whichever is longer;
4) Vulnerable – 10% probability of extinction within 100 years.

For species with overlapping generations, generation time is defined as the mean age of mothers of all newborn females, assuming a stable distribution (the mean interval between the birth of a mother and the birth of her offspring, weighted by the proportion of individuals in each age class, Caughley, 1977). Generation time was calculated using their Leslie Matrices in Poptools (Version 2.7) (Hood 2006). The generation time for ASL was calculated as 12.4 years.

Seal foraging models and spatial distribution of foraging effort
Simple, distance (and in some cases) direction-based foraging models were developed for different age/gender classes, to enable the spatial distribution of foraging effort (seal days·year^{-1}) to be estimated for each age/gender group, within each subpopulation within the study area. These models assumed that seals within a subpopulation foraged within a set range and in some cases a specific direction from their colony of origin, according to the normal probability density function. Foraging distance and heading parameters used for each age/gender group are detailed in Table 6.

Table 5. Leslie Matrix for ASL populations. The first row indicates the stage (age) of females in years. The second row indicates stage-specific fecundity (proportion of female pups born to each female per stage) and the diagonal cells denote stage-specific survival (proportion of the previous stage surviving to the next stage) (note final stage 25.5 has a survival of 0).

1.5	3	4.5	6	7.5	9	10.5	12	13.5	15	16.5	18	19.5	21	22.5	24	25.5
0.000	0.000	0.100	0.200	0.315	0.370	0.395	0.410	0.415	0.420	0.420	0.400	0.375	0.350	0.300	0.200	0.100
0.558	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0.887	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0.884	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0.881	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0.878	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0.874	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0.871	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0.867	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0.862	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0.858	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0.852	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0.845	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0.837	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0.826	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.809	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.785	0.000

Using the geographic information systems (GIS) software package MapInfo™ (Version 6.0, MapInfo Corporation, Troy New York, USA), continental shelf (0-200m) and slope (200-1000m) waters in South Australia were overlaid with a 10 x 10 km grid, and the coordinates (latitude and longitude) of each node were extracted. The distance and heading (bearing) from each seal colony to each node in the array was then calculated. The probability (f) of an animal from a given colony foraging at a particular node (d) was then calculated based on the distance (D) of the node from the subpopulation, and the designated mean (μ) and standard deviation (σ) of foraging distance (km), using the normal probability density function, where

$$f(D_d) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\left(\frac{D_d-\mu}{2\sigma}\right)^2} \qquad (3)$$

Similarly, the probability (*f*) of an animal from a given colony foraging at a particular node (*d*) was also calculated based on the heading (*H*) of the node from the subpopulation, and the designated mean (μ) and standard deviation (σ) of heading (degrees), where

$$f(H_d) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\left(\frac{H_d-\mu}{2\sigma}\right)^2} \qquad (4)$$

The estimated distribution of seal foraging effort (*FE*) was then calculated based on the average number of days spent at sea per year by each age/gender category (seal days·year^{-1}) (Table 6).

$$FE_{a,s} = 365 N_{a,s} P_{a,s} \qquad (5)$$

Where *P* is the proportion of time spent at sea (Table 6). The overall probability of foraging effort *f(FE)* by each age/gender group from a given subpopulation (*c*) at each node (*d*) was then calculated as

$$f(FE_{c,d}) = \sum_{c=1}^{c=N_c} \left\{ FE_{c,d} \frac{f(D_d) f(H_d)}{\sum_{d=1}^{d=N_d} f(D_d) f(H_d)} \right\} \qquad (6)$$

where N_c is total number of subpopulations and N_d is total number nodes. The actual *FE* (seal days·year^{-1}) of age/gender group from a given subpopulation (*c*) at each node (*d*) was then calculated as

$$FE_{c,d} = f(FE_{c,d}).FE \qquad (7)$$

ASL populations were divided into five age/gender groups; pups (0-1.5 years); juveniles (1.5-3 years); sub-adult males (SAM, 3-7.5 years); adult females (≥4.5 years) and adult males (≥7.5 years). Numbers of individuals present in each age/gender group were calculated using the life-table (Table 4). Estimates of the proportion of time spent at sea and mean foraging distance for and juvenile, SAM, adult female and male ASL were also based on satellite tracking data from the Nuyts Archipelago and Dangerous Reef (Goldsworthy et al. unpublished data) (Table 6-7).

No directionality was imposed on foraging models, with the exception of adult males. Tracking studies of adult males off the west coast of the Eyre Peninsula indicate that animals forage predominantly in outer shelf waters (Goldsworthy et al. unpublished data). Because of the geography of the region, a directionless model for age/gender groups that feed at distance from colonies may project some foraging effort in the reverse direction (eg. into northern Gulf waters) to the regions that these age/sex groups are known to use. Distance and directional parameters used in developing the forging models are detailed in Tables 6-7.

These data were imported into MapInfo™, and then interpolated (triangular irregular network interpolation with 5th order polynomial) and plotted using VerticalMapper™ (Version 2.0, Northwood Geosciences Ltd, Nepean, Ontario, Canada).

Spatial and temporal distribution of fishing effort

Commercial fishing effort data for the gill-net sector of the SESSF within and adjacent to South Australian waters, were derived from AFMA for each year between 1973 and 2004 (32 years) (see Table 8). Fishing effort data for the South Australian southern rock lobster fishery (SARLF) were obtained from SARDI Aquatic Sciences for 35 consecutive years between 1970 and 2004 (see Table 9). Fishing effort data for each fishery has been recorded for individual marine fishery areas (MFAs) that are roughly based around a 1º x1º grid (Figures 6 and 7). In order to present the spatial and temporal changes in fishing effort, each zone was represented by multiple nodes spaced equidistant where possible, to spread fishing effort equally throughout the node. Data were interpolated and plotted using MapInfo™ and VerticalMapper™ (triangular irregular network interpolation with 5th order polynomial).

Spatial and temporal overlap in fishery effort and seal foraging effort

Using the distribution of foraging effort models, which were developed for the different age/gender groups, the total amount of foraging effort within each fishery MFA was calculated by summing the values for each 10 x 10km node within each fishery MFA. The proportion of the total foraging effort (*FE*) calculated, which occurred within each fishery MFA, was then derived. For each year of fishing effort records, the product of the proportion of seal *FE* and fishing effort (*F*) were calculated within each MFA. The extent of overlap between fishing effort and seal foraging (overlap index, *OI*, adapted from Schoener (1968)) within each MFA was then

calculated as a probability (*f*) by dividing the product of the proportion of fishing effort (F_{FMA}) and the proportion of seal foraging effort (FE_{FMA}) within each MFA, by the sum of all the products from all MFAs, as follows:

$$f(OI)_{FMA} = \frac{f(FE_{FMA}).f(F_{FMA})}{\sum_{FMA=1}^{FMA=N_{FMA}} f(FE_{FMA}).f(F_{FMA})} \qquad (8)$$

where N_{FMA} is the total number of MFAs.

Table 6. Mean estimated foraging range, proportion of time at sea and estimated foraging effort (seal days.year^{-1}) for different age/gender groups for ASL populations in South Australia.

Age/gender	ASL Foraging distance (km)		Proportion time at sea	FE
	Mean	sd		Seal days/year
Adult male	80[1]	55	0.58	132,231
Adult female	20[1]	15	0.53	746,563
Sub-adult male	24[1]	15	0.51	200,227
Juvenile	18[1]	9	0.46	244,003
Pup	10[2]	10	0.25	244,003
Total				1,772,395

[1]Mean and standard deviation of foraging distance and the proportion of time spent at sea are based on satellite tracking data (Goldsworthy et al. unpublished data).
[2]Estimated.

Table 7. Estimated mean foraging heading (and sd) for adult male ASL based on satellite tracking studies at several locations in SA (Goldsworthy et al. unpublished data)

Colony	Heading	Sd
North Pages Is.	135	90
Seal Slide (Kangaroo Is.)	160	90
Seal Bay (Kangaroo Is.)	180	90
Peaked Rock	180	90
North Is.	180	90
English Is.	190	90
North Neptune, East	190	90
South Neptune, Main	190	90
Dangerous Reef	190	90
Lewis Is.	190	90
Albatross Is.	190	90
Liguanea Is.	200	90
Four Hummocks Is. (north)	200	90
Price Is.	225	90
Rocky (North) Is.	225	90
Pearson Is.	225	90
Ward Is.	225	90
West Waldegrave Is.	225	90
Jones Is.	225	90
Nicolas Baudin Is.	225	90
Olive Is.	225	90
Lilliput Is.	225	90
Blefuscu Is.	225	90
Gliddon Reef	225	90
Breakwater Is.	225	90
Fenelon Is.	225	90
Masillon Is.	225	90
West Is.	225	90
Lounds Is.	225	90
Purdie Is.	225	90
Western Nuyts Reef	200	90
GAB B1	180	90
GAB B2	180	90
GAB B3	180	90
GAB B5	180	90
GAB B6	180	90
GAB B8	180	90
GAB B9	180	90

Results

Population distribution and size

The location and estimated pup production for colonies of ASL in SA are detailed in Table 3 and Figure 8. Life tables detailing the estimated age-specific survival, total numbers of individual within each age-class (stage), and total population size based on the estimated pup production within SA are detailed in Table 4. Based on this life-table, and a pup production of 2,674 per breeding cycle, the size of the SA ASL populations is estimated at 10,905 individuals, of which 6,617 (61%) are females and 4,288 (39%) are male (Table 4). The life-tables produced population estimates that were 4.08 times that of pup-production in ASL populations.

There are 38 breeding sites of the ASL in SA, where pup production has been recorded to number ≥ 5 (Table 3). Of the 38 breeding sites, only 6 (16%) produce more than 100 pups, accounting for 67% of the State's pup production. The largest population is Dangerous Reef in southern Spencer Gulf (585 pups), followed by The Pages (577 pups) in Backstairs Passage between Kangaroo Island and mainland Australia. The next largest populations are Seal Bay (214 pups) on Kangaroo Island, West Waldegrave (157 pups) and Olive Islands (131 pups) off the west coast of the Eyre Peninsula, and Purdie Island (132 pups) in the Nuyts Archipelago. The median pup production for SA colonies is 25.5, with 60% of breeding sites producing fewer than 30 pups per season, 42% producing fewer than 20 pups, and 13% fewer than 10 pups. These analyses do not take into account at least another 11 breeding sites (termed haul-outs with occasional pupping), where fewer than 5 pups have been recorded at some time (McKenzie et al. 2005).

Distribution of seal foraging effort

The estimated distribution of foraging effort by ASL in SA is presented in Figures 9-14. Not surprisingly, the greatest density of foraging effort in ASL occurs in waters adjacent to breeding colonies, with relative foraging distances increasing from pups, to juveniles, adult females and sub-adult males. Because adult males typically forage in outer shelf waters and range widely (Goldsworthy et al. unpublished), their estimated spatial distribution of foraging effort differs markedly from the other

age/gender groups, because they do not focus their foraging near colonies (Figure 13). The estimated total distribution of foraging effort (age/gender groups combined) is presented in Figure 14, and demonstrates the greatest concentration of foraging effort associated with the larger subpopulation centres, especially The Pages (just east of Kangaroo Island), Seal Bay (south coast of Kangaroo Island), Dangerous Reef (southern Spencer Gulf) and the Nuyts Archipelago (west Eyre Peninsula). With the exception of the south-east and northern Gulf waters, some level of ASL foraging effort occurs in almost all near-coastal waters from Encounter Bay to the West Australian border (Figure 14).

Distribution of fishing effort

Gill-net sector of the SESSF

Data detailing the annual fishing effort (km net-lifts.year^{-1}) for the 29 SA MFAs of the gill-net sector of the SESSF, spanning 32 years between 1973 and 2004, are presented in Table 8 and Figure 15A. Over this period, there was a total of 634,496 km of net-lifts, averaging about 20,000 km of net-lifts per year (Table 8, Figure 15A). Annual effort changed markedly in this region of the fishery, with a steady increase from around 3,000 km to 12,000 km net-lifts per year between 1973-1983, with a very significant increase in fishing effort between 1984-1987 peaking at nearly 43,000 km net-lifts in 1987 (Table 8, Figure 15A). Fishing effort then decreased annually to about 23,000 km net-lifts in 1993 and then increased to just over 32,000 km net-lifts in 1998. Fishing effort reduced to around 17,000 km net-lifts in 2000, and has remained at about this level up until 2004 (Table 8, Figure 15A).

The spatial distribution of fishing effort between 1973 and 2004 is summarised for four-year averages in Figures 16-23, and total and average annual fishing effort are present in Figure 24 and 25, respectively. Essentially these track the increase in fishing effort from the 1970s and the 1980s, with the major regions of fishing effort occurring south and south-east of Kangaroo Island, and off the west coast of the Eyre Peninsula (Figures 16-23). Between 2000-2004, about 42% of total fishing effort occurred south and south east of Kangaroo Island (MFA 149-151, Table 8).

SA Rock lobster fishery (SARLF)

Data detailing annual changes in fishing effort over a 35 year period (1970-2004) in 19 MFAs of the SARLF are presented in Table 9 and Figure 15B. Over this period there was a total of 78.9 million pot-lifts, averaging about 2.3 million pot-lifts/year

(Table 9, Figure 15B). Annual effort in the fishery increased from around 2.2 to 2.5 million pot-lifts per year between the 1970s and 1980s, to a maximum of 2.7 million pot-lifts in 1991. Since then, fishing effort has decreased and in 2003 and 2004, averaged just over 1.5 million pot-lifts (Table 9, Figure 15B).

Changes in the spatial distribution of fishing effort in the SARLF between 1970 and 2004 are presented in Figures 26-34. Over this period, about 70% of the total fishing effort has been concentrated in the south-east of the state in MFAs 55, 56 and 58 (Figures 26-34). Elsewhere, effort is focused close to the shore along the south coast of Kangaroo Island, and the southern and west coasts of the Eyre Peninsula (Figures 26-34).

Table 8. Annual fishing effort (km net-lifts.year^{-1}) for the 29 SA MFAs of the gill-net sector of the Commonwealth SESSF, spanning 32 years between 1973 and 2004.

MFA	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990
101	0	0	0	0	0	0	0	0	0	0	108	0	174	0	560	107	741	443
102	0	0	41	0	0	22	16	0	0	80	0	0	0	0	0	14	66	242
103	0	14	2	0	0	0	41	127	79	275	541	48	182	135	504	344	524	524
104	0	0	0	0	0	0	0	0	0	0	0	0	0	120	315	763	482	791
105	0	0	0	0	0	0	0	0	0	0	267	0	0	0	386	279	581	420
106	0	6	0	0	0	0	0	0	72	10	53	15	0	0	256	923	274	989
107	165	279	139	32	108	0	13	287	336	334	810	404	660	1591	2027	1469	821	1593
108	674	746	438	273	626	1029	725	1467	1117	1315	1258	2991	2467	3059	4049	3201	3421	2070
112	0	0	4	0	0	0	0	0	0	0	20	0	0	189	27	444	570	292
113	0	0	6	0	0	0	0	0	0	202	65	74	150	856	524	1200	753	312
114	13	11	2	0	0	0	0	0	96	389	75	461	606	688	1277	2085	1155	1200
115	216	70	156	60	103	35	319	333	524	667	1155	1072	898	1799	1388	1073	2063	582
122	58	75	117	29	106	115	100	276	512	676	413	381	230	229	288	135	42	16
125	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
126	43	3	1	15	0	20	80	103	830	1045	352	911	1697	1190	1663	2494	2475	2163
128	58	107	0	21	145	284	299	634	888	965	705	1101	870	1035	1108	1095	1579	1094
129	0	0	0	0	206	764	1072	1146	1206	1199	440	419	556	1677	1810	1124	1234	1160
132	52	55	38	14	388	108	181	0	409	478	1209	2782	1260	504	1080	687	853	722
136	78	161	9	0	236	580	165	345	162	122	90	130	38	52	323	372	131	128
138	7	5	43	13	0	0	57	401	345	699	334	1061	923	787	1997	1626	1980	3625
139	57	103	91	92	183	226	233	301	461	1092	558	686	751	1531	3112	1637	2549	1103
140	67	60	74	79	64	445	322	440	519	164	246	462	387	549	1275	1854	2092	1165
144	233	212	415	60	471	471	466	452	417	393	313	303	459	1088	1312	1326	948	1273
148	6	140	0	74	65	84	24	28	51	104	57	345	417	1509	1843	1200	1393	2487
149	124	211	245	93	276	33	0	120	110	183	312	831	965	3417	4620	3975	2425	2871
150	184	236	202	122	40	16	108	274	424	368	694	2093	1388	2991	4329	2327	3120	2568
151	615	233	831	429	805	990	989	1471	996	443	1100	3507	2342	3404	4692	6512	3392	4530
155	313	352	144	183	315	552	1198	328	92	181	123	274	1379	1437	1490	1598	3913	2107
158	165	362	193	344	353	313	288	456	457	220	593	198	187	349	734	488	759	1446
Total	3127	3441	3189	1933	4489	6086	6695	8986	10102	11603	11892	20548	18986	30185	42989	40349	40338	37917

Table. 8 Cont.

MFA	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	Total	%	Average
101	13	168	160	181	25	208	280	505	189	244	222	0	0	4666	0.7%	146
102	299	37	0	267	551	191	248	252	47	65	53	0	0	3320	0.5%	104
103	764	380	245	424	647	200	452	398	101	200	276	0	0	8390	1.3%	262
104	190	70	120	42	13	111	516	284	105	27	4	545	612	5859	0.9%	183
105	600	280	175	580	68	322	628	412	335	247	146	309	115	6515	1.0%	204
106	333	292	284	212	441	232	700	222	205	84	33	393	338	6857	1.1%	214
107	786	982	560	947	729	573	985	1043	273	492	371	579	343	20461	3.2%	639
108	2755	1429	1798	1415	1103	2422	1431	1331	765	771	1055	871	890	51024	8.0%	1594
112	592	253	25	185	63	145	176	97	61	23	53	21	17	3647	0.6%	114
113	823	603	1091	604	764	545	601	291	81	155	8	105	103	10599	1.7%	331
114	1897	1002	1899	1235	827	1497	1587	1239	632	495	194	847	472	23121	3.6%	723
115	1223	632	1133	1795	2342	1745	1712	1866	1017	913	950	1322	1131	30968	4.9%	968
122	67	81	18	179	152	81	50	59	57	25	0	0	0	4583	0.7%	143
125	0	0	0	0	0	0	0	0	0	0	0	0	585	585	0.1%	18
126	839	1616	1458	2040	1161	1372	2124	1225	835	645	699	1288	1411	33014	5.2%	1032
128	965	512	385	304	925	903	760	752	326	320	354	334	816	20226	3.2%	632
129	772	373	1094	991	1275	1278	955	1613	205	184	7	32	9	23430	3.7%	732
132	110	338	142	277	378	569	346	593	307	28	31	2	0	14425	2.3%	451
136	34	137	79	122	101	122	57	26	22	66	2	2	5	3901	0.6%	122
138	1284	1334	1282	1570	1099	1127	1530	728	388	448	639	829	771	29424	4.6%	919
139	605	703	942	735	553	809	820	535	420	260	445	733	490	23845	3.8%	745
140	590	444	1282	877	707	1218	830	732	478	404	516	521	282	20058	3.2%	627
144	910	502	746	564	871	1217	1672	1575	1102	1239	437	485	541	24023	3.8%	751
148	1377	865	366	1178	389	1712	1101	700	435	478	406	600	1044	22660	3.6%	708
149	1418	2117	2368	2756	1554	2243	2947	1598	1410	1132	1178	1966	1835	47947	7.6%	1498
150	1847	2461	2028	2764	3511	3593	5250	3539	3312	3559	3114	3026	2833	65986	10.4%	2062
151	3170	3358	2992	2781	2443	3203	3470	2994	3220	2940	2928	1872	1771	78650	12.4%	2458
155	1114	1338	1351	453	1053	1114	846	1066	729	746	1114	979	1042	31621	5.0%	988
158	200	556	769	1190	531	765	404	442	320	311	387	339	207	14692	2.3%	459
Total	25575	22862	24793	26665	24274	29516	32481	26114	17376	16501	15620	17999	17663	634496		19828

Spatial overlap in fishing and seal foraging effort

The estimated spatial overlap between ASL foraging effort and the mean fishing effort in the gill-net sector of the SESSF (1973-2004) and the SA RLF (1970-2004) are presented in Figures 35-43. These figures represent the expected spatial distribution of ASL-fishery interactions, assuming that the probability, or risk of interaction, is directly proportional to the extent of seal foraging and commercial fishing effort in each region. Hence areas where seals forage, but no fishing occurs, or vice versa, have a zero probability of interactions. As such, the expected level of interaction will be highest in regions with high seal foraging and high commercial fishing effort.

Figures 35 and 36, indicate the overlap index (*OI*) for combined age/gender groups in the ASL for the SESSF gill-net and SA RLF, respectively. Because fishing effort in the SESSF gill-net sector occurs in most continental shelf and gulf waters, the *OI* highlights that regions where interaction are most likely to occur are closely associated with the main population centres of ASL, namely, The Pages and Seal Bay (east and south of Kangaroo Island), Dangerous Reef (southern Spencer Gulf) and the west coast of the Eyre Peninsula (especially the Nuyts Archipelago) and the GAB (Figure 35).

This contrasts with the expected spatial interaction with the SARLF. Because most of the fishing effort in SA RLF is concentrated in the south-east of the State, and in near coastal waters south and east of Kangaroo Island, and along the southern and west coasts of the Eyre Peninsula, the *OI* suggests relatively low interaction rates with The Pages, and Dangerous Reef ASL subpopulations (Figure 36). However, interactions with the latter subpopulation occur where their foraging effort intersects fishing effort in southern Spencer Gulf (Figure 36). As with the SESSF gill-net sector, interactions rates are expected to be high on the west coast of the Eyre Peninsula, especially in the Nuyts Archipelago (Figure 36).

Table 9. Annual fishing effort (x1,000's pot-lifts.year^{-1}) for the 19 Marine Fishing Areas (MFAs) of the SARLF, spanning 35 years between 1970 and 2004

MFA	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988
7	5.8	6.2	10.4	11.7	13.0	12.3	12.0	12.5	12.2	9.6	5.9	4.0	6.1	12.6	13.5	5.9	3.7	10.4	7.8
8	23.5	27.7	24.3	25.7	28.7	51.0	40.9	20.8	21.5	16.4	6.8	1.6	5.2	7.9	15.9	22.9	18.4	5.4	16.1
10	16.4	18.0	18.4	10.6	12.3	19.7	12.7	13.7	9.8	4.9	0.9	3.6	0.4	3.1	1.8	1.1	0.5	3.2	3.6
15	32.9	44.6	37.0	51.9	52.2	46.4	52.5	38.7	38.8	49.3	28.8	23.1	25.5	38.4	60.5	43.1	69.2	79.3	74.2
18	0.0	0.0	0.0	0.8	2.7	0.0	0.0	0.9	3.1	0.0	0.0	2.0	0.0	0.0	0.2	3.2	3.0	3.4	3.1
27	0.5	3.4	2.1	0.3	3.8	1.5	7.1	5.5	11.9	28.3	32.2	38.2	32.8	43.2	42.7	47.7	45.5	36.6	41.2
28	76.5	73.2	84.4	70.7	75.8	65.6	66.8	61.3	69.6	57.4	70.9	106.4	97.5	123.0	138.6	135.5	138.8	126.8	119.1
39	91.6	89.3	97.8	81.4	77.1	70.4	82.2	92.1	107.9	110.7	99.2	94.1	114.3	134.3	121.6	117.4	114.4	159.6	135.2
40	6.2	8.1	2.8	7.1	14.8	14.7	10.1	31.8	32.3	25.7	43.7	41.8	42.8	47.6	57.8	54.8	53.5	50.0	73.1
44	0.0	0.0	0.0	0.1	0.3	0.8	0.0	0.3	0.3	0.0	0.5	1.0	0.7	7.5	2.4	2.2	2.8	5.2	3.9
45	13.6	16.6	19.8	12.7	14.9	14.3	11.4	14.2	13.8	9.5	11.8	14.4	11.0	4.1	2.8	2.5	2.7	4.4	0.5
46	0.0	0.0	6.7	4.7	0.5	3.2	2.1	1.3	0.4	0.3	0.0	0.0	1.4	0.3	0.0	7.9	3.9	0.0	2.0
48	12.0	18.8	22.2	15.9	17.2	21.1	14.3	11.9	16.0	27.2	42.9	38.7	50.4	45.8	57.7	45.3	37.6	38.8	70.1
49	23.2	21.9	44.3	22.7	18.1	16.2	12.4	22.1	20.6	26.4	35.0	44.1	61.7	63.3	51.6	51.8	51.6	47.0	47.1
50	1.6	4.5	2.0	0.6	0.9	0.1	7.7	3.5	5.9	8.0	2.4	7.3	13.7	42.3	40.9	39.3	38.0	22.4	33.2
51	219.5	285.3	305.1	279.7	221.1	244.3	225.6	234.2	210.9	203.3	214.6	218.0	238.0	151.5	155.9	141.4	109.2	143.4	121.9
55	489.2	509.6	600.2	570.3	456.2	517.2	472.6	492.5	487.8	505.6	508.0	612.7	692.3	905.9	793.4	789.0	788.4	809.7	712.3
56	615.6	663.4	671.6	675.6	567.3	690.7	675.8	683.0	656.7	598.9	744.7	751.3	710.3	609.1	537.6	558.1	509.2	625.9	515.5
58	499.0	528.6	542.8	591.0	481.2	543.0	484.4	455.5	437.2	481.5	553.4	520.1	509.1	537.8	462.7	481.5	467.7	517.9	503.9
Total	2127	2319	2492	2433	2058	2333	2191	2196	2156	2163	2402	2522	2613	2778	2558	2551	2458	2689	2484

Table 9. Cont.

MFA	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	Total	%	Average
7	3.7	9.0	7.8	8.6	24.9	17.6	29.1	19.2	14.3	13.7	15.1	17.5	15.6	13.8	12.6	5.2	410	1%	11.4
8	20.6	18.4	17.8	31.2	38.7	33.9	35.6	32.4	24.7	21.8	24.3	30.2	23.1	19.9	20.9	26.6	809	1%	22.5
10	1.5	7.1	7.6	5.5	10.9	9.3	9.9	10.9	4.1	6.0	5.1	9.6	9.7	6.3	2.4	2.5	273	0%	29.1
15	65.1	93.3	103.8	80.6	78.9	69.2	107.3	106.7	86.9	93.8	73.8	72.4	68.3	54.2	43.8	35.4	2135	3%	59.3
18	2.7	4.1	9.2	7.3	5.9	5.2	9.9	8.3	6.8	9.8	6.1	5.9	5.5	5.7	1.1	1.6	136	0%	16.8
27	48.4	37.4	38.2	41.7	35.5	38.9	36.2	22.4	29.9	32.4	36.2	22.4	30.9	19.7	24.1	16.5	962	1%	26.7
28	130.2	108.8	150.6	136.6	118.1	131.9	119.5	139.2	170.0	143.5	110.6	126.9	119.2	110.2	101.9	85.2	3788	5%	105.2
39	138.1	133.7	143.7	125.5	107.1	117.8	90.4	110.7	115.6	122.0	146.6	116.0	97.8	105.3	107.6	126.6	3934	5%	109.3
40	72.4	73.0	88.8	78.5	68.8	64.7	76.3	69.5	65.6	66.8	67.4	55.8	41.5	43.8	62.4	67.5	1722	2%	47.8
44	4.3	3.2	3.6	3.9	3.6	4.4	6.3	5.2	2.7	4.1	6.8	5.7	5.1	8.9	4.5	4.7	1.6	0%	4.1
45	0.9	1.9	3.1	1.9	0.8	2.9	1.9	1.7	2.0	0.7	0.0	0.2	2.7	1.9	3.4	1.6	267	0%	23.9
46	4.9	3.2	2.3	6.3	4.1	0.7	0.9	2.5	2.0	2.2	1.6	0.0	0.3	0.0	0.0	1.0	113	0%	10.3
48	62.9	67.0	78.4	80.3	62.8	48.3	43.3	42.4	46.8	48.9	58.9	50.6	47.5	48.5	53.1	58.7	1551	2%	43.1
49	60.9	65.5	81.5	70.0	76.9	85.2	80.0	69.4	68.5	67.3	83.9	86.1	90.0	75.2	101.8	67.1	1959	2%	54.4
50	49.4	17.1	24.2	21.2	30.2	26.7	29.7	26.6	24.6	21.7	20.8	29.5	28.5	18.9	20.9	24.8	689	1%	19.7
51	124.2	139.1	176.0	119.6	111.5	72.2	73.9	78.0	58.8	45.0	27.2	42.0	21.2	14.8	16.0	18.3	5112	6%	142.0
55	729.0	803.3	811.2	730.4	683.3	584.2	610.3	656.5	682.8	573.9	437.3	342.4	299.8	276.1	349.0	326.1	20664	26%	574.0
56	487.4	515.2	561.6	462.8	422.7	442.7	482.8	539.0	552.6	473.4	359.2	333.0	287.6	256.0	322.0	321.6	18936	24%	526.0
58	407.8	440.4	474.6	431.5	403.9	394.7	410.2	471.4	449.0	429.9	330.5	312.2	284.3	287.2	346.0	370.7	15901	20%	441.7
Total	2414	2541	2784	2443	2289	2150	2254	2412	2408	2177	1811	1658	1479	1366	1593	1562	78865		2253

For ASL, over 60% of the estimated foraging effort occurs in four SESSF gill-net sector MFAs: MFA 129 (19%, southern Spencer Gulf), MFA 108 (18%, Nuyts Archipelago), MFA 144 (16%, southern Fleurieu Peninsula) and MFA 149 (8%, south of Kangaroo Island) (Table 10). Importantly, <1% of the estimated ASL foraging effort, occurs outside SA MFAs, although at least part of this is in waters to the west of the SA/WA border, where the SESSF gillnet sector fishery also occurs. Similarly, over 40% of the estimated ASL foraging effort occurs in four SARL MFAs: MFA 8 (15%, Nuyts Archipelago), MFA 44 (13%, southern Fleurieu Peninsula), MFA 15 (8%, mid-west coast Eyre Peninsula) and MFA 49 (8%, southern Kangaroo Island). However, in contrast to the SESSF gill-net sector, 36% of ASL foraging effort occurs outside of SA RLF MFAs where historical catches have been reported in the SARLF (Table 10).

Estimated *OI* between foraging effort in ASL adult females, adult males, sub-adult males, juveniles and pups with fishing effort in the SESSF gill-net sector and SA RLF are presented in Figures 37-46. These estimates of spatial overlap indicate that the probability of interactions is highest close to breeding colonies, where foraging effort is most focused, especially for pups, juveniles and adult females. Because adult males do not utilise waters in close proximity to their colonies, the spatial distribution of *OI* is relatively dispersed. Because fishing effort in the SARLF has been

concentrated in near coastal waters, the *OI* is most highly concentrated in ASL pups and juveniles, especially near breeding colonies (Figures 44 and 46).

Table 10. Average percentage fishing effort among all MFAs in the SESSF gill-net sector and SARLF, relative to the estimated percentage of foraging effort by different age/sex classes of ASL within each MFA of each fishery. 'Outside' refers to the percentage of seal foraging effort that occurs outside the listed MFAs.

Gill-net MFA	%Fishery Effort	ASL % Foraging Effort					
		Females	Males	SAM	Juveniles	Pups	All ASL
101	0.7	3.2	0.5	3.1	3.6	3.6	2.8
102	0.5	1.9	0.5	1.9	2.0	2.1	1.7
103	1.3	0.4	0.5	0.4	0.4	0.4	0.4
104	0.9	0.3	1.7	0.4	0.0	0.0	0.5
105	1.0	0.1	1.6	0.1	0.0	0.0	0.4
106	1.1	0.2	1.9	0.2	0.2	0.2	0.5
107	3.2	2.4	5.2	2.7	1.5	1.0	2.5
108	8.0	18.5	4.0	17.9	19.9	20.4	16.1
112	0.6	0.0	0.6	0.0	0.0	0.0	0.1
113	1.7	0.0	4.2	0.0	0.0	0.0	0.8
114	3.6	1.7	7.4	1.9	1.2	0.9	2.6
115	4.9	8.1	4.0	7.9	8.4	8.8	7.4
122	0.7	0.0	0.5	0.0	0.0	0.0	0.1
125	0.1	0.1	2.0	0.1	0.0	0.0	0.4
126	5.2	1.0	6.5	1.2	0.7	0.5	2.0
128	3.2	4.6	3.4	4.7	4.2	3.6	4.1
129	3.7	18.4	2.8	17.4	21.4	23.1	16.6
132	2.3	0.1	2.1	0.2	0.0	0.0	0.5
136	0.6	0.0	0.9	0.0	0.0	0.0	0.2
138	4.6	2.8	7.5	3.1	2.0	1.7	3.4
139	3.8	6.1	7.9	6.6	4.5	3.8	5.8
140	3.2	2.3	4.1	2.9	0.6	0.2	2.0
144	3.8	15.5	3.2	14.2	19.7	20.9	14.7
148	3.6	0.7	3.7	0.8	0.2	0.1	1.1
149	7.6	8.4	7.2	8.4	8.5	8.4	8.2
150	10.4	3.1	9.4	3.7	1.0	0.4	3.5
151	12.4	0.0	5.2	0.0	0.0	0.0	1.1
155	5.0	0.0	0.5	0.0	0.0	0.0	0.1
158	2.3	0.0	0.0	0.0	0.0	0.0	0.0
Outside		0.2	1.0	0.2	0.0	0.0	0.3
SARLF MFA							
7	0.5	2.4	3.2	2.7	1.5	1.0	1.3
8	1.0	15.3	1.9	14.9	16.3	17.1	16.6
10	0.3	1.3	0.4	1.2	1.7	1.6	1.6
15	2.7	8.1	0.3	7.9	8.4	8.8	8.5
18	0.1	0.3	0.1	0.3	0.1	0.0	0.1
27	1.2	0.4	0.2	0.4	0.5	0.6	0.5
28	4.8	2.6	2.4	2.7	2.4	2.4	2.4
39	4.9	5.8	18.5	6.3	4.3	3.7	4.1
40	2.1	0.6	0.0	0.7	0.2	0.1	0.2
44	0.1	11.4	0.0	9.6	17.2	19.8	18.1
45	0.3	4.0	0.0	4.4	2.5	1.1	2.0
46	0.1	0.0	1.7	0.0	0.0	0.0	0.0
48	1.9	0.7	8.1	0.8	0.2	0.1	0.1
49	2.4	8.4	0.1	8.4	8.5	8.4	8.5
50	0.9	3.1	1.0	3.7	1.0	0.4	0.8
51	6.4	0.0	4.6	0.0	0.0	0.0	0.0
55	26.1	0.0	2.8	0.0	0.0	0.0	0.0
56	23.9	0.0	0.0	0.0	0.0	0.0	0.0
58	20.1	0.0	18.5	0.0	0.0	0.0	0.0
Outside		35.6	43.8	35.8	35.2	35.0	35.1

Population viability analysis

Because of the relative long generation time, subpopulations were classified as vulnerable (10% probability of quasi-extinction within 100 years) before they were classified as *endangered* (20% probability of quasi-extinction in 10 generations; 124 years in ASL). As such, the vulnerable category was superfluous. Further, the critical and *endangered* risk categories were grouped because in most simulations, subpopulations went directly from *endangered* to *quasi-extinct*, with no transition through a critical risk category. As such, population viability analyses delineated subpopulations as not-threatened, *endangered* or *quasi-extinct*, with the *endangered* category being inclusive of vulnerable, *endangered* and critical risk categories.

Results from the population viability analysis for ASL subpopulations based on results from simulations which assessed the level of additional (ie. anthropogenic) female pre-recruit mortality (modelled as removals of 0-1.5 year olds per year) required to place individual subpopulations into different risk categories, with three population trajectory scenarios (stable r= 0.00, decreasing r =-0.01, and increasing r =0.05), are presented in Table 11. Results indicate that assuming no additional female mortalities with the stable and decreasing models developed, 13-27 subpopulations (34-71% of total) respectively, are classed as *endangered* (20% probability of extinction within 10 generations, Table 11). Small increases in pre-recruit female mortality markedly increased the numbers of *endangered* subpopulations. An additional mortality of one female per subpopulation/year resulted in 71% of stable, 84% of decreasing and 5% of increasing subpopulation being categorised as *endangered*. Two additional female mortalities per/subpopulation/year resulted in 84% of stable, 92% of decreasing and 13% of increasing subpopulation being classed as *endangered*. The lowest additional mortality level to result in a subpopulation becoming *quasi-extinct* was at 0.7 pre-recruit female seals/year (or 1 female per breeding cycle, 1.5 years). At this level of theoretical harvest, 2-5 (5-13%) subpopulations became *quasi-extinct* with the stable and decreasing population models, respectively. Q_t times were very short (<2 years, Table 11).

Because of the large number of very small ASL populations, small increases in additional mortality resulted in increasing numbers of subpopulations reaching the *quasi-extinct* threshold. For example, an additional pre-recruit mortality of only two females/subpopulation/year resulted in 16 subpopulations (42%) becoming *quasi-*

extinct in the stable model, or 27 (71%) becoming *quasi-extinct* in the decreasing model (Table 11, Figure 47). With an additional mortality level of only 3.3 pre-recruit females/subpopulation/year (5 females/1.5 year stage), 74% of decreasing, 63% of stable and 11% of increasing subpopulations became *quasi-extinct* (Table 11, Figure 47). Q_t times ranged from as little as 1.5 to 43.1 years in these scenarios. These population viability simulations suggest that even in the best scenario (populations increasing at around 5% per/year) many subpopulations are highly vulnerable to becoming *quasi-extinct* from low-level additional mortality (Figure 47).

Effects of mortality directed at different stages

The above scenarios for ASL demonstrate the susceptibility of subpopulations to *quasi-extinct*ion relative to different rates of female mortality directed at the youngest age group (0-1.5 years). To investigate how these results may vary in response to mortalities being directed at other ages/stages, a range of simulated removals of 20 female seals per year was undertaken on a hypothetical population of 1000 female ASL (slightly larger than the ASL population at Seal Bay, Kangaroo Island) using the stable population model over 50 reproductive cycles (75 years) (Figure 48). Results indicate that the highest rate of population reduction is achieved following removal of females between 3 and 12 years of age, with the greatest impacts achieved from removal of 4.5-6, 6-7.5 and 7.5-9 age-groups (Figure 48). These are females that are breeding for their first, second or third times (R. McIntosh pers comm.). In fact the rate of population decline resulting from mortalities directed at 6-7.5 year olds (-3.4%/year) was more than three times that of mortalities directed at pups (-1.1%/year) (Figure 48). Rates of decline were lowest when mortality was directed towards females older than 18 years of age (Figure 48).

Using the stable (r=0) population model, a comparison was made of the proportion of subpopulations that reached quasi-extinction under five different scenarios of female age-groups being subjected to mortality, from ages 0-1.5, 1.5-3, 3-4.5, 4.5-6, and 6-7.5 (Table 12). Results indicate the increasing vulnerability of subpopulations if mortality is directed at recruiting-age females. For example, if mortality is directed at pups, annual mortalities of one female per subpopulation per year result in 5% of subpopulations becoming *quasi-extinct* (Table 12). However, when mortalities are directed at the 6-7.5 year age group, annual mortalities of one female per subpopulation per year result in 26% of subpopulations becoming *quasi-extinct* (a five-fold increase compared to mortality directed at pups) (Table 12). Based on these estimates, additional mortalities of 1-2 female seals per subpopulation per year could

result in between 5-26% and 42-71% of subpopulations in South Australia becoming *quasi-extinct*, respectively, depending on the age of females removed from subpopulations (Table 12).

Risk classification of subpopulations

To examine whether subpopulations could be grouped according to extinction risk, a Bray-Curtis similarity matrix and hierarchical clustering analysis procedure (Primer V5.2.2) was undertaken based on PVA outputs using the stable population model (number of additional female deaths to change status from *not threatened* to *endangered*, *endangered* to *extinct* and Q_t). This analysis produced four major groupings, and seven minor groups (Figure 49 and 50). The first major grouping, termed *Very High Risk* included 4 subpopulations (11%) (in two subgroups) that were characterised by fewer than 9 pups, and categorised as *endangered* in the PVA analysis with no additional mortality, and had low thresholds of quasi-extinction (0.7-1.3 additional females/year) and Q_t (<10 years). The difference between these two subpopulations is attributed to slight differences in minimum number of pre-recruit (aged 0-1.5 years) female deaths/year to bring about extinction (0.7 and 1.3) and Q_t value (Figure 49 and 50).

The next group was termed *High Risk*, and was the largest group in the analysis with 23 subpopulations (61%). This group contained two subgroups that differed slightly in risk and Q_t. The first subgroup was characterised as having between 9 and 17 pups, low thresholds of quasi-extinction (≤ 2.0 additional females/year) and moderate Q_t 14-26 years). The second subgroup was characterised by larger pup production (21-43) and slightly greater quasi-extinction threshold (≤ 4.0 additional females/year) and Q_t (<38 years) (Figure 49, Table 11 and 13).

The *Moderate Risk* group included nine subpopulations (24%), and also contained two subgroups. The first subgroup was characterised by moderate pup production (56-84) and extinction thresholds achieved with between 4.7 – 8.7 additional female deaths/year and Q_t times ranging between 39-43 years. The second subgroup was characterised by producing more than 100 pups (131-214), with quasi-extinction thresholds between 11.3 – 23.3 additional female deaths/year and Q_t times ranging between 42-47 years (Figure 49, Table 11 and 13).

The *Low Risk* groups included the two largest subpopulations (Dangerous Reef and The Pages), which are at least twice the size as any other subpopulation. Quasi-

extinction thresholds and Q_t times were relatively high, requiring 50 additional female deaths/year over a 53-58 year period (Figure 49, Table 11 and 13).

Spatial distribution of subpopulation risk category

There is no clear pattern to the geographical distribution of different risk category subpopulations, with the four *very high risk* sites equally spaced along the lower and western Eyre Peninsula, the Nuyts Archipelago and the Bunda Cliffs (west of the Head of the Bight). Similarly, *high-risk* subpopulations are distributed along the same stretch of coastline, although there is greater density of these subpopulations in the Bunda Cliffs and lower Eyre Peninsula regions, with one site at Kangaroo Island. *Moderate risk* subpopulations are focused in the Nuyts Archipelago and Western Eyre Peninsula regions (seven sites), with two additional subpopulations in southern Spencer Gulf and one on Kangaroo Island. The two *low risk* subpopulations, Dangerous Reef and The Pages are located in southern Spencer Gulf and east of Kangaroo Island, respectively. In terms of proximity to particular MFAs within each fishery, those MFAs in the region of the Bunda Cliffs, the Nuyts Archipelago and western and lower Eyre Peninsula all contain *high* and *very high* risk subpopulations.

Fishery bycatch scenarios

The approach here is to firstly present the expected distribution of bycatch for ASL at the subpopulation, region and MFA level for both fisheries based on historic distribution of fishing effort and the expected distribution of seal foraging effort. Secondly, temporal variation in expected bycatch levels (by region and MFA) are examined based on historic changes in the distribution of fishing effort in each fishery. Thirdly, scenarios of different bycatch levels within each fishery are examined with respect to the historic average distribution of fishing effort to examine the potential outcomes of different catch rates and how bycatch rates may have varied among different MFAs. Finally, different scenarios of bycatch level in each fishery are examined relative to their expected impact on individual subpopulations, in terms of placing them in different risk categories based on the PVA outputs. These outputs provide the most coherent presentation of risk-assessment to all subpopulations from each fishery, and essentially pull together the subpopulation PVAs, the spatial and temporal overlap in fishing and seal foraging effort in conjunction with different scenarios of bycatch in each fishery.

Spatial distribution of historic bycatch: gill-net sector SESSF

Based on 32 years (1973-2004) of spatial and temporal variability in fishing effort in the SESSF gillnet sector, the expected apportioning of bycatch (with a breakdown by sex) among the different ASL subpopulations is presented in Figure 50. These analyses indicate that most seals would have been taken from the large populations of The Pages, Dangerous Reef and Seal Bay, with many of the colonies in the Nuyts Archipelago and western Eyre Peninsula making up the remainder. From a regional perspective, The Pages, southern Spencer Gulf (including Dangerous Reef, Peaked Rocks, North, English, North Neptune (East), South Neptune, Lewis and Albatross Islands) and the greater Nuyts Archipelago (including Lilliput, Blefuscu, Breakwater, Fenelon, Masillon, Purdie, and Lounds Islands, Gliddon and Western Nuyts Reef) make up about 67% of the expected bycatch, followed by Kangaroo Island (Seal Bay and the Seal Slide)and the Chain of Bays (including Olive, Nicolas Baudin and Jones islands) (Figure 51A). With respect to the expected breakdown of historical bycatch in different MFAs, the most prominent is MFA 108 (Nuyts Archipelago), which is expected to have accounted for more than a quarter of the total historic ASL bycatch in the fishery (Figure 52). MFAs 149 and 144 (Kangaroo Island and The Pages) and MFA 129 (southern Spencer Gulf) are expected to have each accounted for about 10% of the overall historic ASL bycatch (Figure 52).

Spatial and temporal distribution of historic bycatch: gill-net sector SESSF

Figures 54, 55, 56 and 57 present the expected breakdown of bycatch per colony and region, by proportion and number based on historic variability in the amount and distribution of fishing effort in the SESSF gillnet sector (1973-2004). Due to variability and the amount and location of fishing effort, the potential impact on ASL in different regions has changed markedly. The marked increase in fishing effort between the mid-1980s and 1990s is likely to have resulted in greater levels of bycatch for many regions (Figure 55, 57). For the most part, the Greater Nuyts and Southern Spencer Gulf Regions have been the most impacted (in terms of numbers) based on historic fishing effort data. However, with increased fishing effort in the southeast of SA since the late 1990s, the relative contribution of The Pages subpopulation to overall bycatch numbers has likely increased during this period (Figures 54, 56). Predicted temporal variation in historic bycatch contributions for the six most significant MFAs (in terms of estimated contribution of ASL bycatch, MFAs 108,115,129, 144, 149 and 150) are presented in Figures 58 and 59. MFA 108 is predicted to have contributed the highest proportion of ASL bycatch between 1973-2004, even though fishing

effort, and the relative contribution of bycatch in most of the other MFAs increased throughout the period.

At the end of the historic time series (2004), the proportion and number of seals expected to have been derived from The Pages, greater Nuyts and southern Spencer Gulf regions was similar (Figures 54-57). Furthermore, in 2004 MFA 108 is predicted to have accounted for about 26% of bycatch, with MFA 149, 144 and 129 accounting for between 11-13%, and MFA 150 and 115 about 7% (Figure 58).

MFA bycatch scenarios: gill-net sector SESSF

Table 14 presents for each gillnet SESSF MFA the average (1973-2004) annual fishing effort and the proportion of fishing effort and expected ASL bycatch. A range of possible bycatch scenarios is presented, ranging from and average of 0.0005 to 0.0400 seals per km net-lift/year. Average bycatch rates are calculated by dividing the number of seals caught by the total fishing effort in those MFAs where seals where caught. Based on the ASL foraging effort models, the only MFA in SA where ASL are not expected to become bycatch is in MFA 158. At a bycatch rate of 0.0005 seals/km lift/year (approximately 10 seals, ie.1 seal/2,000km net-lift), the bycatch rates among MFAs ranges from 0 to 0.08 (Table 14). At an average bycatch rate of 0.04 seals/km-lift/year (775 seals, ie. 1 seal/25km net-lift), the MFA bycatch rates vary from 0 to 0.128 (Table 14). In all bycatch scenarios, the proportion of bycatch apportioned to each MFA is the same.

Subpopulation PVA with bycatch scenarios: gill-net sector SESSF

Figure 60 indicates the overall bycatch number and average rate required to place different ASL subpopulations into different risk categories. These are based on the number of female mortalities attributed to individual subpopulations, as determined by the overlap in estimated foraging effort and the average (1973-2004) distribution of fishing effort (see Table 14). The bycatch number refers to the total number seals caught per year, of which about 52% are female that are apportioned out among the 38 subpopulations. The estimated number of additional female mortalities per year required to place subpopulations into the various risk categories (based on PVA using the stable population model, see Table 11) was combined with the bycatch scenario analysis to provide an integrated risk assessment analyses. The PVA indicates which subpopulations can least afford to lose individuals, but it does not

indicate whether those subpopulations are likely to lose individual seals based on the distribution of seal foraging and fishing effort. Figure 60 integrates the spatial bycatch analysis with the PVA approach, and therefore identifies which subpopulation should be classified as most at risk under different bycatch scenarios.

With no additional bycatch mortalities, 24% of ASL subpopulations in South Australian are categorised as *endangered*. However, if bycatch mortality in the demersal shark fishery was 50, 100, 150 and 200 seals per year, the percentage of *endangered* subpopulations would increase to 45%, 68%, 84% and 92%, respectively (Figure 60). These results highlight just how vulnerable subpopulations are to small increases in additional mortality. The ten most at risk subpopulations (risk of quasi-extinction from bycatch mortality) in the demersal shark fishery, occur in the Nuyts Archipelago and Kangaroo Island regions. Seven subpopulations occur within the Nuyts Archipelago, all within demersal gillnet marine fishing area (MFA) 108 (Olive, Lilliput, West, Purdie, Blefuscu, and Lounds Island and Breakwater Reef). Based on population viability analyses, subpopulation foraging models and fishery interaction probabilities, these seven subpopulations would become *quasi-extinct* if total annual bycatch levels in the whole gillnet fishery were between 262 and 346 seals (Figure 60). For individual subpopulations this equated to as little as between two (Breakwater Reef) and 12 (Purdie Island) female bycatch mortalities per year (Table 11). The next three most vulnerable subpopulations were in the Kangaroo Island region (Seal Bay, Seal Slide and The Pages), which were estimated to become *quasi-extinct* when total annual bycatch in the fishery reached between 349 and 392 seals (Figure 60). For these three subpopulations, this equated to between 1.3 (Seal Slide) and 50 (The Pages) female bycatch mortalities per year (Table 11).

Table 11. Summary of population viability analysis (PVA) for ASL subpopulations in South Australia. The table presents results from simulations assessing the level of additional female pre-recruit mortality (modelled as annual removal of 1.5 year olds) required to place individual subpopulations into different risk categories (E+C= *endangered* and *critical*, Extinct = *quasi-extinct*), based on the three population trajectory scenarios (stable *r*= 0.00, decreasing *r* =-0.01, and increasing *r* =0.05). Q_t represents quasi-extinction time (years). The estimated pup production of each subpopulation is given (see Table 3) and subpopulations are ranked according to risk.

		Amount of annual additional pre-recruit female mortality to change subpopulation risk								
		Decreasing			Stable			Increasing		
Subpopulation	Pup	λ= 0.9801, r=-0.01			λ =1, r=0			λ = 1.0985, r=0.05		
	No.	E+C	Extinct	Q_t	E+C	Extinct	Q_t	E+C	Extinct	Q_t
GAB B2	5	0.0	0.7	1.5	0.0	0.7	1.7	0.7	2.7	1.8
South Neptune Is.	6	0.0	0.7	1.5	0.0	0.7	1.8	0.7	2.7	1.8
Gliddon Reef	7	0.0	0.7	9.9	0.0	1.3	7.5	1.3	3.3	10.5
Ward Is.	8	0.0	0.7	9.9	0.0	1.3	9.5	1.3	3.3	10.5
Masillon Is.	9	0.0	0.7	16.8	0.0	1.3	14.6	2.0	4.0	17.0
Seal Slide	11	0.0	1.3	15.2	0.0	1.3	19.2	2.7	4.7	17.0
Four Hummocks Is.	12	0.0	1.3	15.2	0.0	2.0	15.8	2.7	4.7	17.0
GAB B6	12	0.0	1.3	15.2	0.0	1.3	19.2	2.7	4.7	17.0
North Neptune (East) Is.	14	0.0	1.3	20.3	0.0	2.0	19.2	3.3	5.3	19.4
Western Nuyts Reef	14	0.0	1.3	20.3	0.0	2.0	19.2	3.3	6.0	19.5
Albatross Is.	15	0.0	1.3	22.8	0.0	2.0	21.8	3.3	6.7	12.5
Jones Is.	15	0.0	1.3	22.8	0.0	2.0	21.8	3.3	6.7	18.8
GAB B1	15	0.0	1.3	22.8	0.0	2.0	21.8	3.3	6.7	18.8
Rocky (North) Is.	16	0.0	1.3	22.8	0.1	2.0	23.7	3.3	6.7	18.8
GAB B9	17	0.0	2.0	21.3	0.1	2.0	25.1	4.0	7.3	21.0
Breakwater Reef	17	0.0	2.0	21.3	0.1	2.0	25.7	3.3	6.0	20.6
Fenelon Is.	21	0.0	1.3	33.9	0.2	2.7	25.1	4.7	8.7	22.8
Peaked Rock	24	0.0	1.3	36.2	0.4	2.7	30.3	5.3	8.7	23.3
Price Is.	25	0.0	2.0	28.5	0.3	2.7	30.3	5.3	10.0	23.3
Lounds Is.	26	0.0	2.0	28.5	0.3	2.7	31.2	5.3	10.0	23.4
Pearson Is.	27	0.0	2.0	33.9	0.4	3.3	30.0	6.0	10.0	26.6
English Is.	27	0.0	2.0	34.2	0.4	3.3	29.7	5.3	10.0	24.0
North Is.	28	0.0	2.0	34.2	0.4	3.3	29.7	5.3	10.0	24.0
GAB B3	31	0.0	2.0	36.6	0.5	3.3	32.3	6.7	11.3	25.4
GAB B8	38	0.0	2.0	39.6	1.0	4.0	34.8	8.0	13.3	28.1
Liguanea Is.	43	0.0	2.0	45.9	1.0	4.0	37.2	9.3	16.0	26.1
GAB B5	43	0.0	2.0	45.9	1.0	4.0	37.2	8.7	15.3	27.0
West Is.	56	0.3	3.3	43.1	1.3	4.7	42.8	11.3	23.3	25.1
Lilliput Is.	67	0.3	4.0	45.3	1.3	6.0	41.9	14.0	23.3	31.7
Nicolas Baudin Is.	72	0.0	4.0	47.3	2.0	7.3	39.3	14.7	26.7	0.0
Lewis Is.	73	0.3	4.0	46.7	2.0	7.3	40.5	14.0	24.7	32.4
Blefuscu Is.	84	0.4	5.3	44.1	2.0	8.7	39.3	20.0	30.0	30.6
Olive Is.	131	1.3	6.0	55.7	3.3	11.3	46.5	26.7	43.3	34.8
Purdie Is.	132	1.3	6.7	52.5	3.3	12.0	45.0	26.0	45.3	33.0
West Waldegrave Is.	157	2.0	8.0	52.8	4.0	14.0	45.6	33.3	53.3	33.8
Seal Bay	214	2.7	10.0	59.6	5.3	21.3	44.9	42.0	72.7	34.1
The Pages	577	6.7	27.3	62.7	16.7	50.7	53.9	120.0	183.3	39.2
Dangerous Reef	585	6.7	28.0	60.3	16.7	48.7	55.1	117.3	190.0	38.1

Table 12. Comparison of the proportion of subpopulations of ASL reaching quasi-extinction subject to increasing additional female mortality, under five scenarios of different age-groups subjected to mortality. The age-groups are 0-1.5, 1.5-3, 3-4.5, 4.5-6, and 6-7.5 years. Calculations are based on the stable population model (r=0).

Number of additional female deaths/ subpopulation/year	Percentage of subpopulations that become *quasi-extinct* relative to age-group from which additional females are removed				
	Stages/Age-group				
	0.-1.5	1.5-3	3-4.5	4.5-6	6-7.5
0.10	0	0	0	0	0
0.22	0	0	0	0	0
0.33	0	0	0	0	0
0.44	0	0	0	0	0
0.50	0	0	0	0	0
0.67	5	13	13	21	26
1.00	5	13	16	26	26
1.33	5	13	16	26	26
2.00	42	63	71	71	71
2.7	53	71	74	74	79
3.3	53	71	74	74	79
4.0	71	82	84	84	84
4.7	74	84	84	84	89
5.3	74	84	84	84	89
6.0	76	84	89	89	89
6.7	76	84	89	92	92
7.3	76	84	89	92	92
8.0	82	89	92	92	92
8.7	84	92	92	92	95
9.3	84	92	92	92	95
10.0	84	92	92	95	95
10.7	84	92	95	95	95
11.3	84	92	95	95	95
12.0	89	95	95	95	95
12.7	89	95	95	95	95
13.3	89	95	95	95	95
14.0	92	95	95	95	95
16.7	92	95	95	95	95
20.0	92	95	95	95	95
23.3	92	95	95	95	100
26.7	95	95	100	100	100
30.0	95	97	100	100	100
33.3	95	97	100	100	100
36.7	95	100	100	100	100
40.0	95	100	100	100	100
43.3	95	100	100	100	100
46.7	97	100	100	100	100
50.0	100	100	100	100	100

Table 13. Summary of PVA outcomes for SA ASL subpopulations, grouped in risk categories.

				Additional female mortalities to change subpopulation status		
Risk Category	Group	No. pups	No. subpops	*Endangered* /critical	Extinct	Q_t (years)
Very High Risk (11%)	1	5-6	2	0.0	0.7	1.7-1.8
	2	7-8	2	0.0	1.3	7.5-9.5
High Risk (61%)	3	9-17	12	0.0-0.1	1.3-2	14.6-25.7
	4	21-43	11	0.2-1.0	2.7-4	25.1-37.2
Moderate Risk (24%)	5	56-84	5	1.3-2.0	4.7-8.7	39.3-42-8
	6	131-214	4	3.3-5.3	11.3-21.3	42-46.5
Low Risk (5%)	7	577-585	2	16.7	18.7-50.9	53.6-57.5

Spatial distribution of historic bycatch: SA Rock lobster fishery

Based on 35 years (1970-2004) of spatial and temporal variability in fishing effort in the SA RLF, the estimated apportioning of bycatch among the different ASL subpopulations is presented in Figure 50. This analysis suggest that in terms of numbers, most bycatch seals would have been taken from Seal Bay (20%) and West Waldegrave Island (16%) (Figure 50). Many of the subpopulations in the southern Spencer Gulf region, including Dangerous Reef, Lewis, Liguanea and Price Islands were also likely to have contributed significantly to bycatch in the SA RLF (Figure 50). These southern Spencer Gulf subpopulations likely contributed to most of the bycatch (24%), followed by Kangaroo Island (21%), Western Eyre Peninsula (195) and the Greater Nuyts Archipelago (13%, Figure 51B).

With respect to the estimated spread of historical bycatch across different MFAs, five MFAs would have accounted for most (94%) of it (Figure 53. The main ones being MFA 15 (24%), MFA 39 (20%) and MFA 49 (21%), followed by MFA 8 (17%) and MFA 28 (12%) (Figure 53).

Spatial and temporal distribution of historic bycatch: SA Rock lobster fishery

Figures 56 and 57 present the expected breakdown of bycatch per region by proportion and number based on historic variability in the amount and distribution of fishing effort in the SA RLF (1970-2004). The proportion and number of seals taken from the different regions are likely to have varied considerably over this period, with a general increase in the importance of Kangaroo Island, especially since the late 1990s (Figures 56 and 57). Southern Spencer Gulf and the western Eyre regions are

also likely to have been significant in their contribution to bycatch, and to a lesser extent, the Greater Nuyts Archipelago (Figures 56 and 57).

Predicted temporal variation in historical bycatch contributions from the five most significant MFAs (MFAs 49, 39, 28, 15, and 8), is presented in Figures 7.27a and 7.28a. There is a clear trend for increasing bycatch contribution (both percentage and number) from MFA 49, especially since the late 1990s, when the contribution from MFA 15 began to decline markedly.

MFA bycatch scenarios: SA Rock lobster fishery

Table 15 presents the average (1970-2004) annual fishing effort per SA RLF MFA, and the proportion of fishing effort and expected ASL bycatch for each MFA. A range of possible bycatch scenarios is presented, ranging from an average of 0.005 to 0.500 seals/1,000 pot-lifts/year. Average bycatch rates were calculated by dividing the number of seals caught by the total fishing effort in those MFAs where seals where caught. Based on the ASL foraging effort models and these bycatch scenarios, there are five MFAs in SA where ASL are not expected to become bycatch. These are MFA 46, 51, 55, 56 and 58 (Table 15). At a bycatch rate of 0.005 seals/1,000 pot-lifts/year (ie.1 seal/200,000 pot-lifts), the bycatch rates among MFAs ranges from 0 to 0.02 seals/1,000 pot-lifts (Table 15). At an average bycatch rate of 0.500 seals/1,000 pot-lifts/year (ie. 1 seal/2,000 pot-lifts), the MFA bycatch rates vary from 0 to 2.2 seals/1,000 pot-lifts (Table 15).

Subpopulation PVA with bycatch scenarios

If bycatch mortality in the southern rock lobster fishery was 50, 100, 150 and 200 ASL per year (Figure 60), then the percentage of subpopulations in South Australian categorised as *endangered* would increase from 24% (zero bycatch) to 53%, 66%, 79% and 82%, respectively. The ten ASL subpopulations at greatest risk of extinction to bycatch in the South Australian southern rock lobster fishery included Price Island, Peaked Rocks, South and North Neptune Islands, North and Liguanea Islands in the southern Spencer Gulf/lower Eyre Peninsula region, West Waldegrave and Jones Island (west Eyre Peninsula) and Seal Bay and the Seal Slide (Kangaroo Island) (Figure 60). Based on population viability analyses, subpopulation foraging models and fishery interaction probabilities, the most at-risk subpopulations would become extinct if total annual bycatch levels in the fishery reached between 127 and 254

seals (Figure 60). For individual subpopulations, this equated to as little as between 0.7 (South Neptune Island) and 23 (Seal Bay) female bycatch mortalities per year.

Table 14. Hypothetical ASL bycatch scenarios in the gillnet sector of the SESSF off SA. Average (1973-2004) annual fishing effort per MFA, the proportion of fishing effort and expected proportion of ASL bycatch per MFA are presented with a range of possible bycatch scenarios, ranging from an average of 0.0005 to 0.0400 seals per km net-lift/year. Individual MFA bycatch rates are given for each scenario.

Gillnet SESSF			Prop. seal bycatch	Average bycatch rate gillnet SESSF (seals/km net-lift)													
				0.0005		0.0010		0.0020		0.0030		0.0040		0.0050		0.006	
MFA	Fishing effort (km lifts)	Prop. fishing effort		No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate
101	146	0.007	0.004	0.0	0.0003	0.1	0.0006	0.2	0.0011	0.2	0.0017	0.3	0.0022	0.4	0.0028	0.5	0.0033
102	104	0.005	0.002	0.0	0.0002	0.0	0.0003	0.1	0.0007	0.1	0.0010	0.1	0.0013	0.2	0.0017	0.2	0.0020
103	262	0.013	0.001	0.0	0.0000	0.0	0.0001	0.0	0.0002	0.1	0.0002	0.1	0.0003	0.1	0.0004	0.1	0.0005
104	183	0.009	0.001	0.0	0.0000	0.0	0.0001	0.0	0.0002	0.1	0.0003	0.1	0.0004	0.1	0.0005	0.1	0.0006
105	204	0.010	0.001	0.0	0.0000	0.0	0.0001	0.0	0.0001	0.0	0.0002	0.1	0.0003	0.1	0.0004	0.1	0.0004
106	214	0.011	0.001	0.0	0.0001	0.0	0.0001	0.0	0.0002	0.1	0.0003	0.1	0.0004	0.1	0.0005	0.1	0.0006
107	639	0.032	0.017	0.2	0.0003	0.3	0.0005	0.6	0.0010	1.0	0.0015	1.3	0.0020	1.6	0.0025	1.9	0.0030
108	1594	0.080	0.264	2.6	0.0016	5.1	0.0032	10.2	0.0064	15.3	0.0096	20.5	0.0128	25.6	0.0160	30.7	0.0192
112	114	0.006	0.000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0001	0.0	0.0001	0.0	0.0001	0.0	0.0001
113	331	0.017	0.003	0.0	0.0001	0.1	0.0002	0.1	0.0003	0.2	0.0005	0.2	0.0007	0.3	0.0008	0.3	0.0010
114	723	0.036	0.019	0.2	0.0003	0.4	0.0005	0.7	0.0010	1.1	0.0016	1.5	0.0021	1.9	0.0026	2.2	0.0031
115	968	0.049	0.074	0.7	0.0007	1.4	0.0015	2.9	0.0030	4.3	0.0044	5.7	0.0059	7.2	0.0074	8.6	0.0089
122	143	0.007	0.000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0001	0.0	0.0001	0.0	0.0001	0.0	0.0001
125	18	0.001	0.000	0.0	0.0000	0.0	0.0001	0.0	0.0002	0.0	0.0003	0.0	0.0003	0.0	0.0004	0.0	0.0005
126	1032	0.052	0.021	0.2	0.0002	0.4	0.0004	0.8	0.0008	1.2	0.0012	1.6	0.0016	2.0	0.0020	2.4	0.0024
128	632	0.032	0.026	0.3	0.0004	0.5	0.0008	1.0	0.0016	1.5	0.0024	2.0	0.0032	2.6	0.0040	3.1	0.0049
129	732	0.037	0.125	1.2	0.0017	2.4	0.0033	4.8	0.0066	7.3	0.0099	9.7	0.0132	12.1	0.0165	14.5	0.0198
132	451	0.023	0.002	0.0	0.0000	0.0	0.0001	0.1	0.0002	0.1	0.0003	0.2	0.0004	0.2	0.0005	0.3	0.0006
136	122	0.006	0.000	0.0	0.0000	0.0	0.0000	0.0	0.0001	0.0	0.0001	0.0	0.0001	0.0	0.0002	0.0	0.0002
138	919	0.046	0.032	0.3	0.0003	0.6	0.0007	1.2	0.0014	1.9	0.0020	2.5	0.0027	3.1	0.0034	3.7	0.0041
139	745	0.038	0.044	0.4	0.0006	0.9	0.0011	1.7	0.0023	2.6	0.0034	3.4	0.0046	4.3	0.0057	5.1	0.0069
140	627	0.032	0.013	0.1	0.0002	0.3	0.0004	0.5	0.0008	0.8	0.0012	1.0	0.0016	1.3	0.0020	1.5	0.0024
144	751	0.038	0.113	1.1	0.0015	2.2	0.0029	4.4	0.0058	6.6	0.0088	8.8	0.0117	11.0	0.0146	13.1	0.0175
148	708	0.036	0.008	0.1	0.0001	0.2	0.0002	0.3	0.0004	0.5	0.0007	0.6	0.0009	0.8	0.0011	0.9	0.0013
149	1498	0.076	0.126	1.2	0.0008	2.4	0.0016	4.9	0.0033	7.3	0.0049	9.8	0.0065	12.2	0.0081	14.6	0.0098
150	2062	0.104	0.074	0.7	0.0003	1.4	0.0007	2.9	0.0014	4.3	0.0021	5.8	0.0028	7.2	0.0035	8.7	0.0042
151	2458	0.124	0.027	0.3	0.0001	0.5	0.0002	1.0	0.0004	1.5	0.0006	2.1	0.0008	2.6	0.0010	3.1	0.0013
155	988	0.050	0.001	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.1	0.0001	0.1	0.0001	0.1	0.0001	0.1	0.0001
158	459	0.020	0.000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000
Sum	19,828		Total seals	10		19		39		58		77		97		116	

Continued on next page.

Table 14. Cont.

MFA	Average bycatch rate gillnet SESSF (seals/km net-lift)															
	0.007		0.008		0.009		0.010		0.015		0.020		0.030		0.040	
	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate
101	0.6	0.0039	0.6	0.0044	0.7	0.0050	0.8	0.0055	1.2	0.0083	1.6	0.0110	2.4	0.0165	3.2	0.0220
102	0.2	0.0024	0.3	0.0027	0.3	0.0030	0.3	0.0034	0.5	0.0050	0.7	0.0067	1.0	0.0101	1.4	0.0134
103	0.1	0.0005	0.2	0.0006	0.2	0.0007	0.2	0.0008	0.3	0.0012	0.4	0.0016	0.6	0.0023	0.8	0.0031
104	0.1	0.0007	0.1	0.0008	0.2	0.0009	0.2	0.0010	0.3	0.0014	0.3	0.0019	0.5	0.0029	0.7	0.0038
105	0.1	0.0005	0.1	0.0006	0.1	0.0007	0.1	0.0007	0.2	0.0011	0.3	0.0015	0.4	0.0022	0.6	0.0029
106	0.2	0.0007	0.2	0.0009	0.2	0.0010	0.2	0.0011	0.3	0.0016	0.5	0.0021	0.7	0.0032	0.9	0.0043
107	2.3	0.0035	2.6	0.0040	2.9	0.0045	3.2	0.0051	4.8	0.0076	6.5	0.0101	9.7	0.0152	12.9	0.0202
108	35.8	0.0224	40.9	0.0257	46.0	0.0289	51.1	0.0321	76.7	0.0481	102.3	0.0641	153.4	0.0962	204	0.1283
112	0.0	0.0002	0.0	0.0002	0.0	0.0002	0.0	0.0002	0.0	0.0003	0.1	0.0004	0.1	0.0007	0.1	0.0009
113	0.4	0.0012	0.4	0.0013	0.5	0.0015	0.6	0.0017	0.8	0.0025	1.1	0.0034	1.7	0.0050	2.2	0.0067
114	2.6	0.0036	3.0	0.0042	3.4	0.0047	3.7	0.0052	5.6	0.0078	7.5	0.0104	11.2	0.0156	15.0	0.0208
115	10.0	0.0104	11.5	0.0118	12.9	0.0133	14.3	0.0148	21.5	0.0222	28.6	0.0296	42.9	0.0444	57.3	0.0592
122	0.0	0.0002	0.0	0.0002	0.0	0.0002	0.0	0.0002	0.0	0.0003	0.1	0.0004	0.1	0.0007	0.1	0.0009
125	0.0	0.0006	0.0	0.0007	0.0	0.0008	0.0	0.0009	0.0	0.0013	0.0	0.0017	0.0	0.0026	0.1	0.0034
126	2.8	0.0028	3.3	0.0032	3.7	0.0035	4.1	0.0039	6.1	0.0059	8.1	0.0079	12.2	0.0118	16.3	0.0158
128	3.6	0.0057	4.1	0.0065	4.6	0.0073	5.1	0.0081	7.7	0.0121	10.2	0.0162	15.3	0.0243	20.5	0.0324
129	16.9	0.0231	19.4	0.0264	21.8	0.0297	24.2	0.0330	36.3	0.0496	48.4	0.0661	72.6	0.0991	96.8	0.1321
132	0.3	0.0007	0.3	0.0008	0.4	0.0008	0.4	0.0009	0.6	0.0014	0.8	0.0019	1.3	0.0028	1.7	0.0038
136	0.0	0.0002	0.0	0.0003	0.0	0.0003	0.0	0.0003	0.1	0.0005	0.1	0.0007	0.1	0.0010	0.2	0.0014
138	4.4	0.0047	5.0	0.0054	5.6	0.0061	6.2	0.0068	9.3	0.0102	12.5	0.0136	18.7	0.0203	24.9	0.0271
139	6.0	0.0080	6.8	0.0092	7.7	0.0103	8.5	0.0115	12.8	0.0172	17.1	0.0229	25.6	0.0344	34.2	0.0459
140	1.8	0.0028	2.0	0.0032	2.3	0.0036	2.5	0.0040	3.8	0.0061	5.1	0.0081	7.6	0.0121	10.1	0.0161
144	15.3	0.0204	17.5	0.0234	19.7	0.0263	21.9	0.0292	32.9	0.0438	43.8	0.0584	65.7	0.0876	87.6	0.1168
148	1.1	0.0015	1.2	0.0017	1.4	0.0020	1.5	0.0022	2.3	0.0033	3.1	0.0043	4.6	0.0065	6.2	0.0087
149	17.1	0.0114	19.5	0.0130	21.9	0.0146	24.4	0.0163	36.6	0.0244	48.8	0.0325	73.1	0.0488	97.5	0.0651
150	10.1	0.0049	11.5	0.0056	13.0	0.0063	14.4	0.0070	21.6	0.0105	28.9	0.0140	43.3	0.0210	57.7	0.0280
151	3.6	0.0015	4.1	0.0017	4.6	0.0019	5.1	0.0021	7.7	0.0031	10.3	0.0042	15.4	0.0063	20.6	0.0084
155	0.1	0.0001	0.1	0.0001	0.2	0.0002	0.2	0.0002	0.3	0.0003	0.4	0.0004	0.6	0.0006	0.7	0.0007
158	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000
	136		155		174		194		291		387		581		775	

Table 15. Hypothetical ASL bycatch scenarios in the SA RLF. Average (1970-2004) annual fishing effort per MFA, the proportion of fishing effort and expected proportion of ASL bycatch per MFA are presented. A range of possible bycatch scenarios is presented, ranging from an average of 0.005 to 0.500 seals per 1,000 pot-lifts/year and bycatch rates are given for each scenario.

SA Rock Lobster			Prop. seal bycatch	Average bycatch rate SA Rock Lobster Fishery (seals/1,000 pot-lift)																											
				0.005		0.010		0.020		0.030		0.040		0.050		0.06		0.070		0.080		0.090		0.100		0.200		0.300		0.400		0.500	
MFA	Fishing effort (1,000 lifts)	Prop. fishing effort		No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate	No.	MFA bycatch rate
7	11.5	0.005	0.006	0.0	0.0015	0.0	0.0029	0.1	0.0058	0.1	0.0087	0.1	0.0117	0.2	0.0146	0.2	0.0175	0.2	0.0204	0.3	0.0233	0.3	0.0262	0.3	0.0291	0.7	0.0583	1.0	0.0874	1.3	0.1165	1.7	0.1457
8	22.9	0.010	0.1732	0.5	0.0199	0.9	0.0398	1.8	0.0796	2.7	0.1194	3.6	0.1592	4.6	0.1990	5.5	0.2388	6.4	0.2786	7.3	0.3184	8.2	0.3581	9.1	0.3979	18.2	0.7959	27.3	1.1938	36.4	1.5918	45.5	1.9897
10	7.5	0.003	0.0055	0.0	0.0019	0.0	0.0039	0.1	0.0078	0.1	0.0116	0.1	0.0155	0.1	0.0194	0.2	0.0233	0.2	0.0272	0.2	0.0310	0.3	0.0349	0.3	0.0388	0.6	0.0776	0.9	0.1164	1.2	0.1552	1.5	0.1940
15	60.6	0.027	0.2362	0.6	0.0103	1.2	0.0205	2.5	0.0410	3.7	0.0615	5.0	0.0820	6.2	0.1026	7.5	0.1231	8.7	0.1436	9.9	0.1641	11.2	0.1846	12.4	0.2051	24.8	0.4102	37.3	0.6154	49.7	0.8205	62.1	1.0256
18	3.4	0.001	0.0001	0.0	0.0001	0.0	0.0001	0.0	0.0003	0.0	0.0004	0.0	0.0005	0.0	0.0007	0.0	0.0008	0.0	0.0009	0.0	0.0010	0.0	0.0012	0.0	0.0013	0.0	0.0026	0.0	0.0039	0.0	0.0052	0.0	0.0065
27	26.7	0.012	0.0065	0.0	0.0006	0.0	0.0013	0.1	0.0026	0.1	0.0038	0.1	0.0051	0.2	0.0064	0.2	0.0077	0.2	0.0089	0.3	0.0102	0.3	0.0115	0.3	0.0128	0.7	0.0256	1.0	0.0383	1.4	0.0511	1.7	0.0639
28	107.4	0.048	0.1172	0.3	0.0029	0.6	0.0057	1.2	0.0115	1.8	0.0172	2.5	0.0229	3.1	0.0287	3.7	0.0344	4.3	0.0401	4.9	0.0459	5.5	0.0516	6.2	0.0574	12.3	0.1147	18.5	0.1721	24.6	0.2294	30.8	0.2868
39	111.3	0.049	0.2016	0.5	0.0048	1.1	0.0095	2.1	0.0191	3.2	0.0286	4.2	0.0381	5.3	0.0476	6.4	0.0572	7.4	0.0667	8.5	0.0762	9.5	0.0857	10.6	0.0953	21.2	0.1905	31.8	0.2858	42.4	0.3810	53.0	0.4763
40	48.0	0.021	0.0042	0.0	0.0002	0.0	0.0005	0.0	0.0009	0.1	0.0014	0.1	0.0018	0.1	0.0023	0.1	0.0028	0.2	0.0032	0.2	0.0037	0.2	0.0042	0.2	0.0046	0.4	0.0092	0.7	0.0138	0.9	0.0185	1.1	0.0231
44	3.0	0.001	0.0251	0.1	0.0220	0.1	0.0441	0.3	0.0882	0.4	0.1323	0.5	0.1763	0.7	0.2204	0.8	0.2645	0.9	0.3086	1.1	0.3527	1.2	0.3968	1.3	0.4408	2.6	0.8817	4.0	1.3225	5.3	1.7633	6.6	2.2042
45	6.4	0.003	0.0052	0.0	0.0021	0.0	0.0043	0.1	0.0086	0.1	0.0128	0.1	0.0171	0.1	0.0214	0.2	0.0257	0.2	0.0300	0.2	0.0342	0.2	0.0385	0.3	0.0428	0.5	0.0856	0.8	0.1284	1.1	0.1711	1.4	0.2139
46	1.9	0.001	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000
48	42.9	0.019	0.0024	0.0	0.0001	0.0	0.0003	0.0	0.0006	0.0	0.0009	0.0	0.0012	0.1	0.0014	0.1	0.0017	0.1	0.0020	0.1	0.0023	0.1	0.0026	0.1	0.0029	0.2	0.0058	0.4	0.0087	0.5	0.0116	0.6	0.0145
49	54.6	0.024	0.2102	0.6	0.0101	1.1	0.0203	2.2	0.0405	3.3	0.0608	4.4	0.0810	5.5	0.1013	6.6	0.1215	7.7	0.1418	8.8	0.1620	9.9	0.1823	11.1	0.2025	22.1	0.4051	33.2	0.6076	44.2	0.8101	55.3	1.0127
50	19.7	0.009	0.0063	0.0	0.0008	0.0	0.0017	0.1	0.0033	0.1	0.0050	0.1	0.0067	0.2	0.0083	0.2	0.0100	0.2	0.0117	0.3	0.0134	0.3	0.0150	0.3	0.0167	0.7	0.0334	1.0	0.0501	1.3	0.0668	1.6	0.0835
51	144.6	0.064	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000
55	588.8	0.261	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000
56	539.4	0.239	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000
58	452.6	0.201	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000	0.0	0.0000
Sum	2253			3		5		11		16		21		26		32		37		42		47		53		105		158		210		263	

Discussion

Study limitations

This study has compiled and synthesised a considerable amount of information on the demography, size, foraging ecology and extinction risk for South Australia's ASL subpopulations and the historical spatial distribution of fishing effort in the SESSF gillnet sector and SA RLF in order to provide an assessment of the risk to SA seal populations from bycatch in these fisheries. This has been done in the absence of any quantitative data on pinniped bycatch levels in these fisheries. A task such as this inevitably has to make many assumptions and deal with data deficiencies that can impact on the outcomes of analyses, and the degree of certainty placed on the findings. As such, we address the major limitations first, so the broad findings can be viewed in an appropriate context.

Seal population data

For ASL, although the relative size of subpopulations is generally understood, the quality of data on the pup production of different subpopulations is typically poor. There are a number of reasons for this (McKenzie et al. 2005, Shaughnessy et al. 2005). Firstly, because of the asynchronous and non-annual breeding cycle the timing of breeding is not well understood for the majority of ASL subpopulations. Secondly, the species has a protracted (5-7 month) breeding season that means that by the end of the season, some pups will have died, moulted and/or dispersed, making it difficult to determine total pup production. Thirdly, pup production estimates (the only means of estimating subpopulation size) are typically based on the maximum number of live pups seen on single or multiple counts made during a breeding season, and where possible, cumulative numbers of dead pups are added to produce a final estimate. There is uncertainty about the accuracy of these counts, because current methods do no provide estimates of confidence or error. For this reason, the limited time series data available for subpopulations are difficult to interpret and provide little confidence about trends in abundance.

The demographic models used to estimate the size of seal subpopulations were constructed based on limited data and a number of assumptions based on data from

closely related species. The main model used for the ASL assumed that all subpopulations were stable (equilibrium survival and birth rates), and that the vital rates for all subpopulations were identical, regardless of size. This is almost certainly not the case, because trend data for three subpopulations (The Pages, Seal Bay and Dangerous Reef) indicate a spectrum of increasing, decreasing and potentially stable populations. The demographic models assumed density dependence not to be a significant factor regulating the size of subpopulations. Although there may be some basis to this assumption (eg. species below their carrying capacity following significant range and population reductions, as discussed above), it may be an important factor, which could be limiting the recovery of some subpopulations. Similarly, Allee effects were not incorporated into demographic models, primarily because of their unknown role in regulating pinniped populations. The resultant models used are therefore relatively conservative (ie. presenting more positive growth), because density dependence would reduce the rate at which subpopulations can grow, while Allee effects would tend to reduce the growth potential of small and declining subpopulations.

The uncertainties detailed above in terms of the size and demographic structure of subpopulations impact on the ability to undertake realistic population viability analyses (PVAs), and this procedure has been criticised, because there are often large uncertainties involved in predicting the probability of extinction of populations or species (Taylor 1995, Ludwig 1999, Ellner et al. 2002). Because of this, it is important to examine and evaluate (where possible) the major sources of error.

Foraging models and fishing effort

The foraging effort models used were overly simplistic because they did not incorporate differences within and between subpopulations in the direction (and in some cases distance) of travel. Direction of travel was not estimated for subpopulations and as a consequence, the area included in the foraging effort in ASL was likely to be widely spread and therefore may have incorrectly incorporated some areas. For example, foraging data for subpopulations of ASL in the Nuyts Archipelago indicate a marked variability in the distribution of foraging effort between colonies, with animals in some colonies feeding inshore in shallow waters and some offshore, while adjacent subpopulations, which are separated by small distances display marked differences in foraging habit (Goldsworthy et al. unpublished data).

Based on the marked variability in foraging habit, the only way to resolve this is through additional tracking studies at a range of subpopulations.

Because the fishing effort data used here were recorded in spatially broad MFAs (approximately 1˚ x 1˚), the analysis of spatial overlap and risk of interactions with seals was also analysed at this scale. Although both the SESSF gillnet sector and SARL fisheries concentrate fishing effort inshore, the presentation of fishing effort in MFAs distributes effort over a larger area, and as a consequence reduces the degree of overlap with foraging effort of seals, which also concentrate their foraging effort inshore. Greater spatial precision, in both seal foraging effort and fishing effort, would produce more realistic estimates of interaction and risk probabilities.

Assessment of risks to ASL

PVA assessment of subpopulations

Our PVA analysis provides the most sophisticated assessment of quasi-extinction risk for ASL subpopulations. These analyses provide strong support for the recent listing of the species as *Threatened* (*Vulnerable* category) under Commonwealth *EPBC Act*. This study has estimated total SA pup production of approximately 2,674 per breeding cycle and a total SA population size of about 10,905. Almost 70% of this population is accounted for by six breeding sites, which make up 16% of known SA breeding localities. As a consequence there are large numbers of breeding sites where few pups are born, with 60% of sites producing fewer than 30 pups (42% with fewer than 20). The median pup production for SA colonies is only 25.5. Depending on which population trajectory model was being used (*decreasing*, *stable*, *increasing*), PVA results indicated that up to 71% of subpopulations could be classed as *endangered* without any additional (anthropogenic) mortality. Very small increases in mortality resulted in quasi-extinctions, with an additional mortality of 2 pups per subpopulation per year resulting in the quasi-extinction of 71% of subpopulations in 30 years in the decreasing population model, or 42% of subpopulations (in <38 years) in the stable population model. Furthermore, if mortalities were directed at older, recruiting age females, rates of decline could increase by more than three-fold compared with similar mortality rates directed at pups.

All of the PVA simulations indicate that in absence of any anthropogenic mortality, some ASL subpopulations are likely to become *quasi-extinct*. With low levels of additional (anthropogenic) mortalities, many other small subpopulations are expected

to become *quasi-extinct*, and negative growth will become a feature of even the largest subpopulations for the species. However, because the status and trends in subpopulations, their stage-specific survival and fecundity rates, and the actual rates of anthropogenic mortality are unknown, it is not possible to know whether any of the population scenarios are plausible.

One of the many challenges facing management of the species is that the majority of subpopulations are small (and possibly depleted). In the worst-case scenario, most of these subpopulations could be in decline and heading for extinction. If this is the case, we may be seeing a range of subpopulations at different stages in the process of extinction, and other (unknown) subpopulations may have recently gone extinct. The difficulty in detecting declines in subpopulations that have been reduced to low levels, has been identified as a major problem for population managers (Staples et al. 2005). The possibility that the high numbers of small subpopulations have resulted from systemic subpopulation declines is a pressing hypothesis that needs to be addressed. Similarly, the potential that fishery bycatch in gillnet and trap fisheries is the principal cause for subpopulation declines is another critical hypothesis that needs urgent attention.

Evaluating the risk posed by SESSF gillnet sector

Some level of bycatch of ASL occurs in the gillnet sector of the SESSF in SA, but its incidence and spatial occurrence are unknown, because rates of bycatch appear to be under-reported. This study has indicated almost complete overlap between the distribution of effort in the fishery and the spatial extent of foraging effort by all age/gender classes of ASL. Further, fishing effort tends to be focused inshore (in shallower waters) in areas of high ASL foraging effort, and there is evidence for relatively high incidence of entanglement of ASL in demersal gillnet material, at least in parts of their range (see Page et al. 2004). This fact, plus the considerable fishing effort averaging about 20,000 km of net-set per year in SA MFAs, point to there being a high potential for interactions with ASL.

Page et al. (2004) reported 19 individual ASL entangled in monofilament gillnet at Seal Bay (Kangaroo island) over a 15-year period, or about 1.3 entangled seals/year. Most of this subpopulation is monitored daily by SA Department for Environment and Heritage (DEH) staff, providing unique opportunities to monitor the nature and extent of entanglement in fishing gear (Page et al. 2004). The population is currently

declining by about 0.7% per year (Shaughnessy et al. 2006). It is impossible to estimate what proportion of the entire population are entangled in demersal gillnets, free themselves and reach ashore wearing the gillnet material. Fowler (1987) and Fowler et al. (1990) undertook a study of entanglement in northern fur seals (*Callorhinus ursinus*), and determined that entangled seals were less likely to be observed on land because, a) an unknown number drown during or shortly after entanglement; b) entangled seals will be encountered less often on shore because of their lower survival, and c) entangled seals spend longer periods at sea foraging because of the additional drag of entangling material. Fowler et al. (1990) suggested that because of these factors, entanglement-related mortality of juvenile northern fur seals was 35 times that of onshore entanglement rates (ie. entangled seals ashore represent 2.9% of all animals entangled).

Based on the probability of interactions between ASL and gillnet SESSF calculated for all ASL subpopulations in SA, we estimate that about 11.4% of the fishery bycatch would be from the Seal Bay subpopulation. Given this, 1.3 entangled seals ashore/year at Seal Bay would imply a total annual SA bycatch of 23 seals if entangled seals ashore represent 50% of all those entangled, 114 seals (if entangled seals ashore represent 10% of all those entangled), 227 seals (if entangled seals ashore represent 5% of all those entangled), and 376 seals (if entangled seals ashore represent about 3% of all those entangled), as in the study of Fowler et al (1990). If entangled seals ashore represent between 1-10% of all ASL that become entangled, then annual bycatch rates for SA and adjacent waters could number between 100-300+ seals/year.

By combining PVAs with subpopulation fishery interaction probabilities, this study has identified the subpopulations and fishery MFAs that are at most risk from gillnet sector SESSF bycatch, population reduction and quasi-extinction. This analysis identified that subpopulations from two regions were most at-risk. Firstly, the top seven subpopulations at greatest risk were all in the Nuyts Archipelago and Streaky Bay regions off the western Eyre Peninsula in MFA 108 (Olive Island, East Franklin Reef, West Island, Purdie Island, West Franklin Reef, Lounds Islands and Breakwater Reef), with the next three at-risk subpopulations occurring south and east of Kangaroo Island in MFAs 149 and 144 (Seal Bay, the Seal Slide and The Pages). Annual bycatch levels of between 260-400 seals per year would be required for quasi-extinction of these populations in about 50 years. These equate to average bycatch rates of 0.01 –0.02 seals per/km net-lift/year (1-2 seals per 100kms of net-lift

averaged across all SA MFAs). However, even lower levels of bycatch (between 100-150 seals/year, 0.004-0.005 seals/km/net-lift, 0.4-0.5 seals per 100km net-lifts) would cause many subpopulations to decline. These analyses suggest that even with modest levels of bycatch, the extent and distribution of fishing effort could have significantly impacted the viability of numerous ASL subpopulations.

Evaluating the risk posed by SA RLF

As with the gillnet sector SESSF, an unknown level of bycatch of ASL occurs in the SA RLF. Because bycatch involves entrapment and drowning of seals in pots, impact of the fishery is likely to be limited to small seals that can physically fit in pot-openings. The SA RLF is concentrated in the south-east of SA, between the south cost of Kangaroo Island and the lower Eyre Peninsula, and along the west coast of the Eyre Peninsula. As a consequence of the restricted spatial distribution of the fishery, 36% of ASL foraging effort is estimated to occur outside regions where SA RLF catches have been reported. Further, probabilities of interaction are low in the major fishing MFAs (southern zone) of the fishery (55, 56 and 58), which account for over 75% of effort in the SA RLF (about 1 million pot-lifts/yr). Most interactions were predicted to occur in the northern zone of the SA RLF, which accounts for about a third total fishing effort of the fishery (about 500,000 pot-lifts/year).

By combining PVAs with ASL interaction probabilities in the SA RLF, the colonies at highest risk were more spatially spread compared with the risk posed by gillnet SESSF. The ten highest risk subpopulations where distributed across four main regions and MFAs: Price Island (southern Eyre Peninsula, MFA 28) was the most at-risk population, followed by subpopulations in southern Spencer Gulf (Peaked Rocks, North Island, Albatross Island, Lewis Island and South Neptune Island, MFA 39), western Eyre Peninsula (West Waldegrave and Jones Island, MFA 15) and Kangaroo Island (Seal Bay and the Seal Slide, MFA 49). Low levels on annual bycatch (13+ seals, 0.025+ seals/1,000 pot-lifts in MFAs where seals occur) were enough to place some subpopulations into the *vulnerable* category, 40+ seals (0.076+ seals/1,000 pot-lifts) into the *endangered* and *critical* category and 120+ to *quasi-extinct* (0.228+ seals/1,000 pot-lifts). These rates of bycatch are higher than those reported for ASL in the western rock lobster fishery in WA (Campbell et al. 2004). Campbell et al. (2004) estimated annual bycatch rates at about 0.003 seals per 1,000 pot-lifts, but these may be underestimates, because they are based on phone and logbook surveys, and include fishing effort in areas where ASL may not

forage. Because bycatch probabilities are a function of fishing effort and seal foraging effort, it is difficult to compare rates of interaction between sites, unless differences in the level of fishing and seal foraging effort can be quantified.

Potential to develop risk management tools

The study has made considerable progress toward developing spatial tools to assess the potential risk-reduction (risk of extinction) benefits that could arise from a range of spatial management options in both the gillnet SESSF and SA RLF. Such tools could be useful in the gillnet SESSF, where bycatch mitigation options related to gear modification are limited. Spatial management of fishing effort, which could reduce risks to particular subpopulations, is attractive because it provides immediate risk reduction to the targeted subpopulation or region, with minimal impact on fishery catch. For example, the seven most at-risk ASL subpopulations identified in the gillnet SESSF were located in MFA 108, which accounted for about 5% of the total fishery effort and about 8.5% and 3.9% of the catch of gummy and school shark for SA MFAs (based on catch data for 2004). Spatial management options could be investigated to assess how such effort and catch could be reallocated to reduce the impact on high-risk ASL subpopulations.

Enhanced spatial tools for risk assessment will be required if spatial management of fishing effort is to become a major management strategy for mitigating ASL bycatch in the gillnet sector SESSF. Such tools would provide a simple approach for policy makers and managers, enabling them to evaluate the benefits and costs of different spatial allocations of fishing effort, in terms of increasing or decreasing the risk to sea lion subpopulations. However, further development of such tools is required, because current models are limited by the absence of data on the foraging movements of sea lions in some high risk regions, as well as the absence of accurate fishing effort data at appropriate spatial scales. Satellite tracking of ASL subpopulations identified as high-risk should be undertaken to improve the accuracy of spatial foraging models. In addition, because the gillnet sector SESSF fishers now record the positions of each net-set, the spatial resolution of bycatch probabilities could be improved and with them, the risk assessment for each subpopulation.

Such spatial allocations of fishing effort would also need to include the actual rates of ASL bycatch in the fishery, because spatial closures would need to be underpinned by estimates of bycatch rates in targeted regions. With these data the benefits of

spatial closures (in terms of reduced bycatch), could be estimated and compared to the costs to industry. This could be achieved by targeting specific fishing areas for independent observer coverage.

Conclusions and recommendations

This study assessed the risk of bycatch of ASL in two fisheries that occur off the coast of SA: the gillnet sector of the SESSF and the SA RLF. A major constraint in the assessment of the risk of bycatch to seal subpopulations is the absence of quantitative data on bycatch rates in both fisheries.

Risks were assessed based on overlap in the spatial distribution of fishing effort and the estimated spatial distribution of seal foraging effort. The probability of interactions are a function of the extent to which fishing effort and seal foraging effort overlap in space and time. As such, interaction probabilities will change with spatial and temporal variability in fishing and seal foraging effort, and changes in seal population sizes.

ASL showed a high risk of significant depletion and quasi-extinction of SA subpopulations.

Population viability analysis of ASL subpopulations reinforced the recent Australian Government listing of the ASL as a *threatened* species, by identifying that many subpopulations of the species are presently vulnerable to extinction. PVA simulations suggest that in absence of any anthropogenic mortality, some ASL subpopulations will likely become *quasi-extinct* and, in the face of sustained but small additional mortalities (eg. from fishery bycatch), many other small subpopulations will likely become *quasi-extinct*, and negative growth will become a feature of even the largest subpopulations for the species.

The large proportion of small ASL subpopulations may be attributable to declines in their size, and fishery bycatch in gillnet and trap-fisheries may be the principal cause for these declines. These are challenging hypotheses that need urgent attention.

Of the two fisheries investigated (SA component of gillnet sector SESSF and SA RLF), the more significant in terms of bycatch of ASL is likely to be the gillnet SESSF. There are three main reasons for this :

- there is almost complete spatial overlap in fishing effort with the foraging effort of ASL in SA,
- fishing effort is substantial in SA and adjacent waters (about 20,000 km of net-set per year), occurs year-round and in close proximity to most ASL subpopulations,
- bycatch can potentially impact all age-sex classes.

The impact from SA RLF is likely to be less because:

- there is less overlap in fishing effort with seal foraging effort, because about two-thirds of the fishing effort occurs in areas with little ASL foraging,
- fishing is restricted to eight months of the year,
- bycatch is likely to be restricted to pups and juvenile seals.

Although this study investigated the bycatch risks posed to seal subpopulations by the gillnet SESSF and SA RLF, the potential additive and interactive impacts posed by combined bycatch in these fisheries have not been investigated, but they could be significant, especially to ASL.

The combining of PVA outcomes with bycatch scenarios based on interaction probabilities has identified the subpopulations, regions and fishery MFAs that are likely to be most significant in terms of bycatch in each fishery. In the gillnet SESSF, the seven ASL subpopulations at greatest risk occurred in one MFA, highlighting the potential significance of spatial management of fishing effort to mitigate bycatch risk in this fishery. In contrast, the ASL subpopulations at greatest risk were spread over a number of MFAs.

The two fisheries investigated here lend themselves to different mitigation approaches to addressing seal bycatch issues. In the gillnet SESSF, gear modification options are limited, with the possible exception of acoustic deterrent devices (‘pingers’). Spatial management of fishing effort could provide a range of risk-reduction options to management, but this would need to be coupled with independent observer effort to demonstrate and justify the benefits. In contrast, there is significant scope for gear modification options in the SA RLF, with pot-protection devices already used to reduce the incidence of seal bycatch in some parts of the fishery. Quantitative testing of these pot-protection devices and alternate protection measures (as is taking place in the WA WRLF), and industry-wide adoption of best-

mitigation practices may eliminate seal bycatch, without the need for a large and costly independent observer program.

A number of recommendations arise from this study:

1. An independent observer program in the gillnet sector of the SESSF should be implemented to assess the significance of ASL bycatch in the high-risk regions identified in the study.

2. The spatial risk assessment approach developed in this study should be improved using higher resolution fishing effort data (lat/long location of effort in the gillnet SESSF and depth-stratified data in the SA RLF) coupled with higher resolution spatial foraging data in ASL (utilising satellite telemetry) to produce a spatial risk-management tool, which policy makers/managers can use to assess the risk and benefits of different spatial management scenarios.

3. Investigate options for gear modification (such as acoustic deterrents) to reduce the incidence of seal bycatch in the gillnet sector of the SESSF. Of those options that may be feasible, undertake trials to assess their efficacy.

4. Undertake quantitative trials to assess the efficacy of different pot-protection devices at eliminating seal bycatch in the southern rock lobster fishery. These trials should include testing the impact of different protection measures on catch and size selectivity. Once developed, seal excluding/pot-protection devices should be adopted throughout the southern rock lobster fishery, to address broader seal interactions issues in other States (eg. Victoria and Tasmania).

5. Methods and guidelines for measuring and evaluating the performance of systems for monitoring, assessing and mitigating interactions between the fisheries and seals needs to be developed. This would include improving industry reporting of seal interactions, and developing performance indicators to assess the level and effectiveness of risk reduction following implementation of mitigation options.

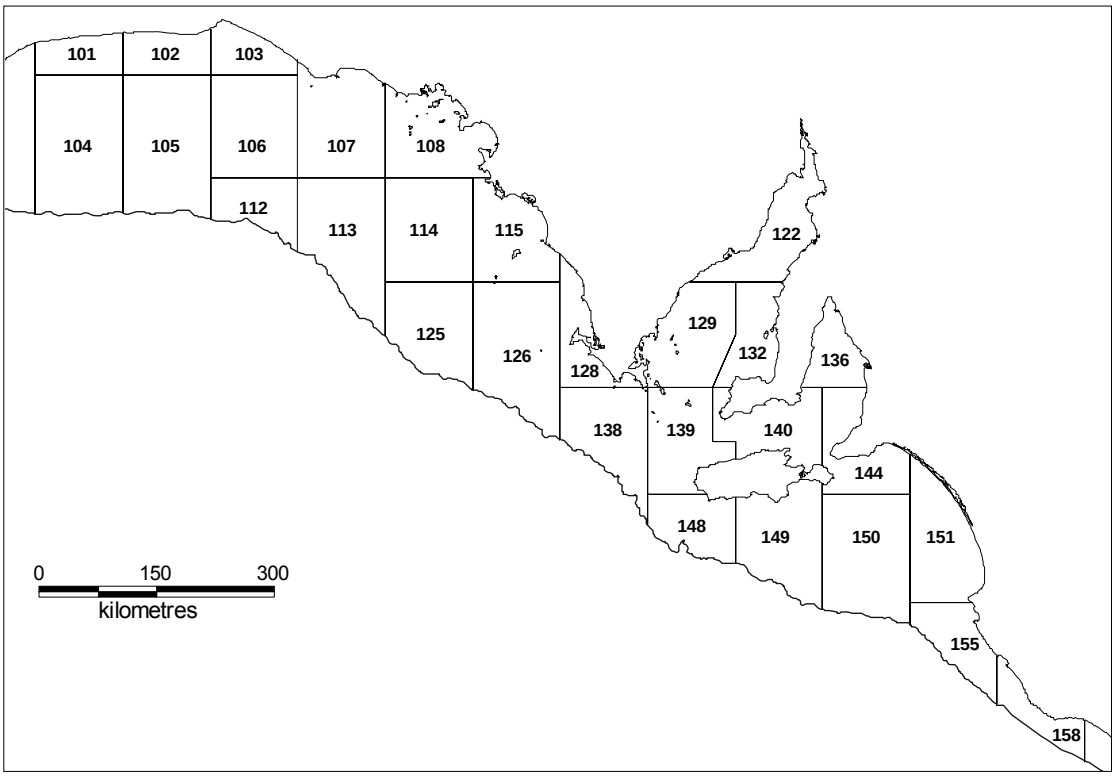

Figure 6. Gill-net sector SESSF Marine Fishing Areas (MFAs) off South Australia for which catch and effort data have been recorded since 1973.

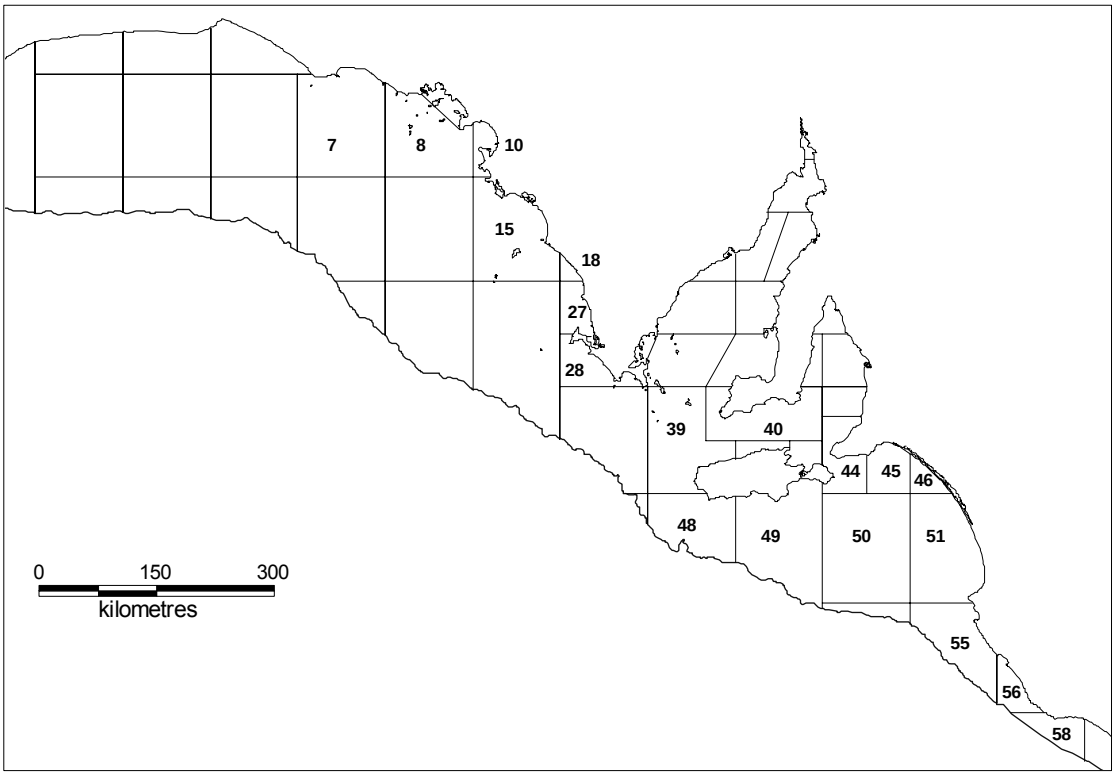

Figure 7. SARLF fishery Marine Fishing Areas (MFAs) off South Australia for which catch and effort data have been recorded since 1970.

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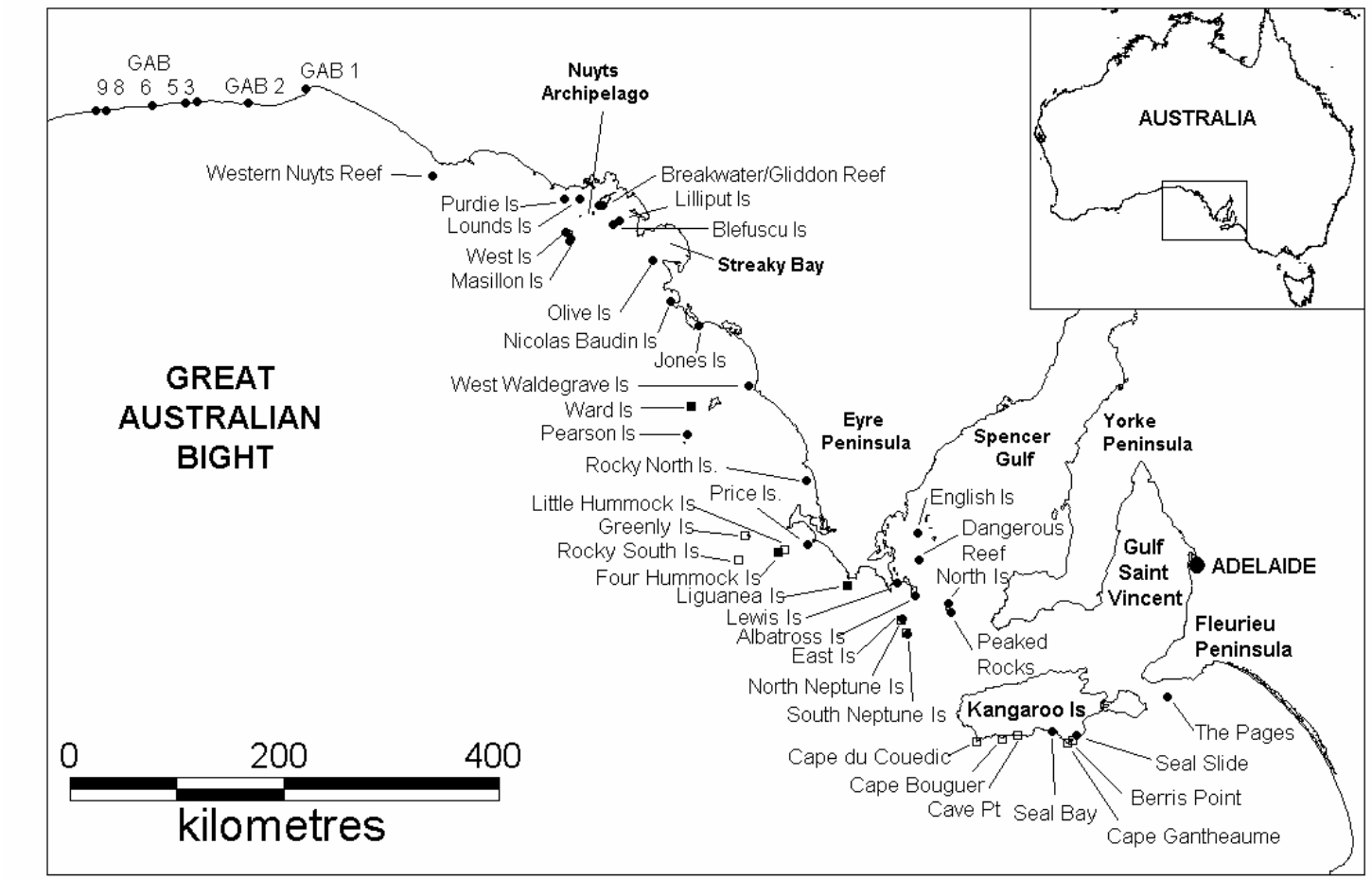


Figure 8. Map of South Australia and the eastern Great Australian Bight (GAB) indicating the location of ASL (closed circles) and New Zealand fur seal (open squares) breeding sites. Black squares indicate sympatric breeding locations.

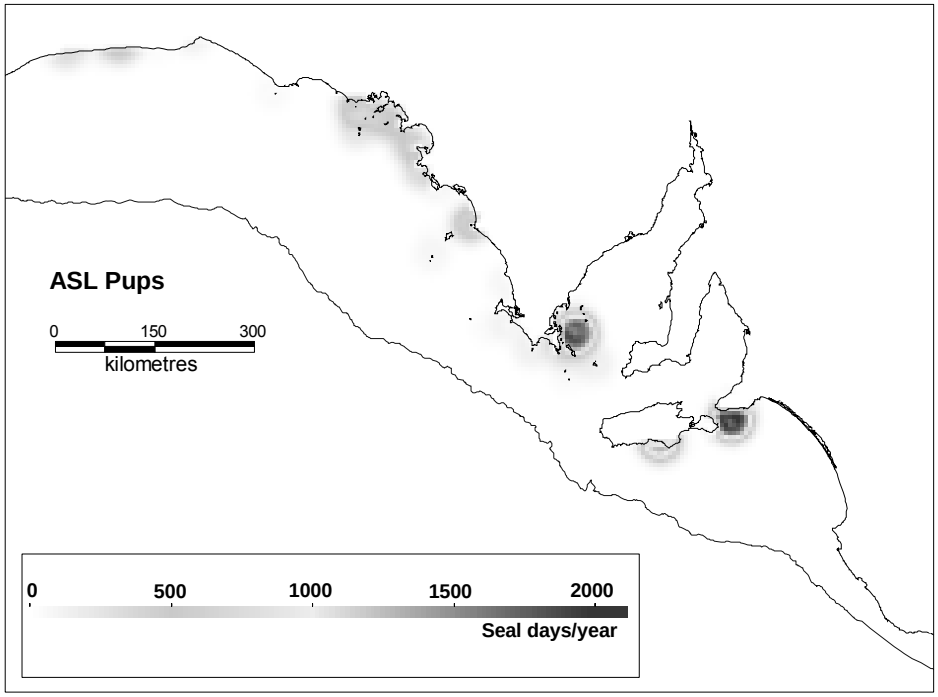


Figure 9. Estimated distribution of foraging effort (seal days.year^{-1}) of ASL pups in South Australia. The line indicates the edge of the continental shelf (200m).

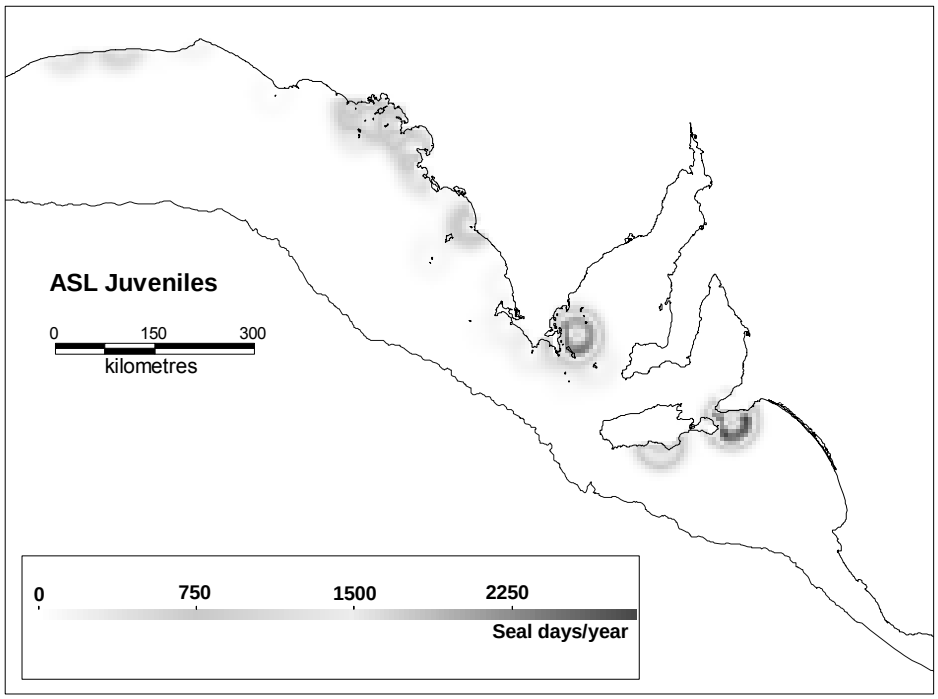


Figure 10. Estimated distribution of foraging effort (seal days.year^{-1}) of ASL juveniles in South Australia. The line indicates the edge of the continental shelf (200m).

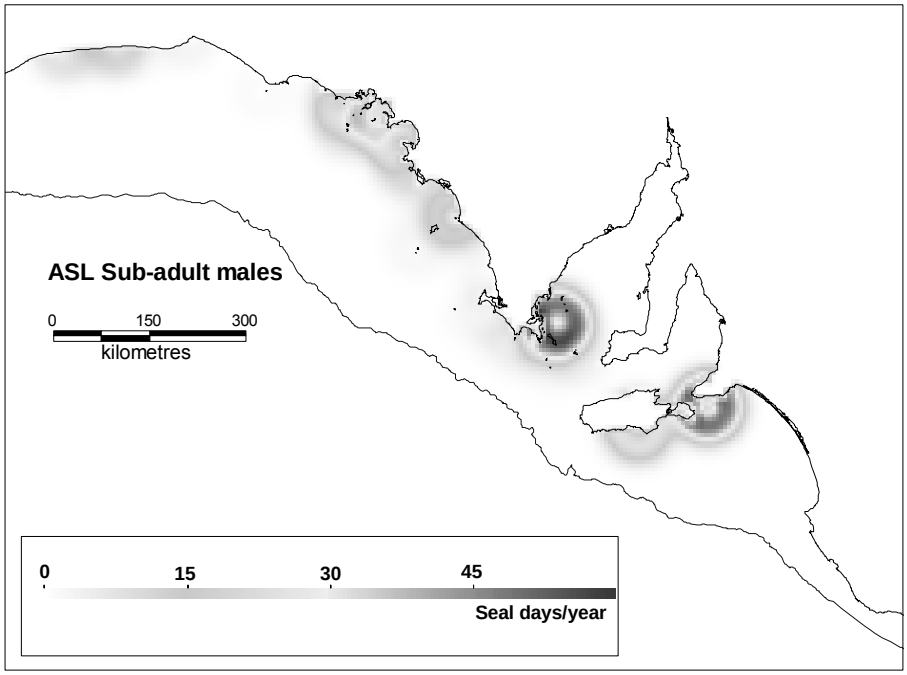


Figure 11. Estimated distribution of foraging effort (seal days.year^{-1}) of ASL sub-adult males in SA. The line indicates the edge of the continental shelf (200m).

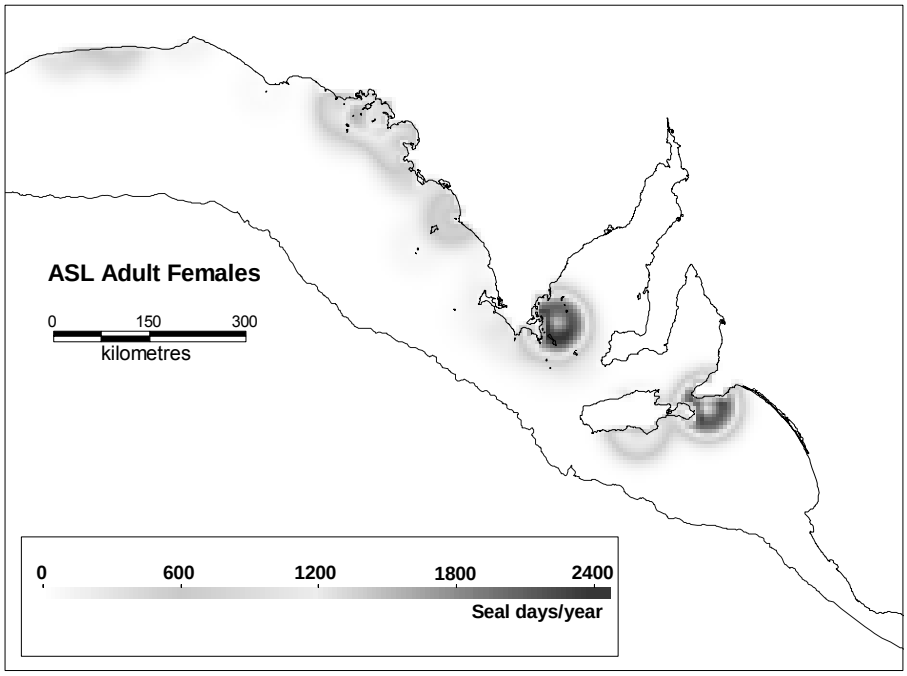


Figure 12. Estimated distribution of foraging effort (seal days.year^{-1}) of ASL adult females in South Australia. The line indicates the edge of the continental shelf (200m).

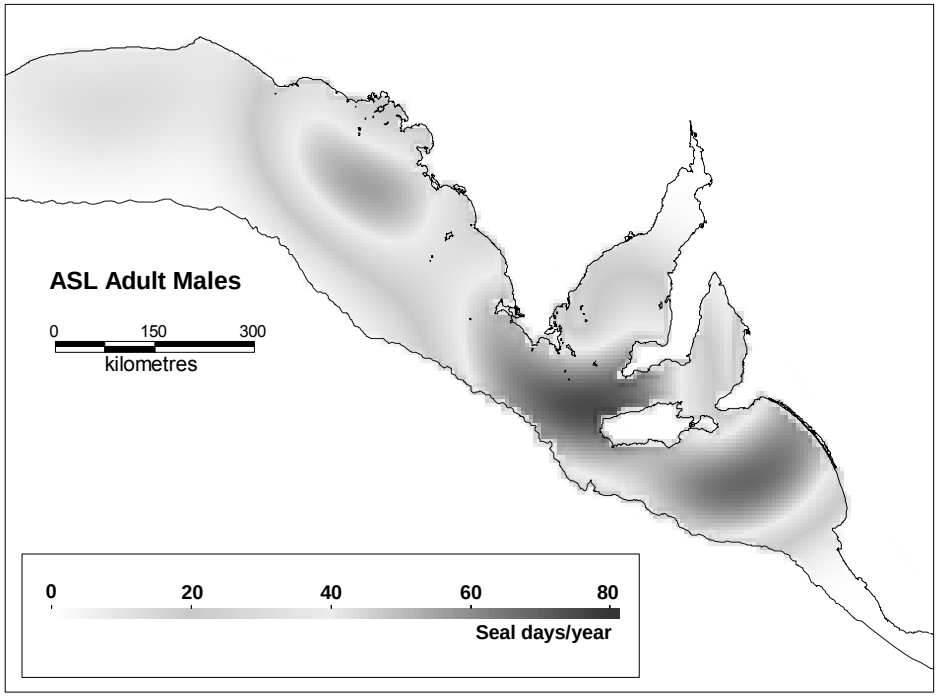


Figure 13. Estimated distribution of foraging effort (seal days.year^{-1}) of ASL adult males in South Australia. The line indicates the edge of the continental shelf (200m).

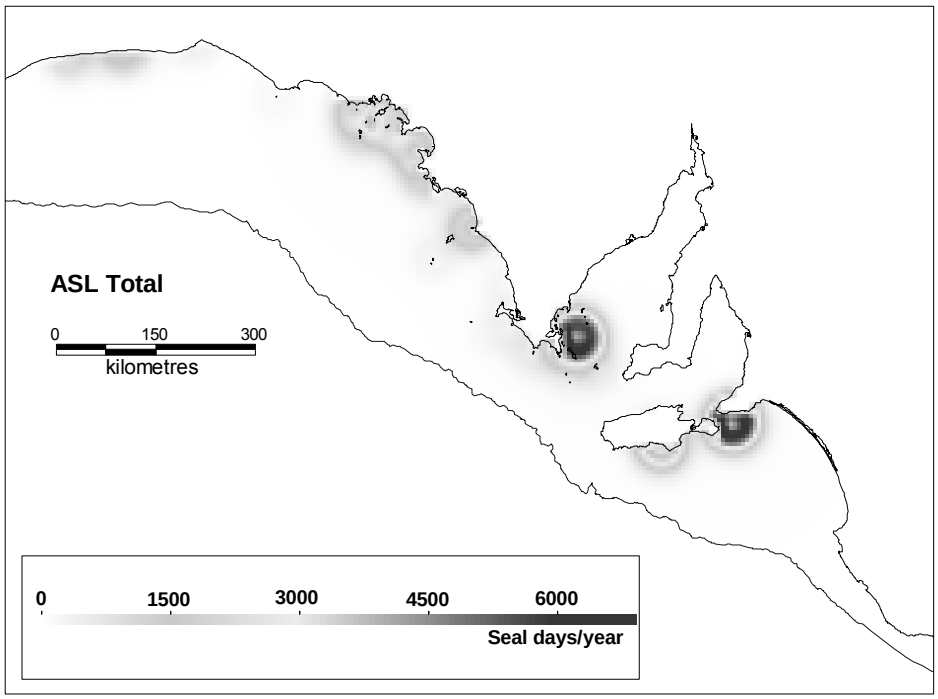
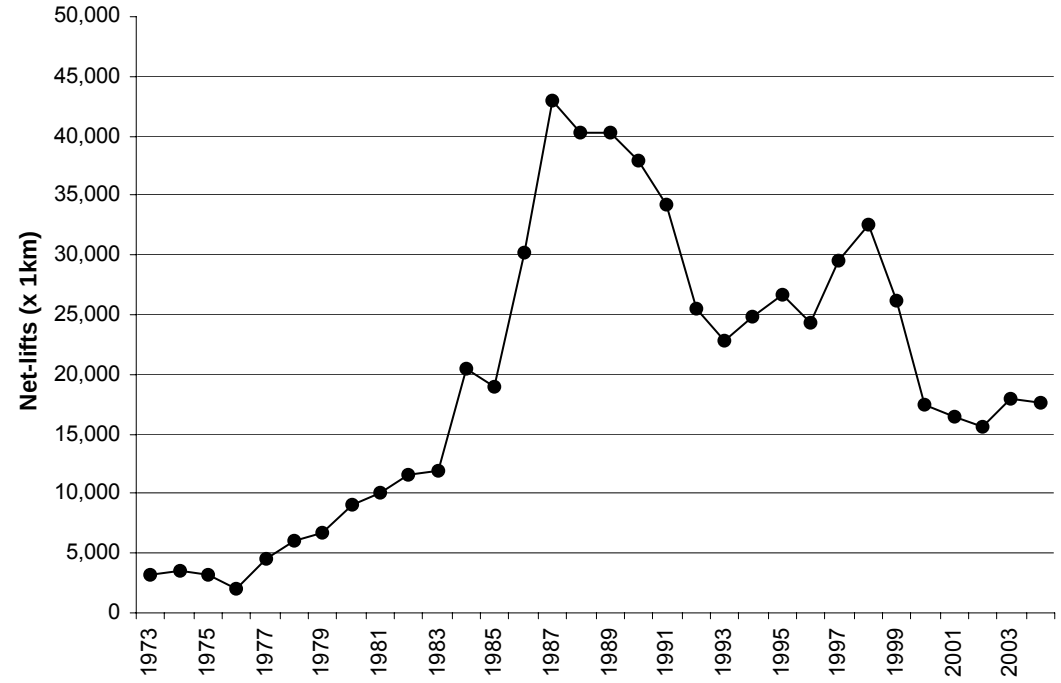


Figure 14. Estimated total distribution of foraging effort (seal days.year^{-1}) of ASL (age/gender groups combined) in South Australia. The line indicates the edge of the continental shelf (200m).

A



B

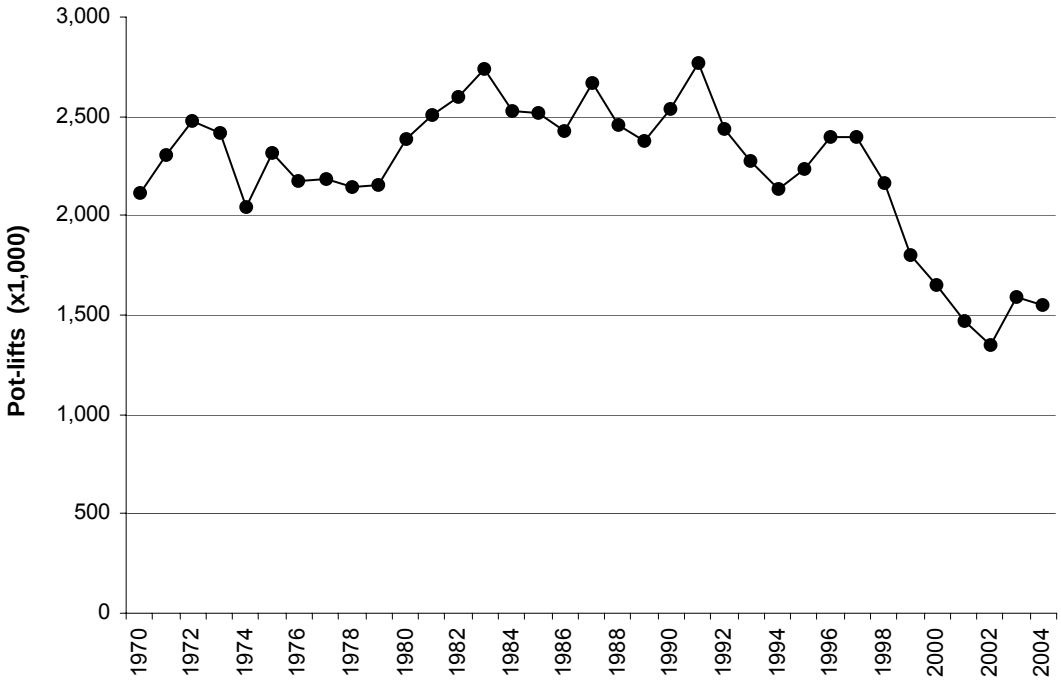


Figure 15. Temporal variation on total fishing effort in SA and adjacent Commonwealth waters in the gillnet sector of the SESSF between 1973-2004 (A), and SARLF (B) between 1970-2004

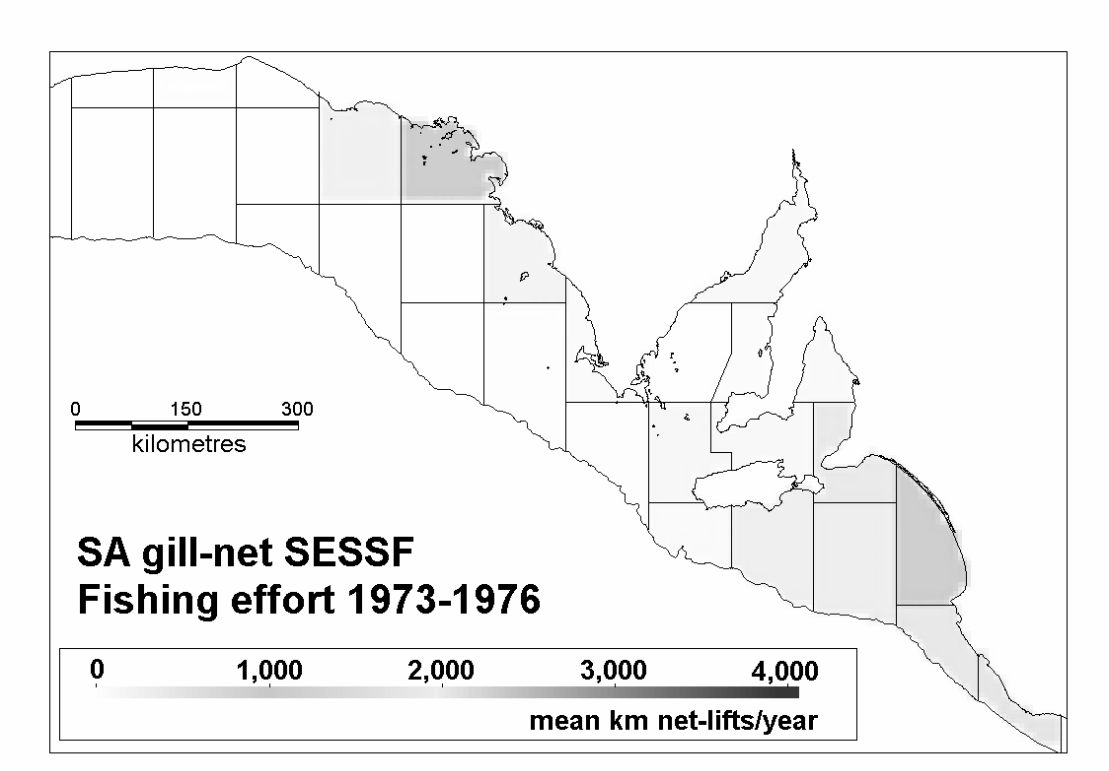


Figure 16. Distribution of fishing effort in the SA component of the gill-net sector of the SESSF, 1973-76.

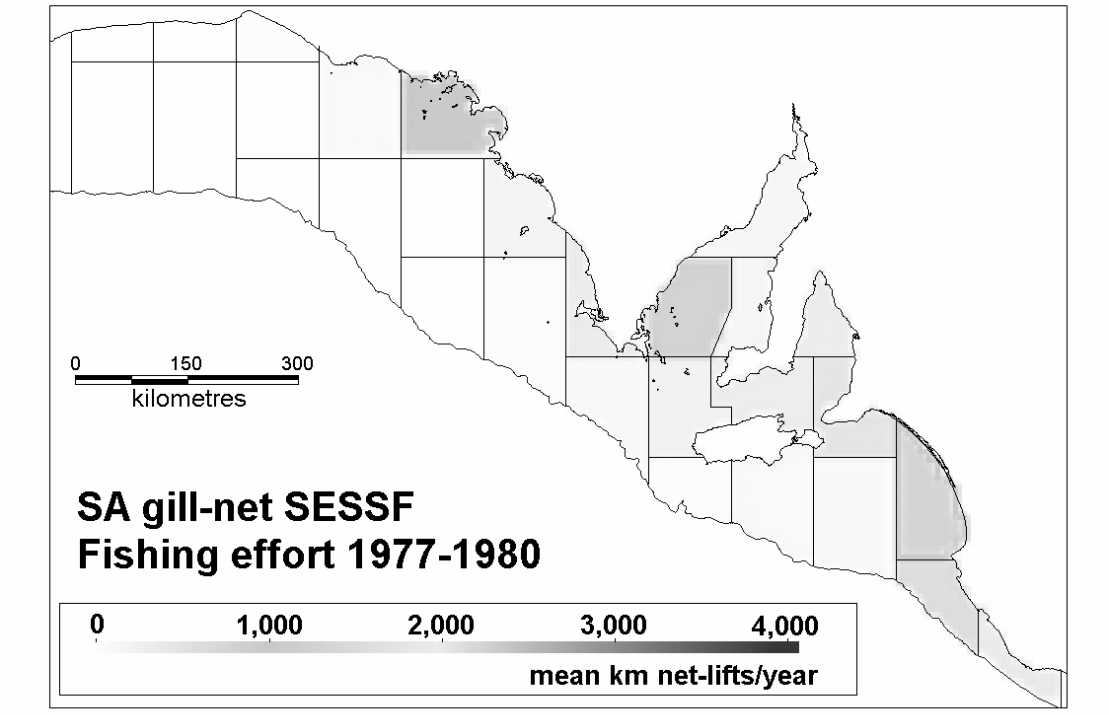


Figure 17. Distribution of fishing effort in the SA component of the gill-net sector of the SESSF, 1977-80.

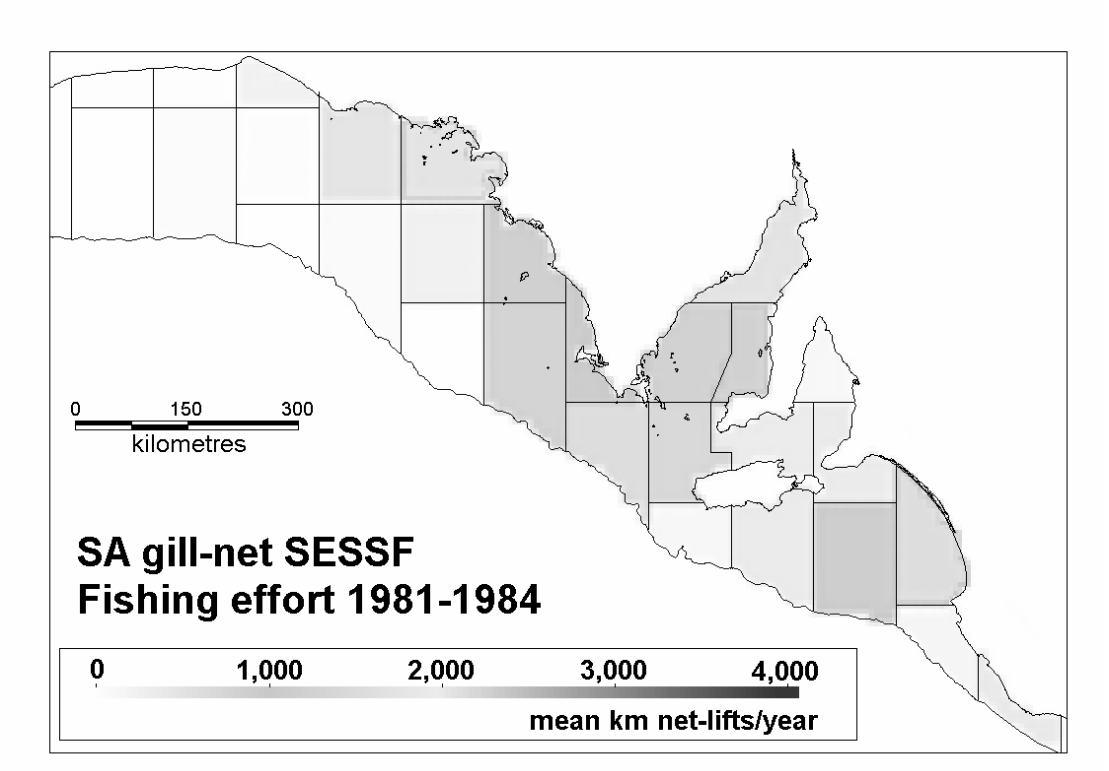


Figure 18. Distribution of fishing effort in the SA component of the gill-net sector of the SESSF, 1981-84.

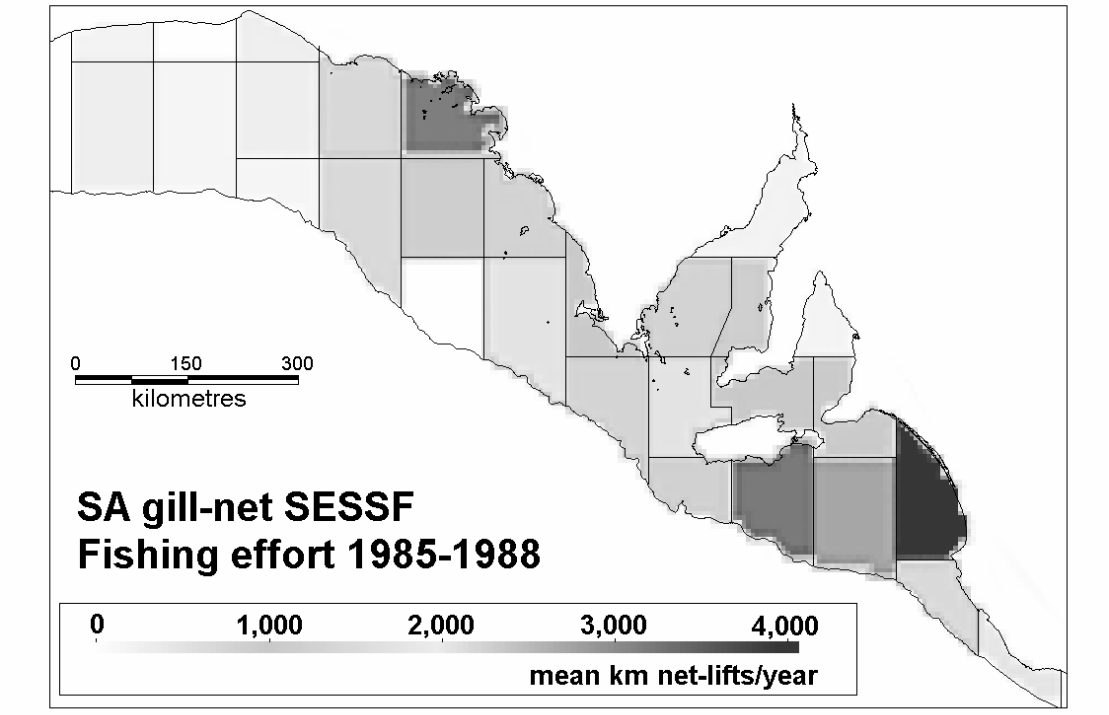


Figure 19. Distribution of fishing effort in the SA component of the gill-net sector of the SESSF, 1985-88.

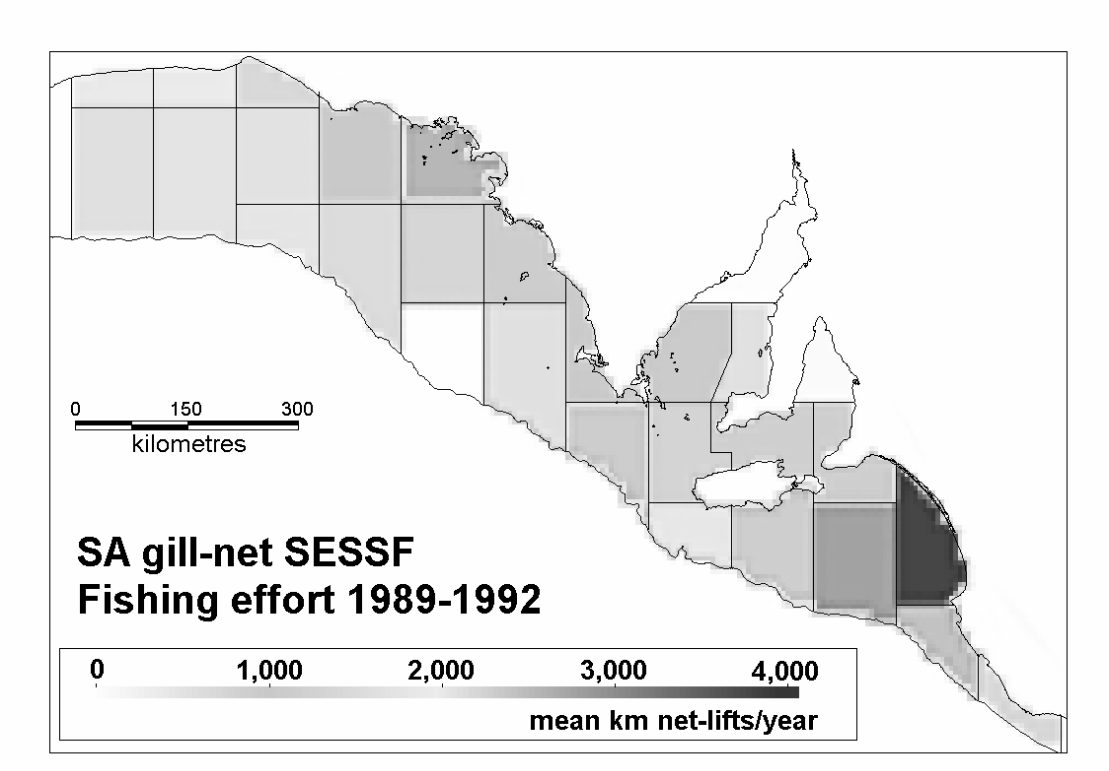


Figure 20. Distribution of fishing effort in the SA component of the gill-net sector of the SESSF, 1989-92.

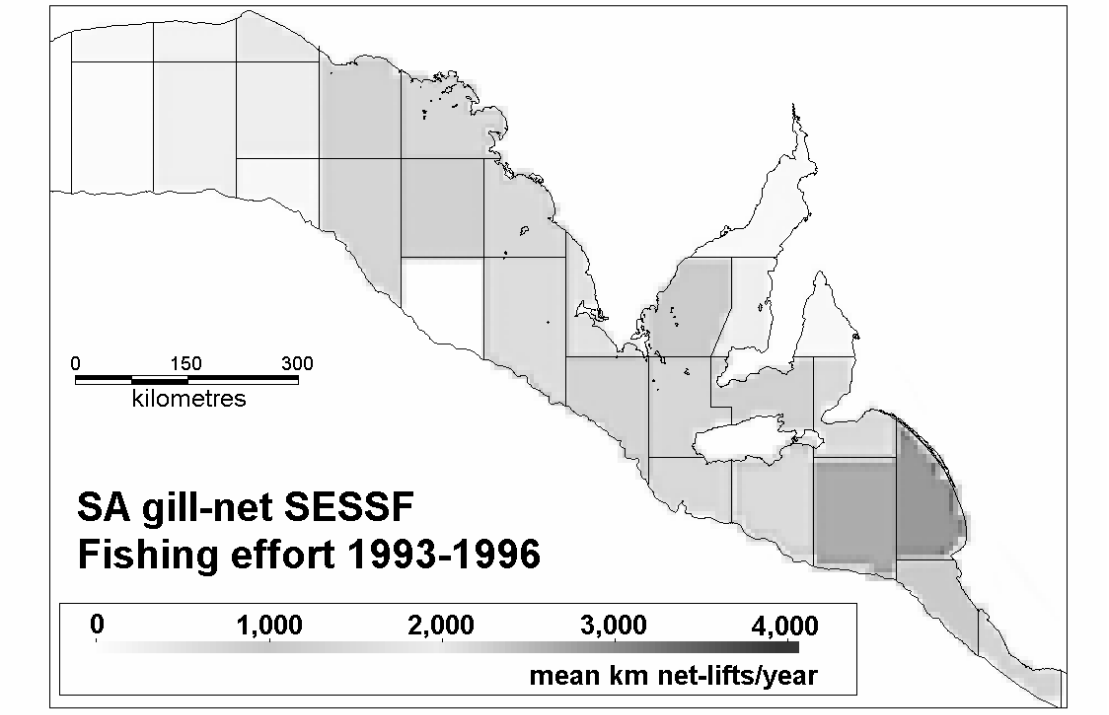


Figure 21. Distribution of fishing effort in the SA component of the gill-net sector of the SESSF, 1993-96.

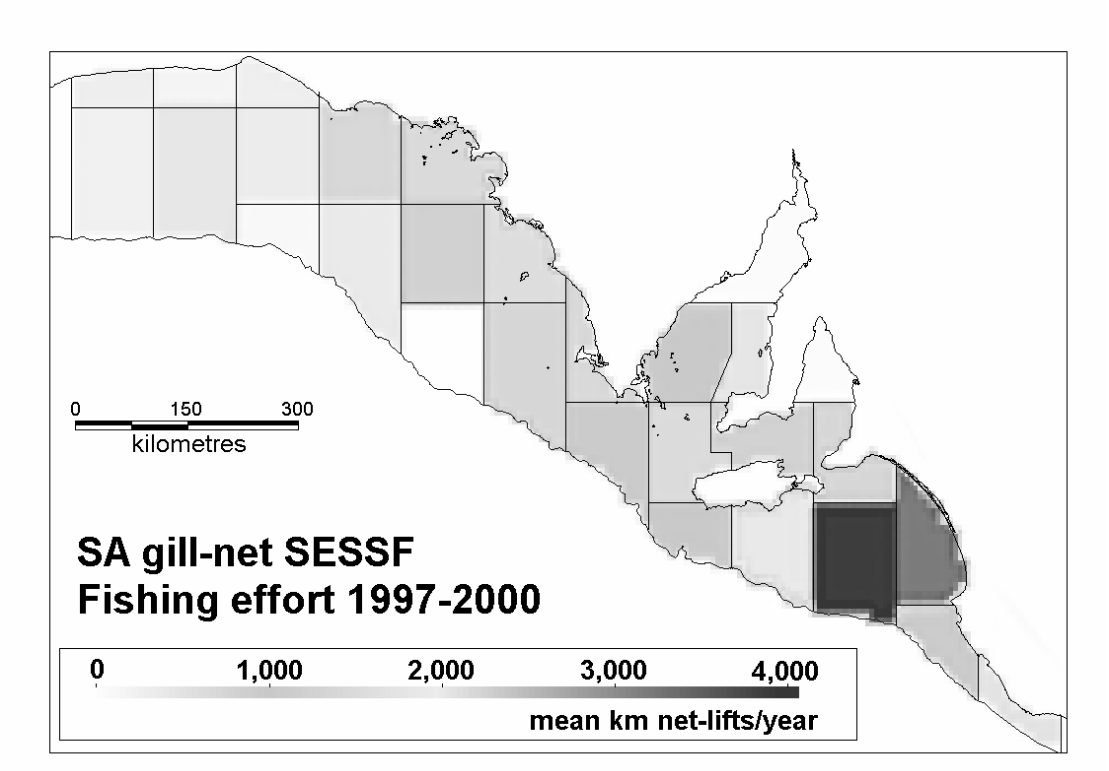


Figure 22. Distribution of fishing effort in the SA component of the gill-net sector of the SESSF, 1997-00.

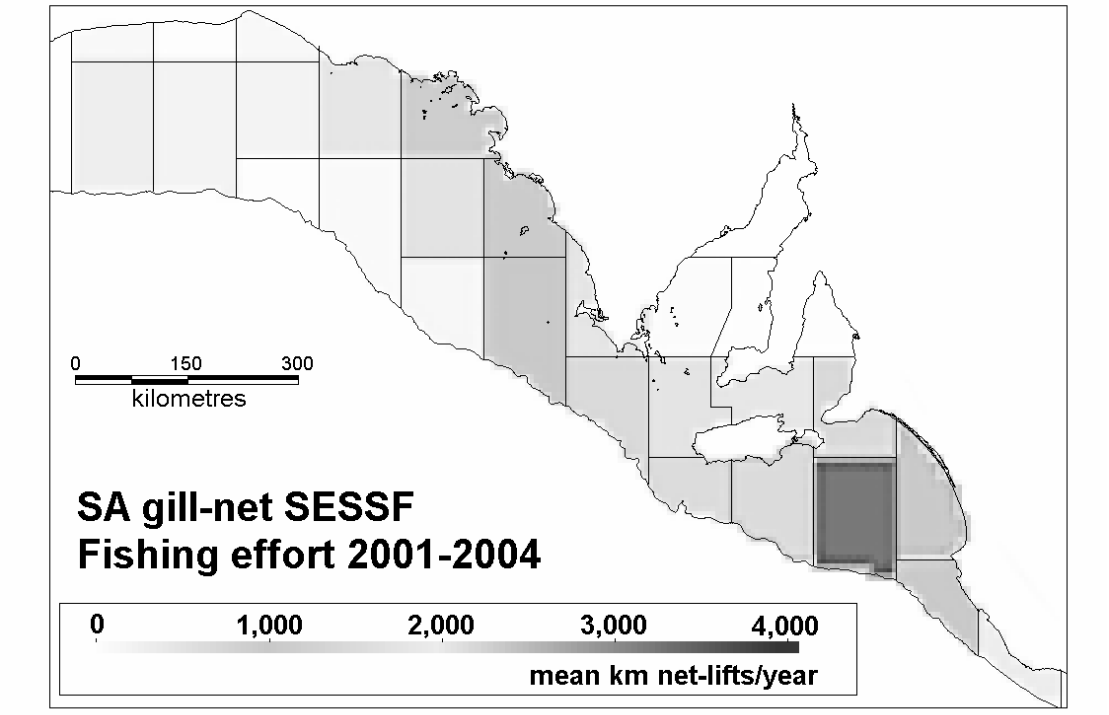


Figure 23. Distribution of fishing effort in the SA component of the gill-net sector of the SESSF, 2001-04.

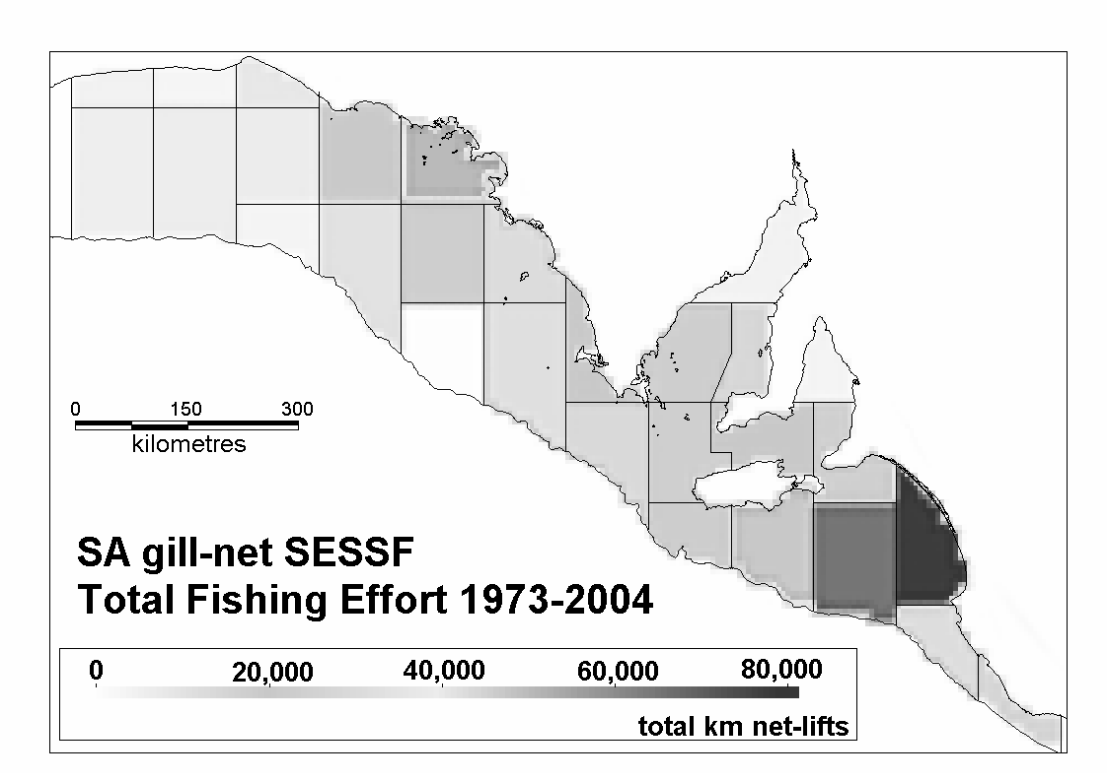


Figure 24. Distribution of fishing effort in the SA component of the gill-net sector of the SESSF, 1973-2004.

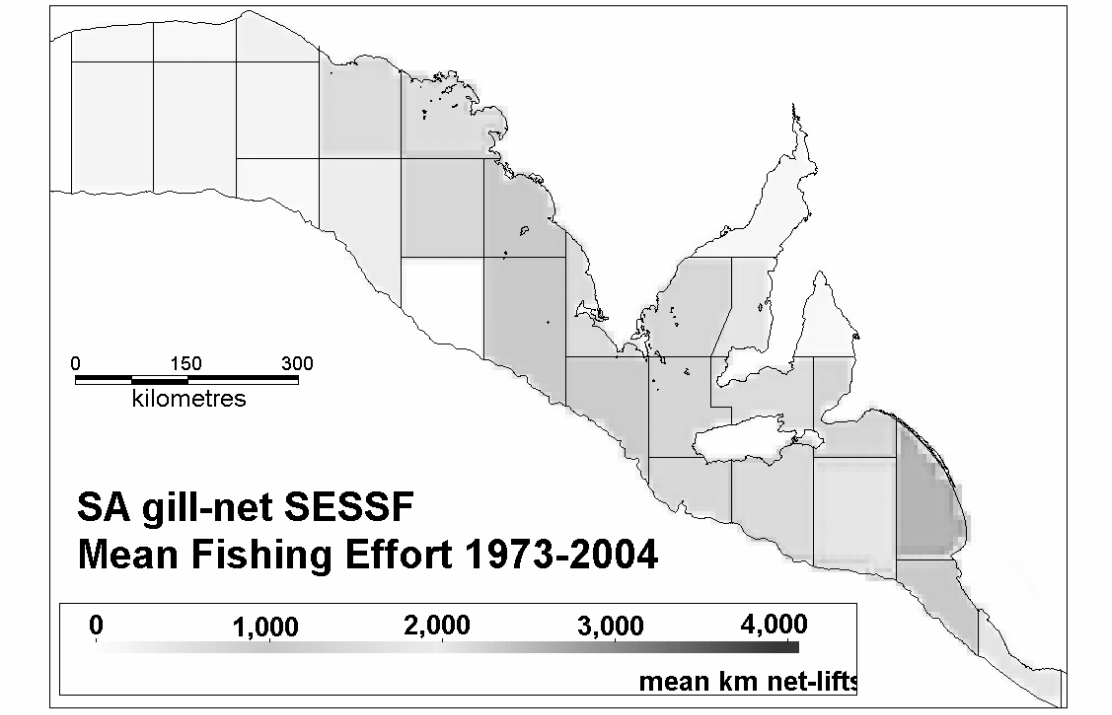


Figure 25. Mean annual distribution of fishing effort in the SA component of the gill-net sector of the SESSF, 1973-2004.

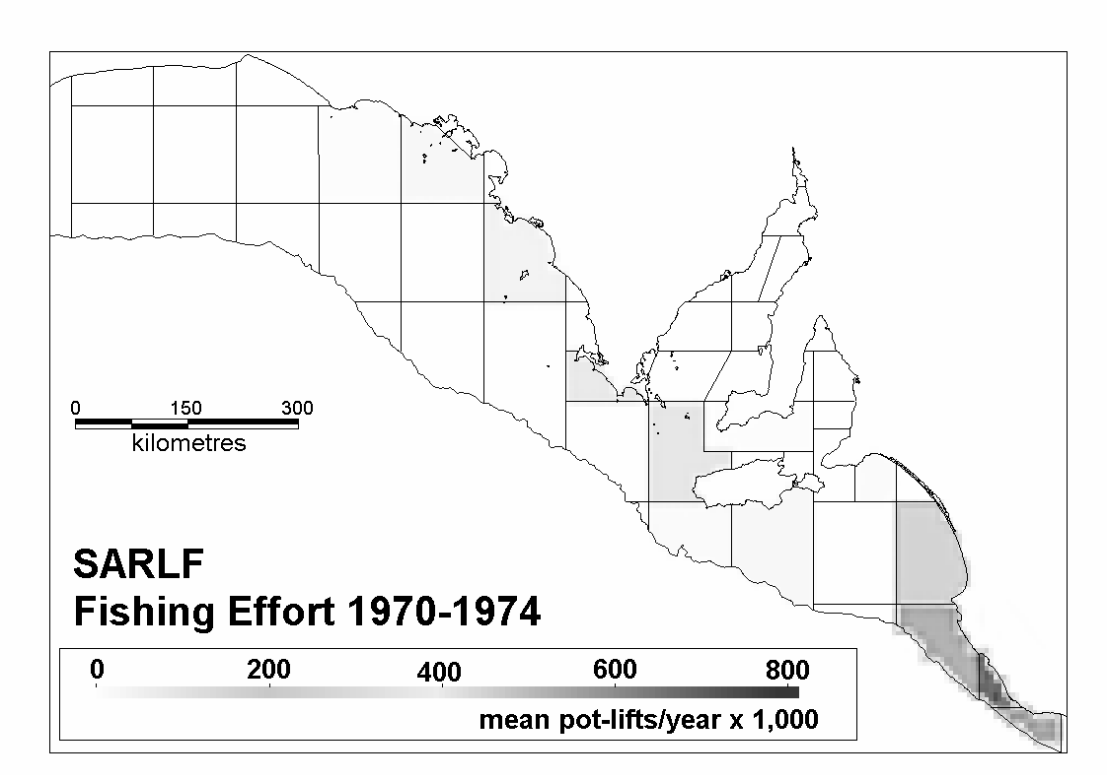


Figure 26. Mean annual distribution of fishing effort in the SARLF, 1970-74.

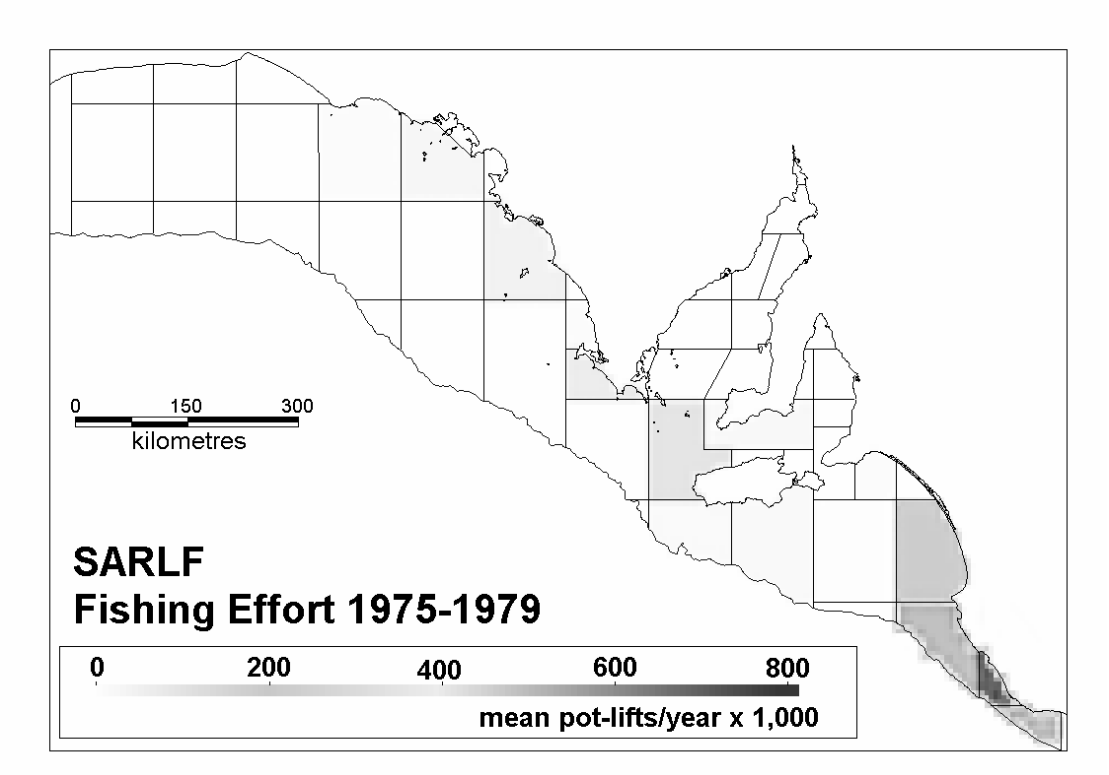


Figure 27. Mean annual distribution of fishing effort in the SARLF, 1975-79.

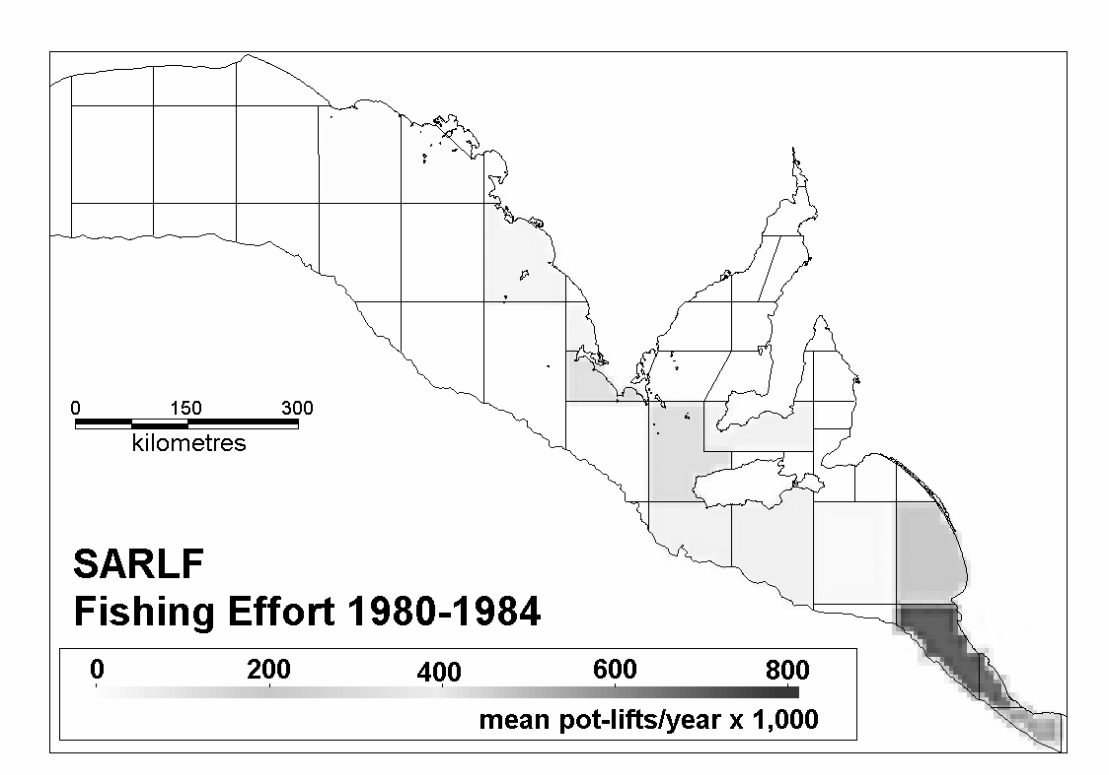


Figure 28. Mean annual distribution of fishing effort in the SARLF, 1980-84.

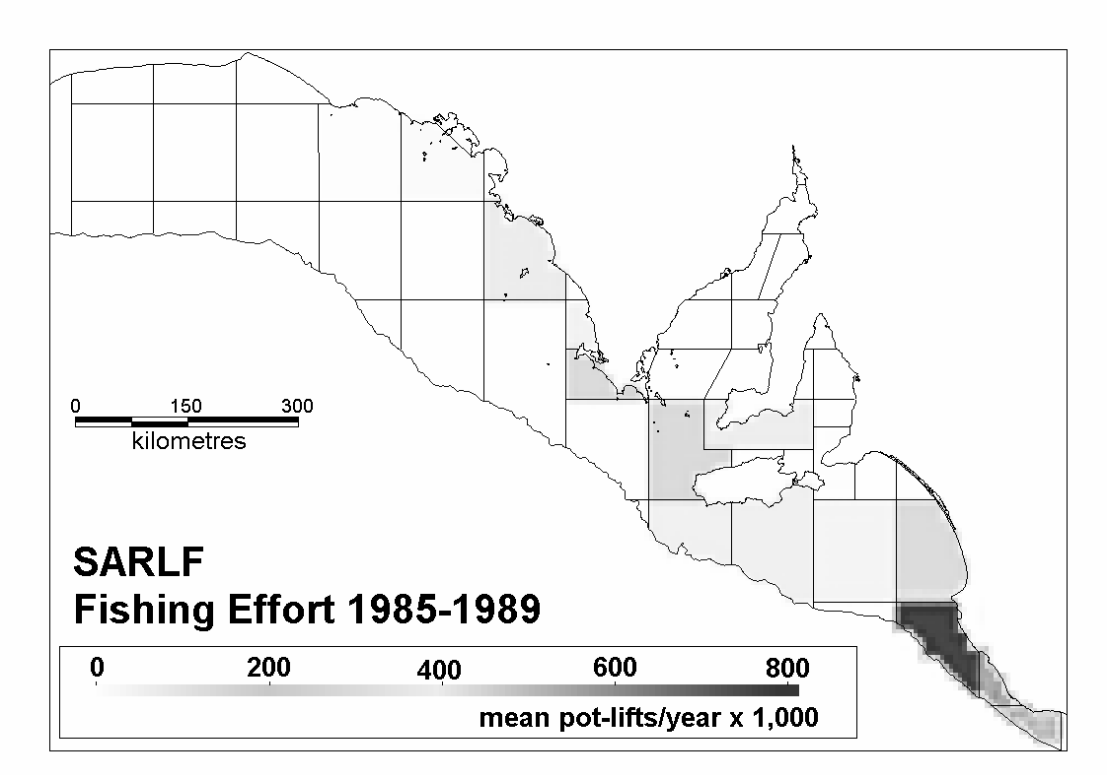


Figure 29. Mean annual distribution of fishing effort in the SARLF, 1985-89.

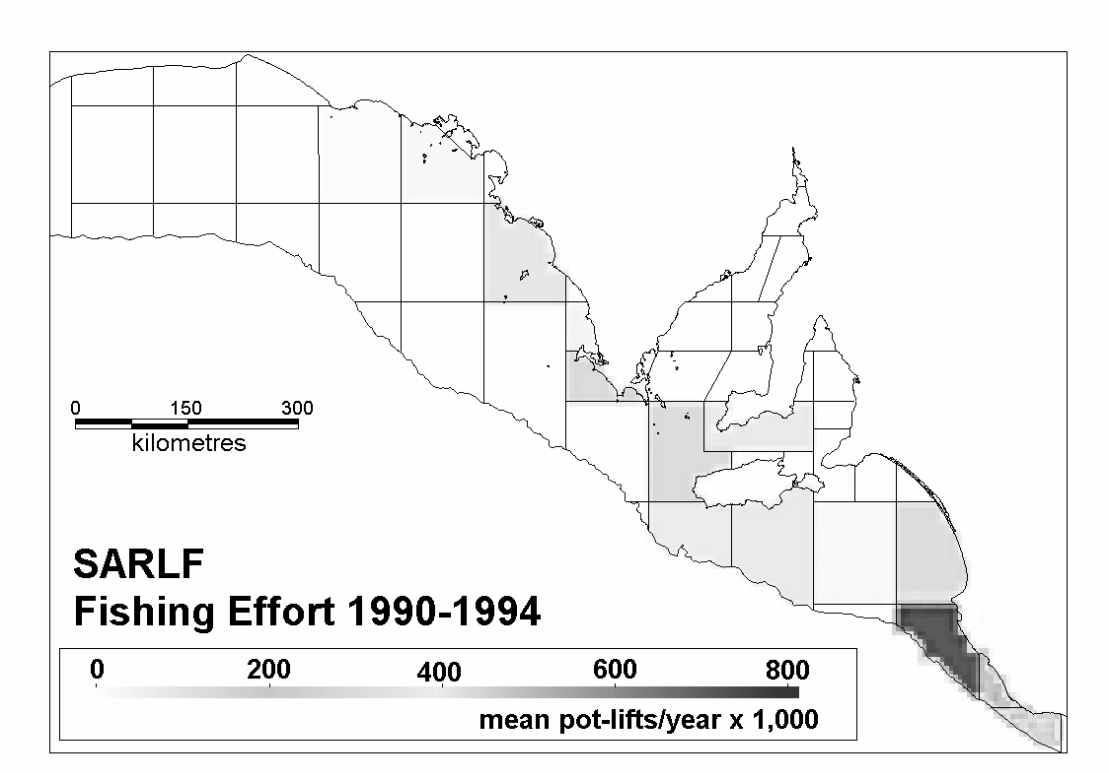


Figure 30. Mean annual distribution of fishing effort in the SARLF, 1990-94.

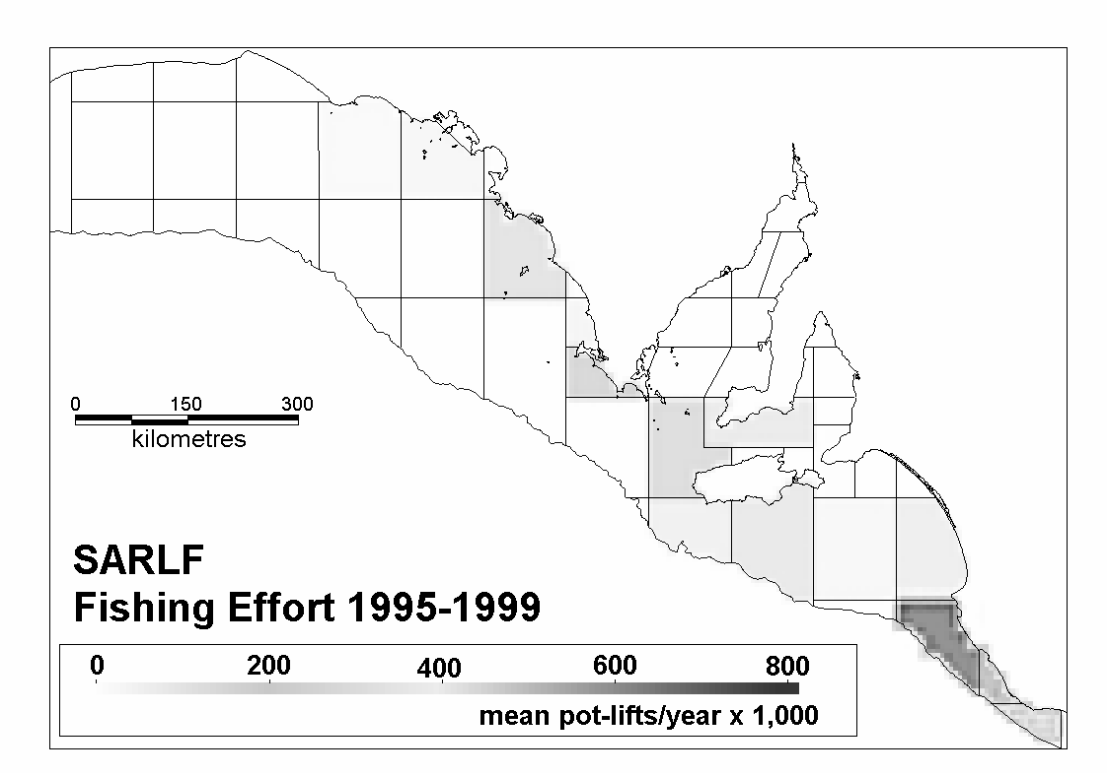


Figure 31. Mean annual distribution of fishing effort in the SARLF, 1995-99.

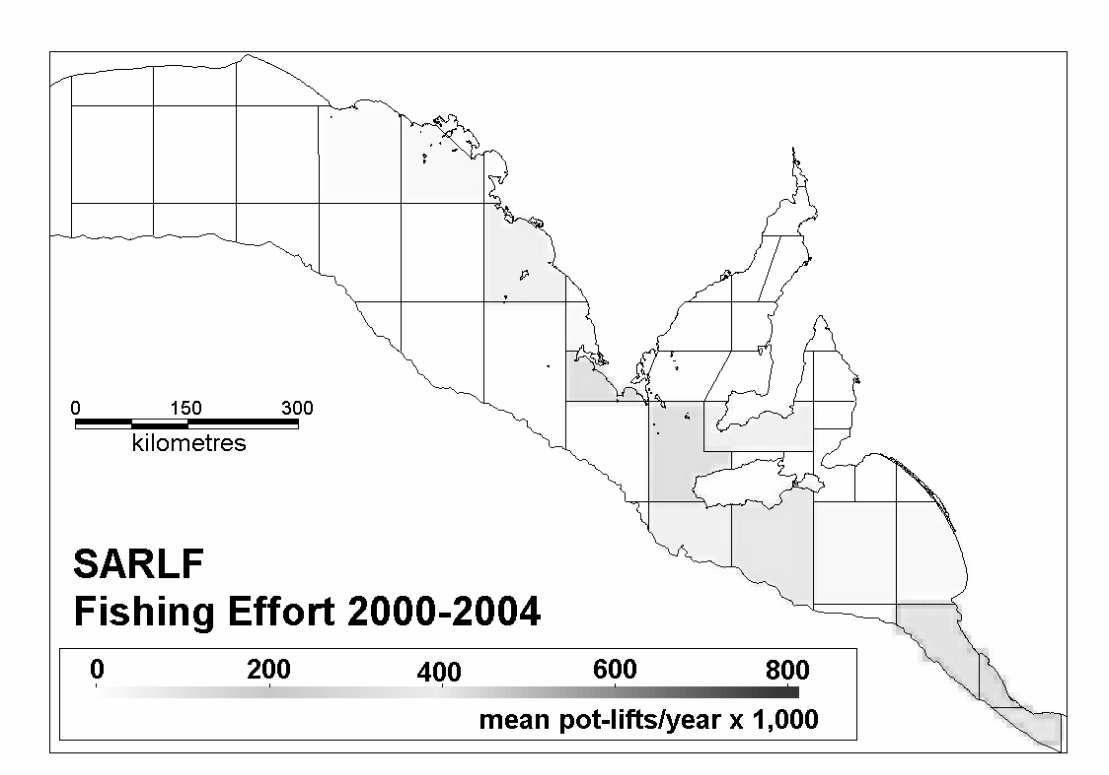


Figure 32. Mean annual distribution of fishing effort in the SARLF, 2000-04.

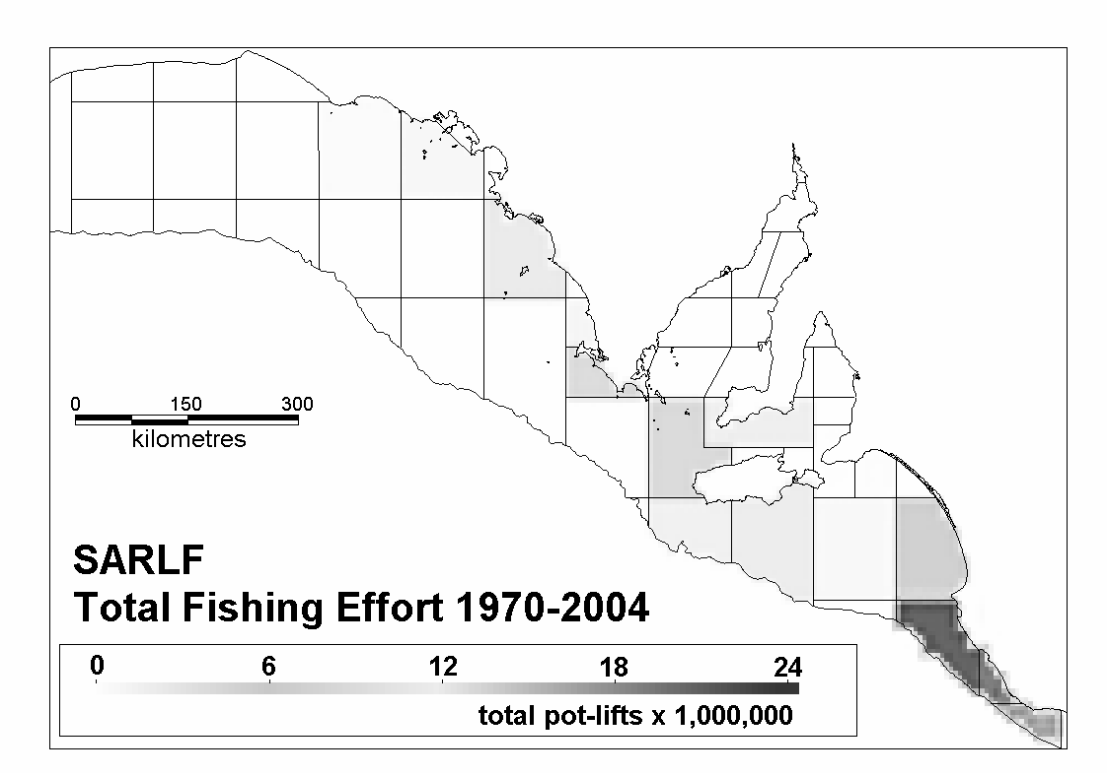


Figure 33. Distribution of total fishing effort in the SARLF, 1970-2004.

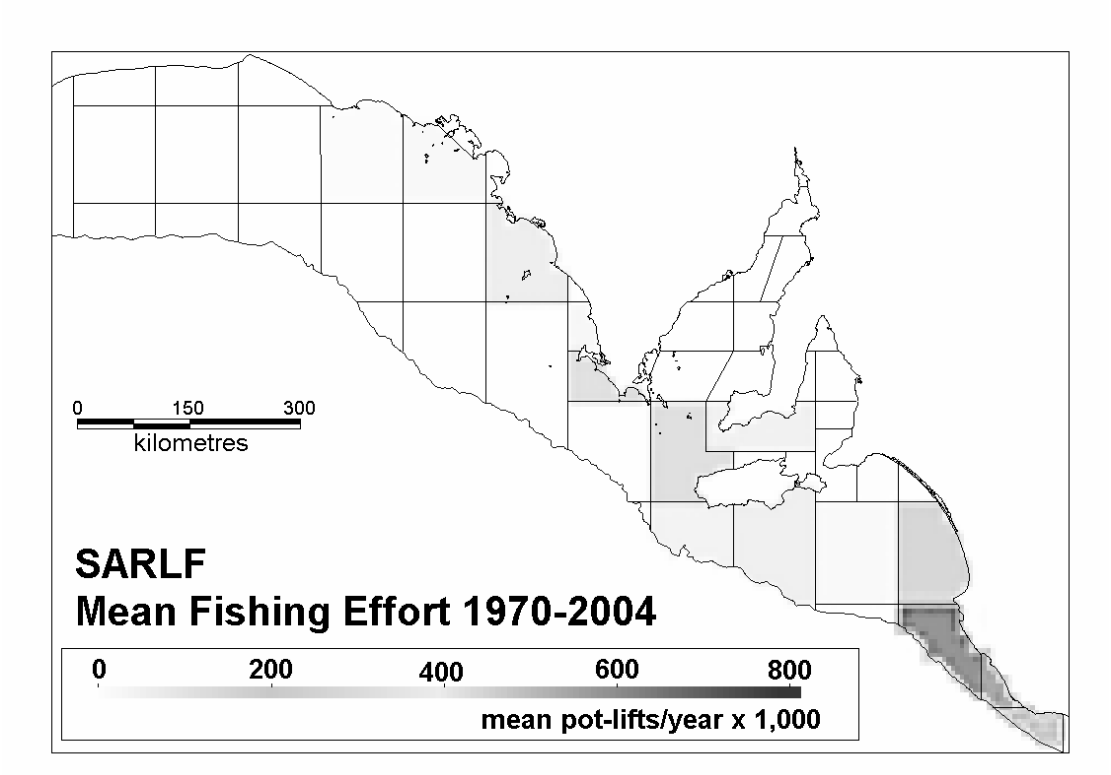


Figure 34. Mean annual distribution of fishing effort in the SARLF, 1970-2004.

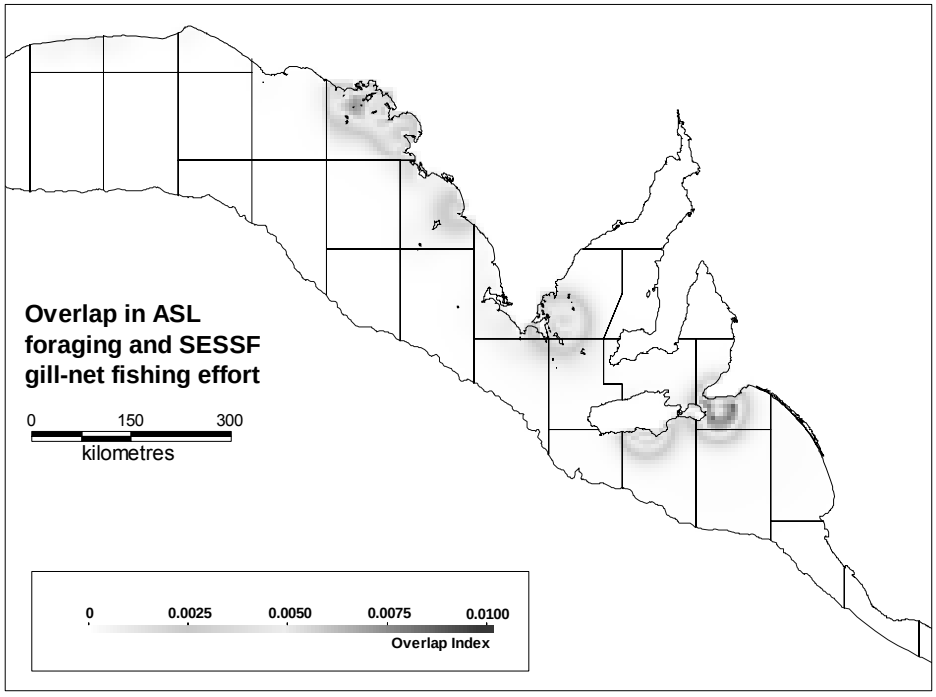


Figure 35. Overlap index in ASL foraging effort and SESSF gill-net fishing effort.

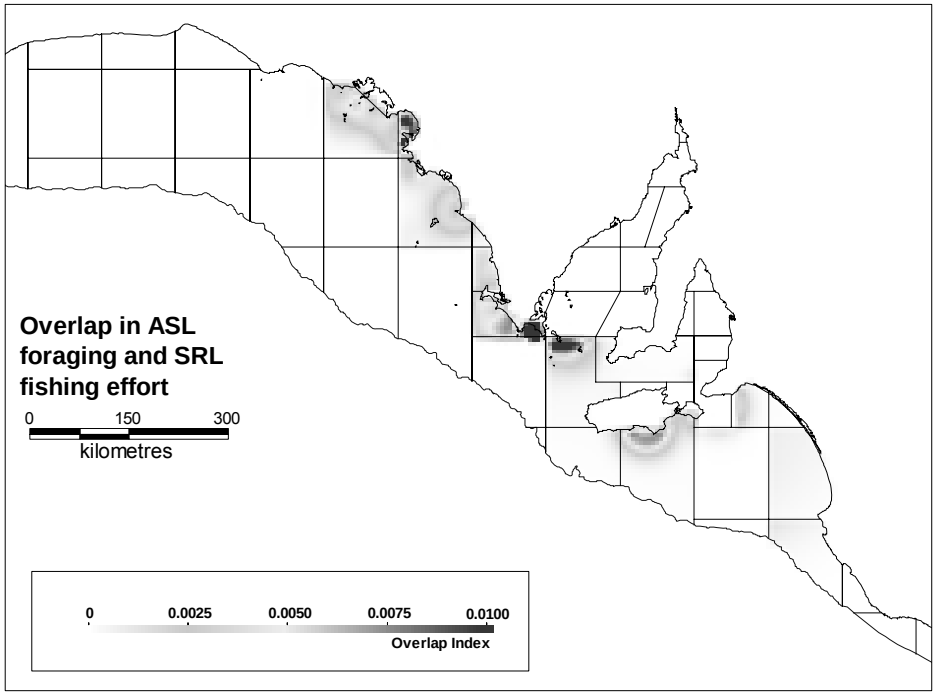


Figure 36. Overlap index in ASL foraging effort and SARLF fishing effort.

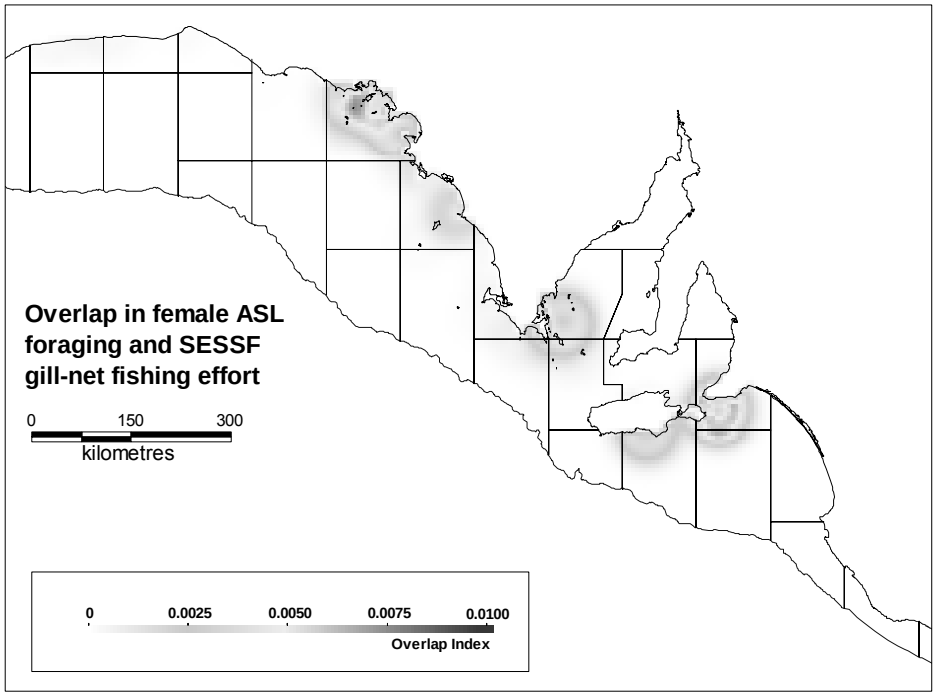


Figure 37. Overlap index between adult female ASL foraging effort and SESSF gill-net fishing effort.

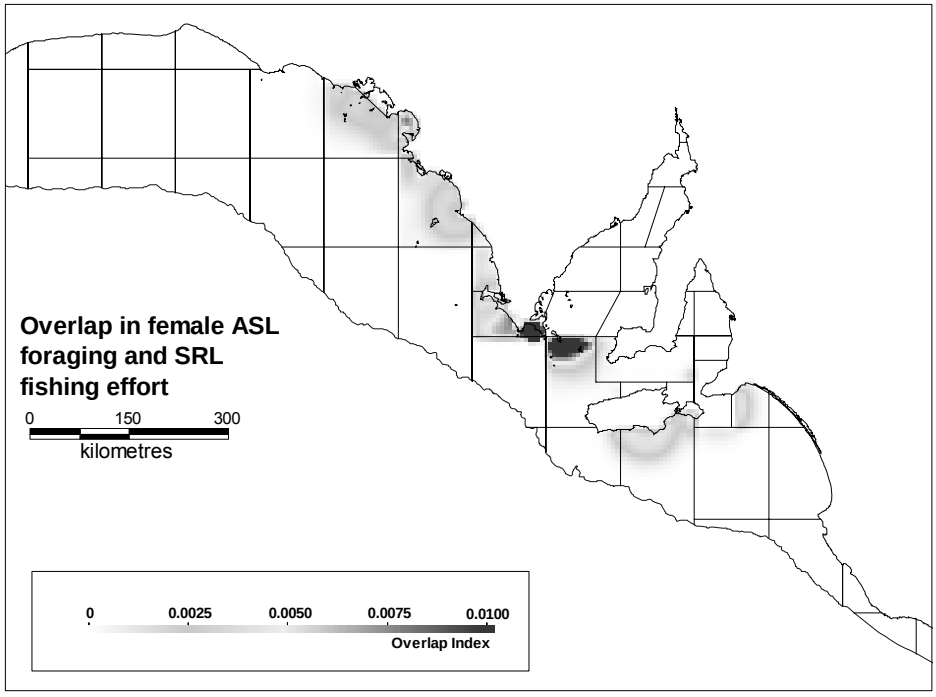


Figure 38. Overlap index between adult female ASL foraging effort and SARLF fishing effort.

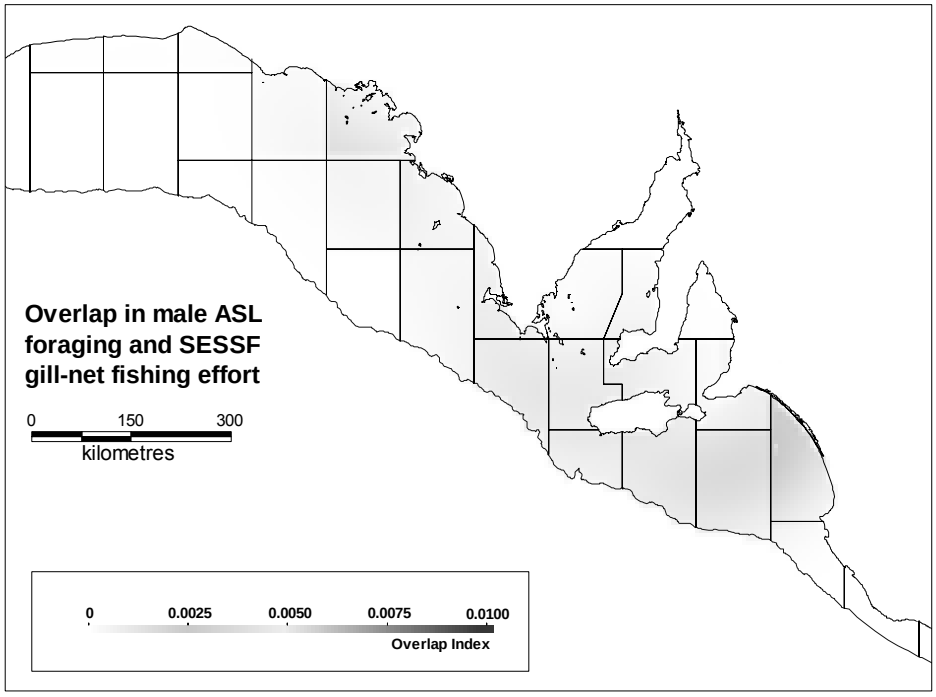


Figure 39. Overlap index between adult male ASL foraging effort and SESSF gill-net fishing effort.

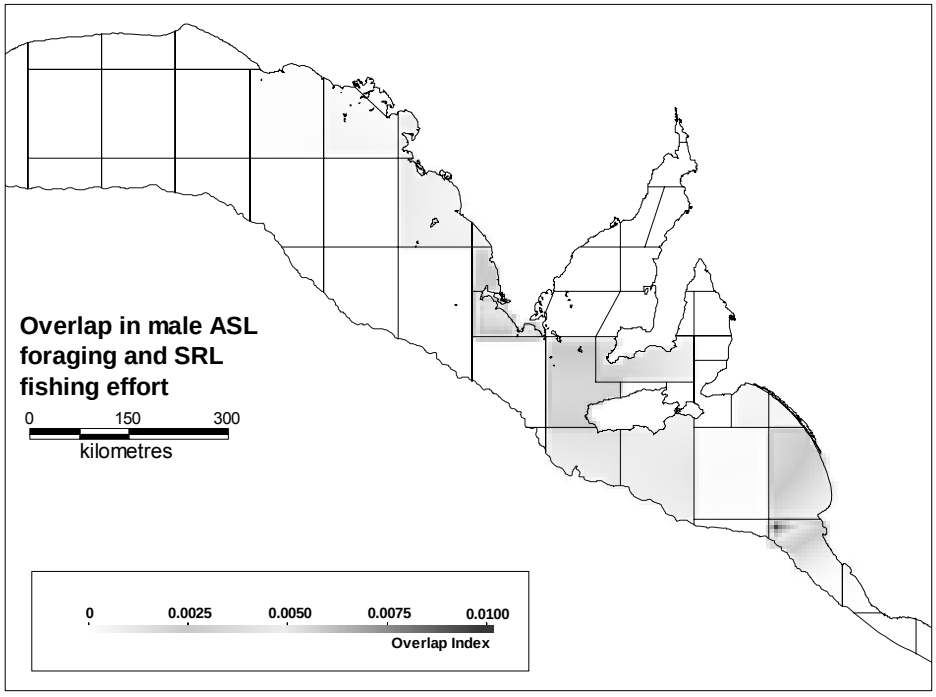


Figure 40. Overlap index between adult male ASL foraging effort and SARLF fishing effort.

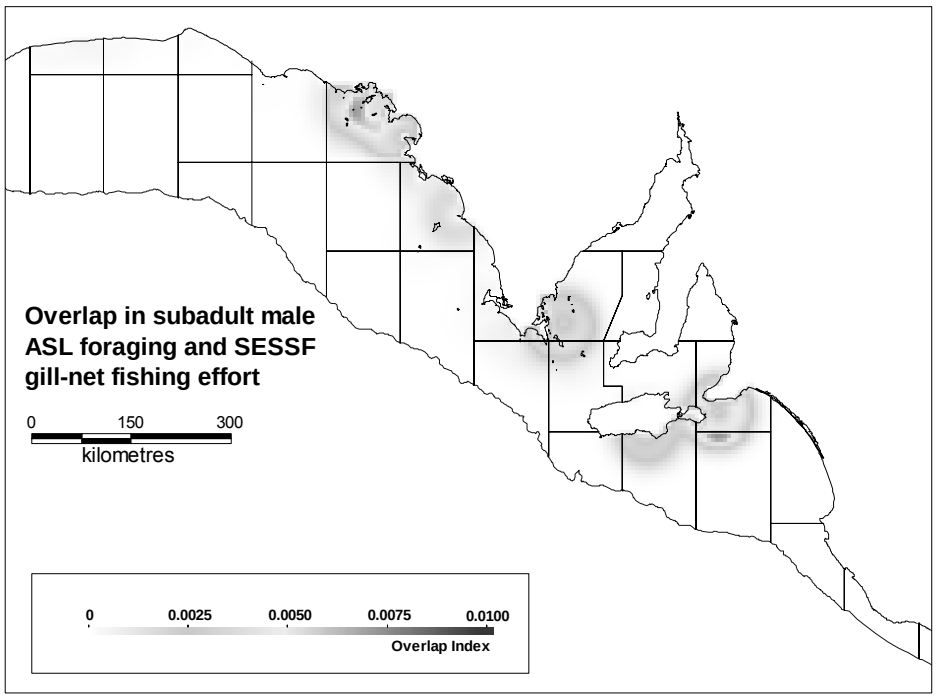


Figure 41. Overlap index between sub-adult male ASL foraging effort and SESSF gill-net fishing effort.

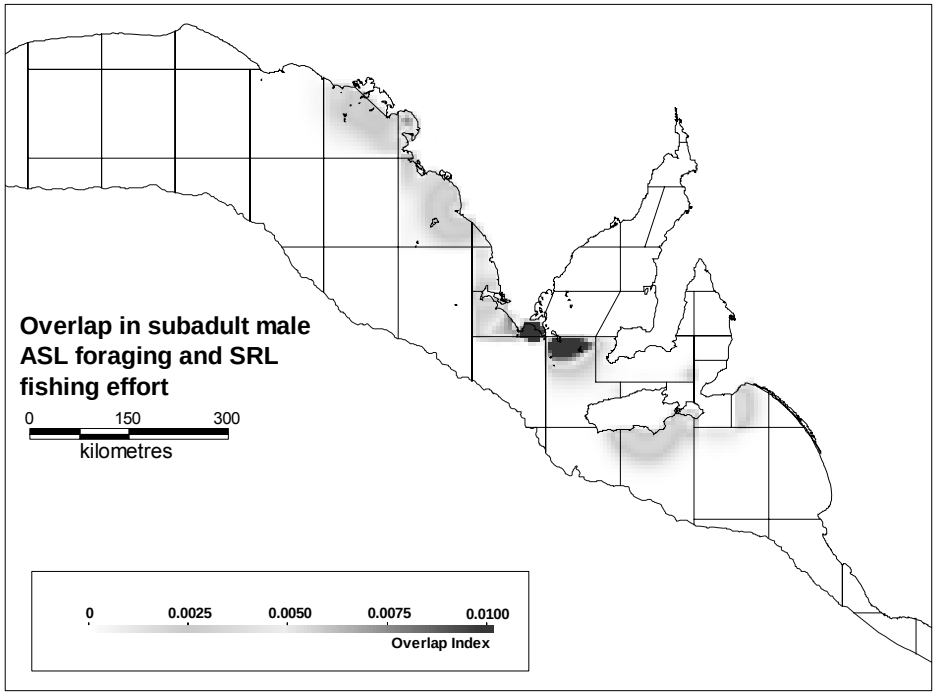


Figure 42. Overlap index between sub-adult male ASL foraging effort and SARLF fishing effort.

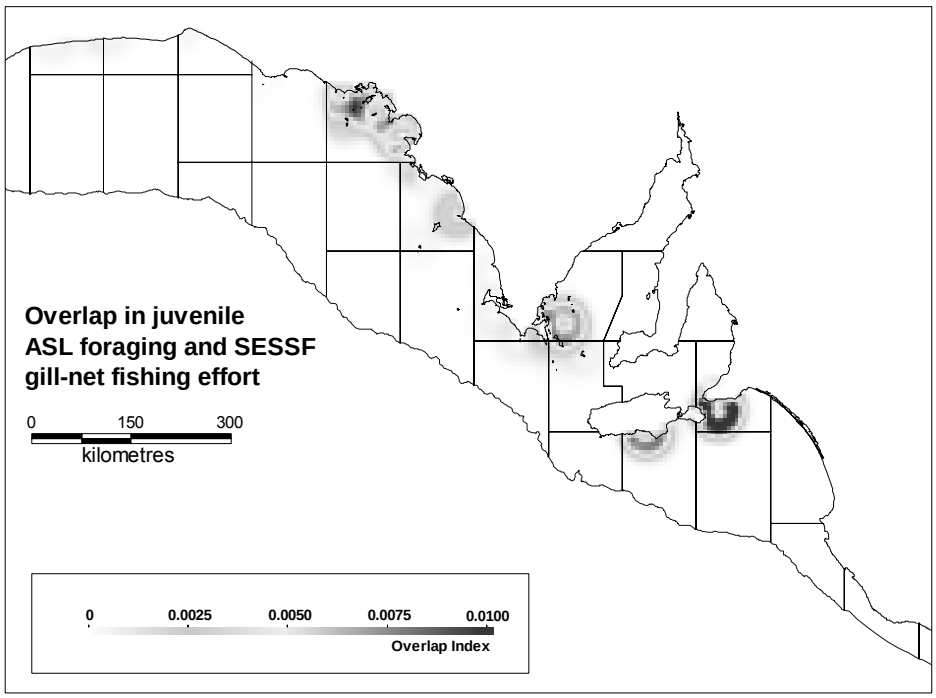


Figure 43. Overlap index between juvenile ASL foraging effort and SESSF gill-net fishing effort.

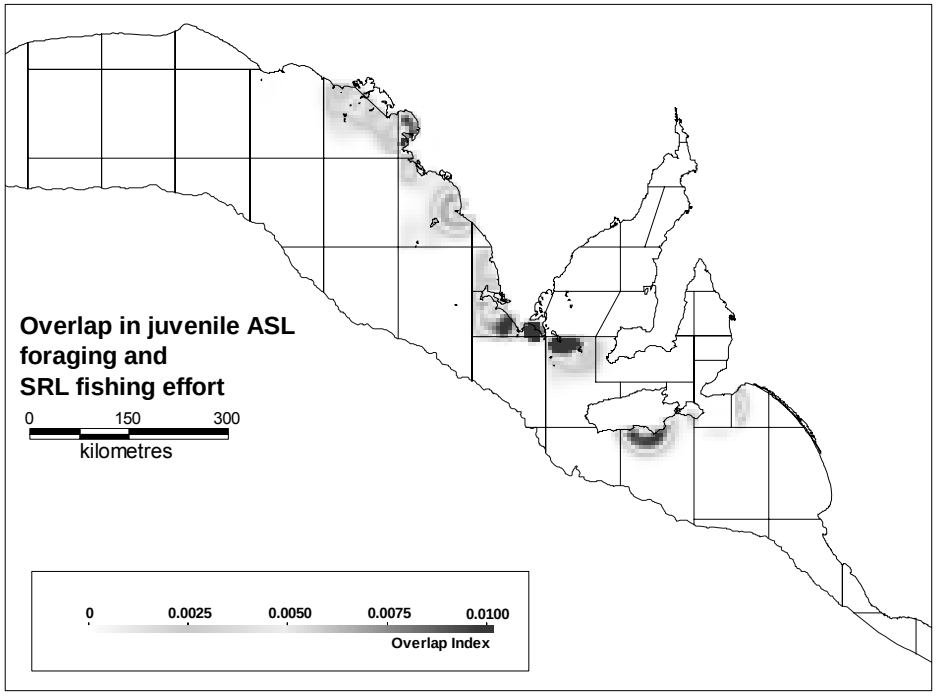


Figure 44. Overlap index between juvenile ASL foraging effort and SARLF fishing effort.

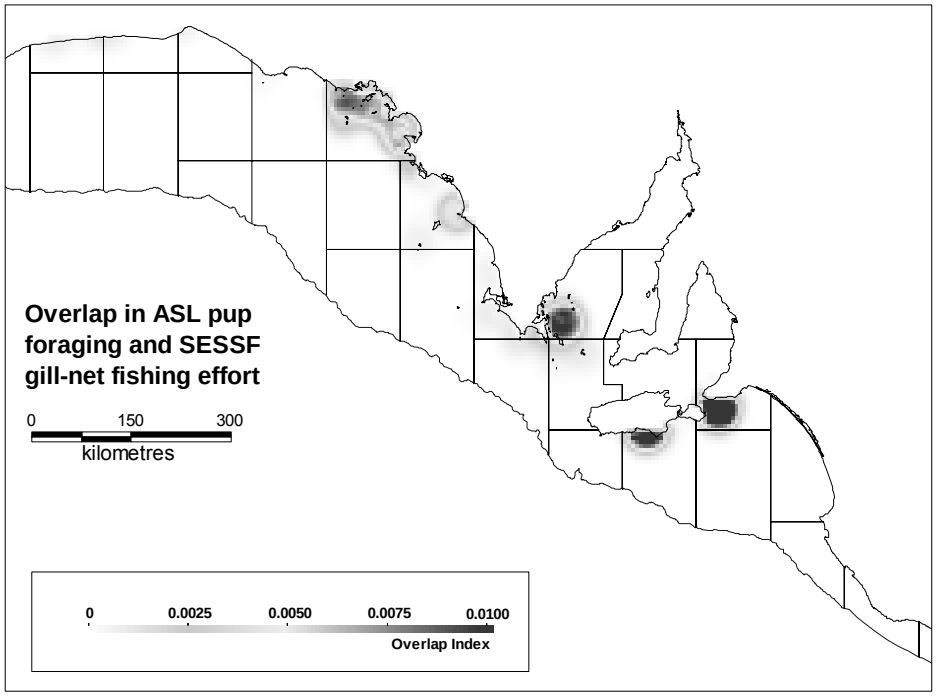


Figure 45. Overlap index between ASL pup foraging effort and SESSF gill-net fishing effort.

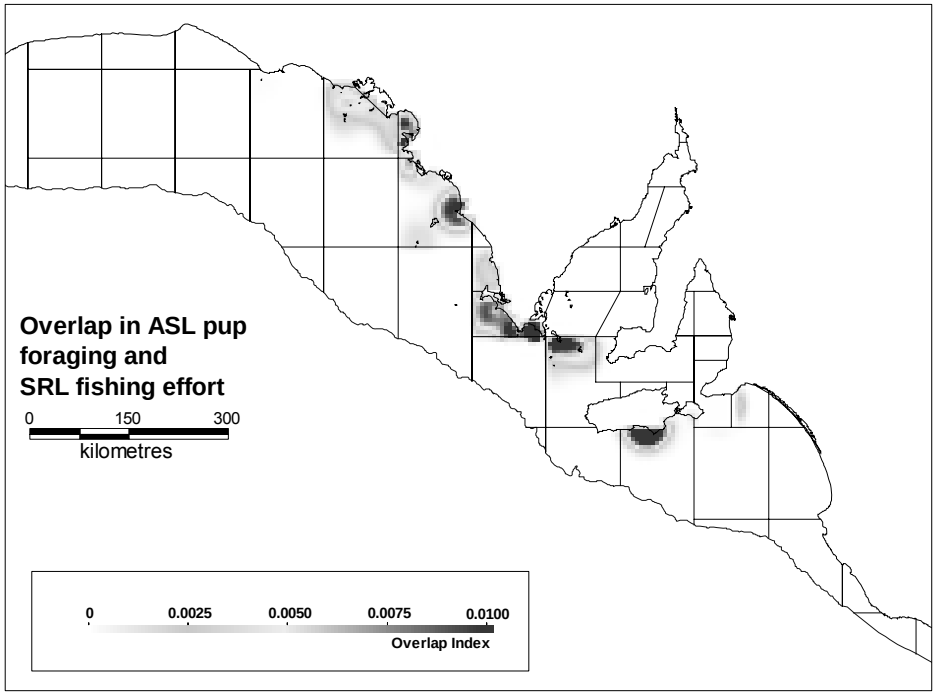


Figure 46. Overlap index between ASL pup foraging effort and SARLF fishing effort.

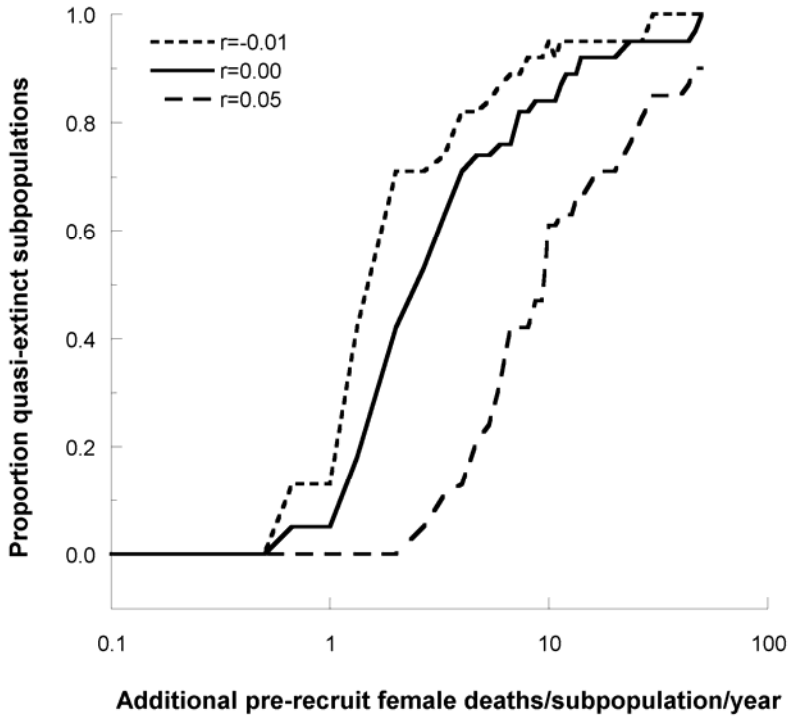


Figure 47. Estimated proportion of ASL subpopulations that achieve quasi-extinction as a function of the number of additional pre-recruit female mortalities/subpopulation/year. Three scenarios are given, based on the increasing (r=0.05), stable (r=0.00) and declining (r=-0.01) population models.

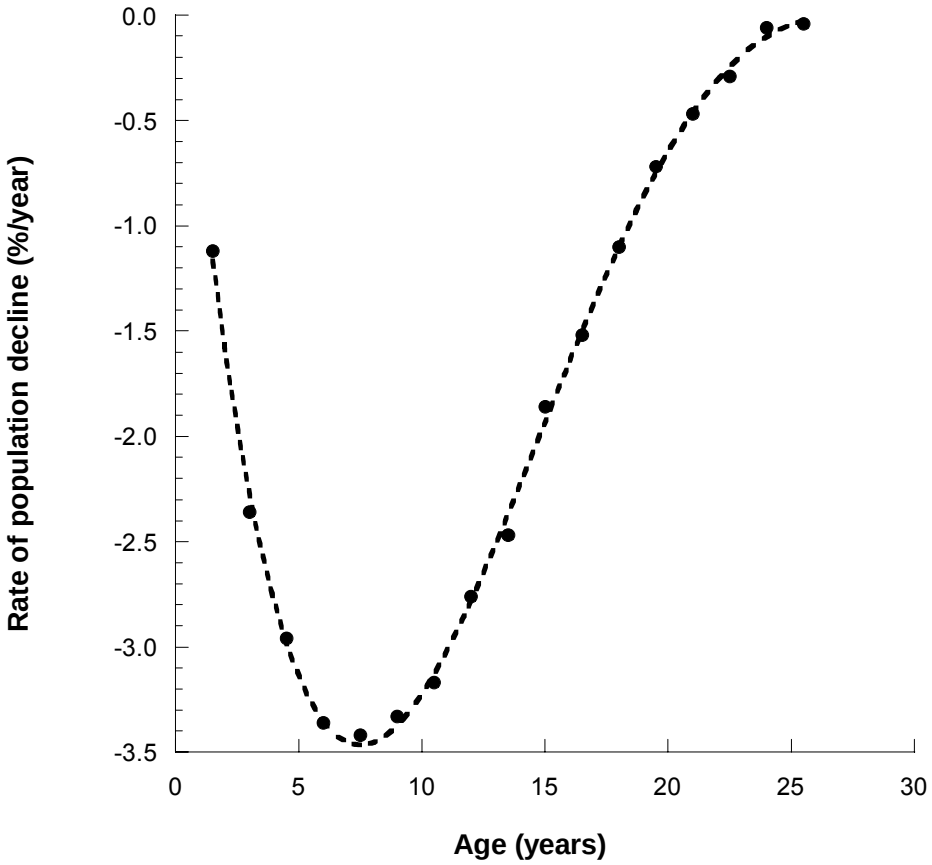


Figure 48. Simulated example of how the stage (age) at which mortalities are taken affects the rate of population change. In this example a subpopulation of 1,000 female ASL has 20 females removed from a particular age-group each year for 50 reproductive cycles (75 years), using the stable population model (r=0). The rate of population decline resulting from each scenario is presented, fitted with a 4th order polynomial curve. The example demonstrates how the rate of decline is affected by the age-group of females removed from the population. The greatest rates of decline are achieved when 4.5-6, 6-7.5 and 7.5-9 age-group females are removed.

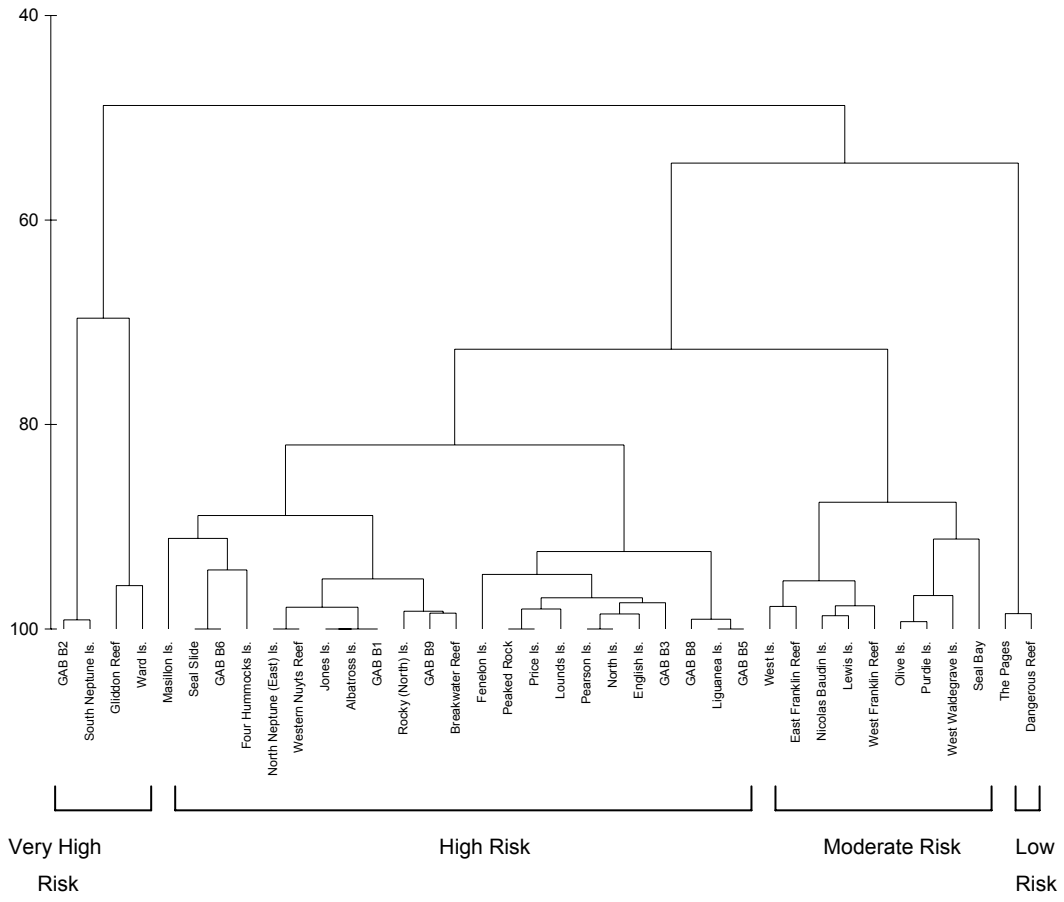


Figure 49. Bray-Curtis similarity matrix dendrogram of SA ASL subpopulations, which are clustered according to percentage similarity of quasi-extinction risk (from PVA outputs). Four main groups are identified as the different Risk Categories (dendrogram produced using Primer V5.2.2).

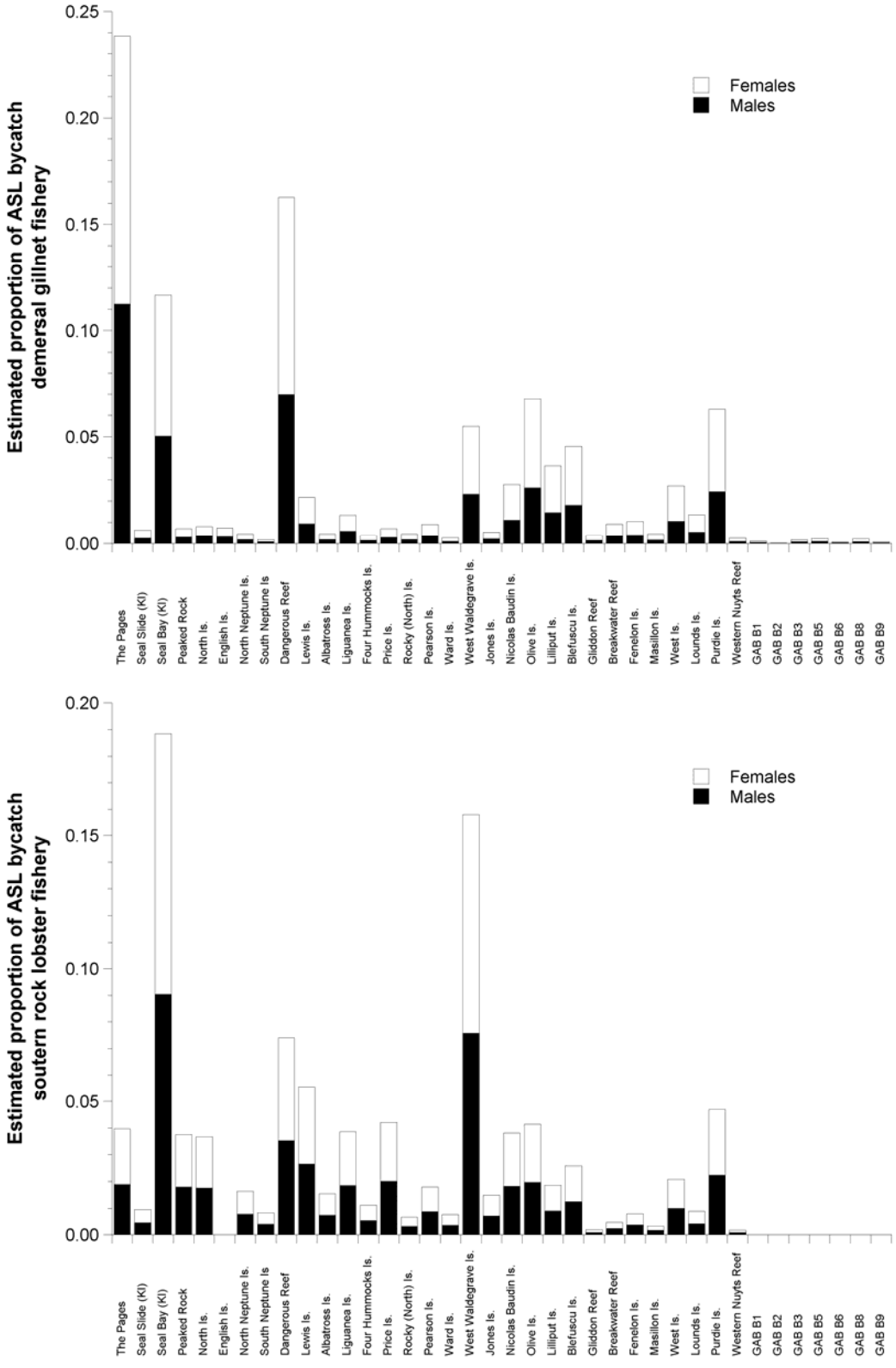
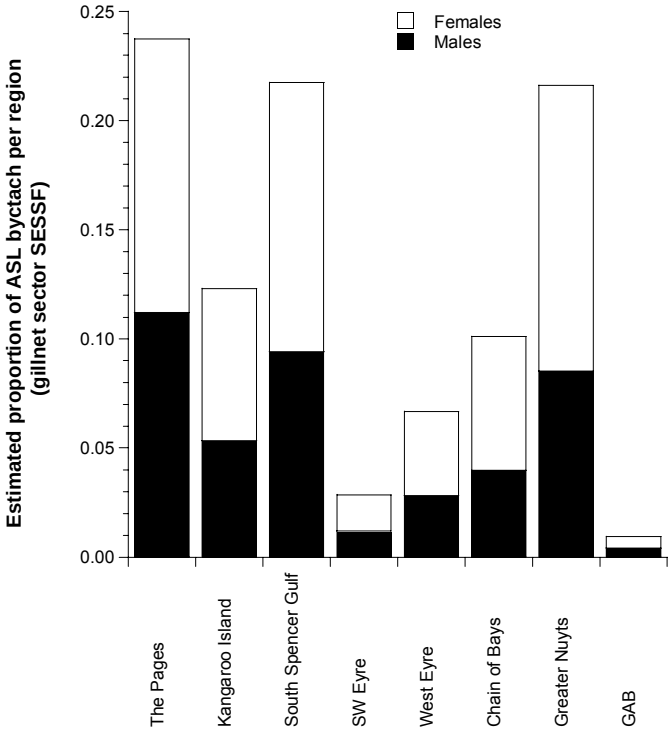


Figure 50. Estimated proportion of historic bycatch (broken down by seal sex) accounted for by each SA ASL subpopulation in the SESSF gillnet sector (top plot) and the SARLF (bottom plot).

A.



B.

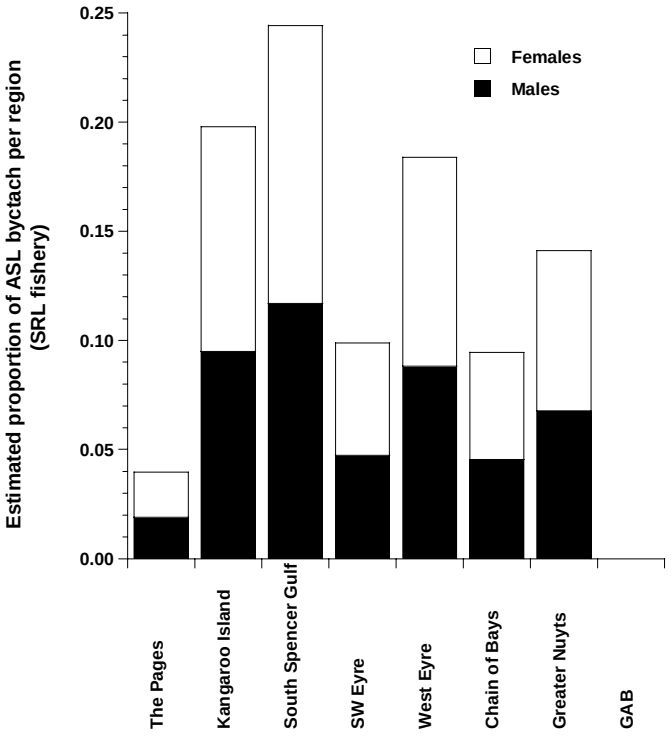


Figure 51. Estimated proportion of historic bycatch (broken down by seal sex) accounted for by regional groupings of SA ASL subpopulations in the SESSF gillnet sector (top plot) and the SARLF (bottom plot).

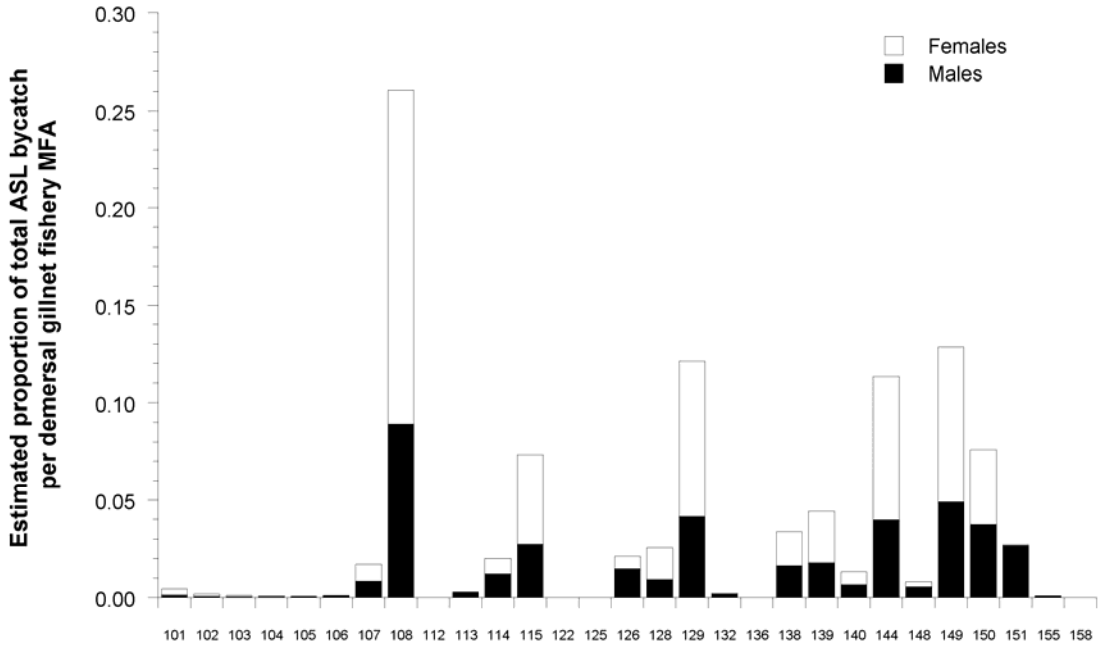


Figure 52. Estimated proportion of historic bycatch (broken down by seal sex) (1973-2004) in SA for ASL accounted for by each SESSF gillnet sector MFA.

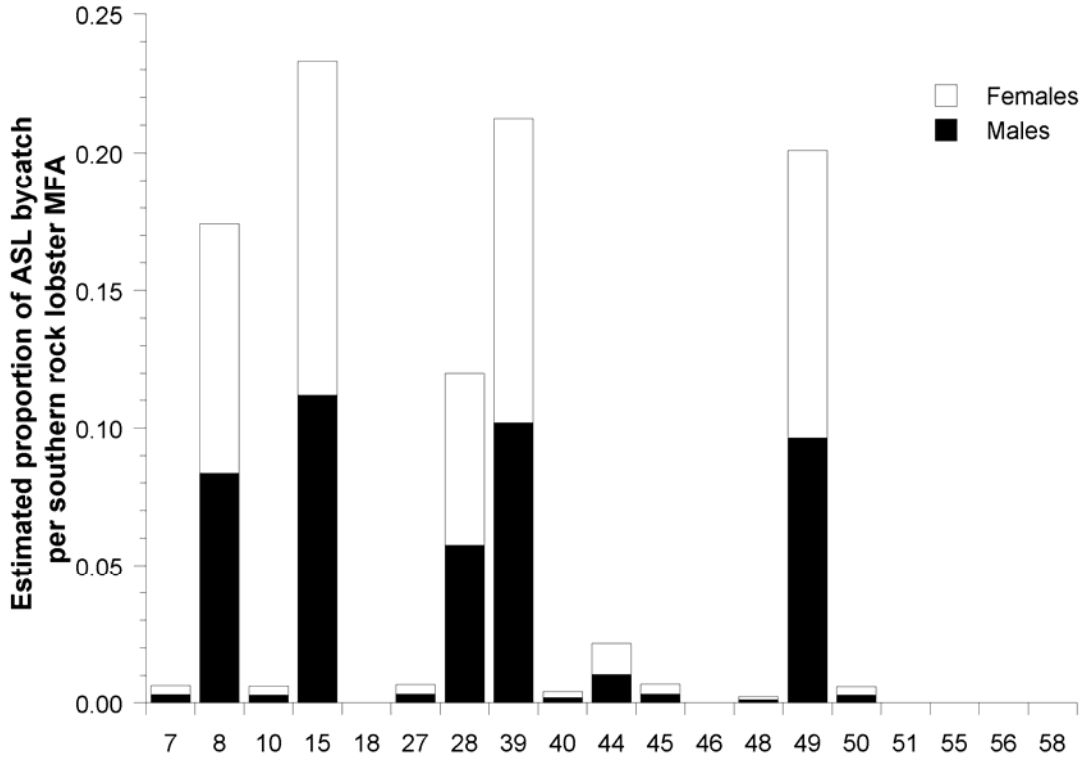


Figure 53. Estimated proportion of historic (1970-2004) bycatch (broken down by sex) in SA for ASL accounted for by each SA RLF MFA.

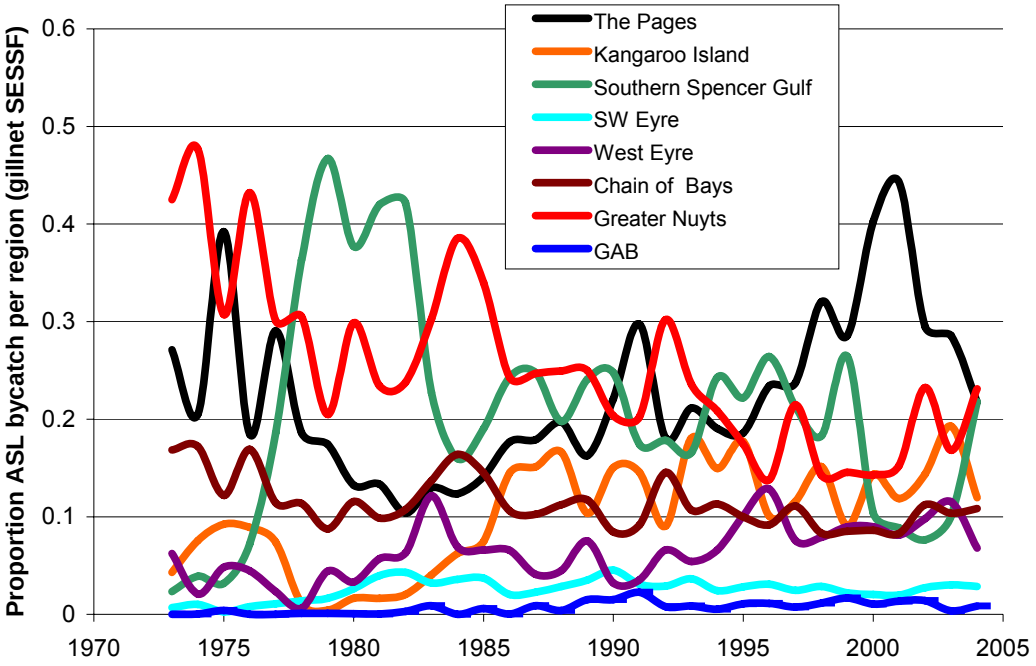


Figure 54. Estimated temporal change in the proportion of historic (1973-2004) SA SESSF gillnet sector bycatch, of ASL from different geographic regions.

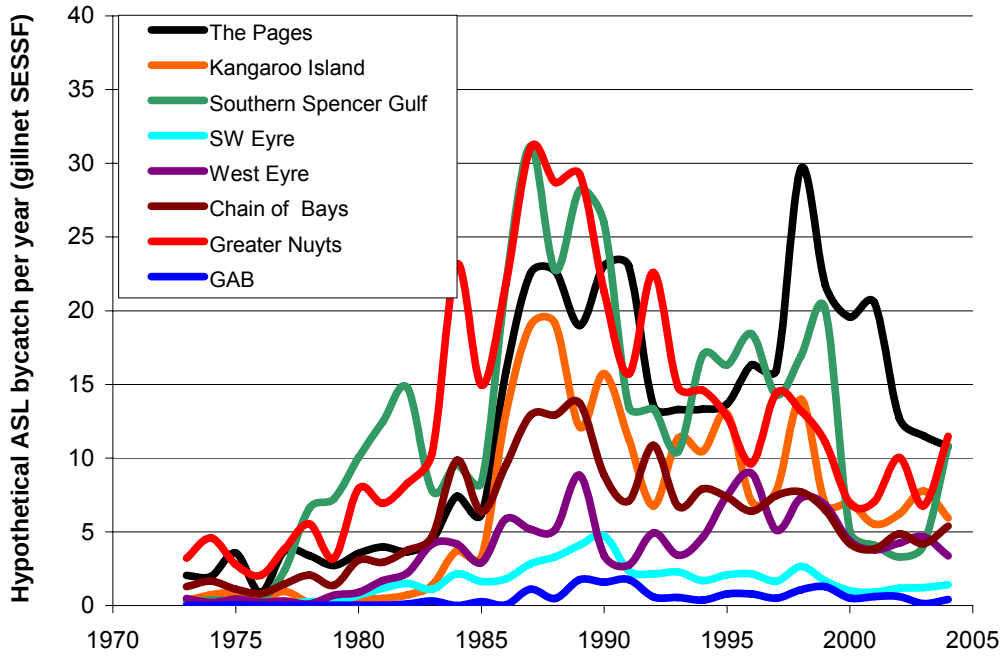


Figure 55. Hypothetical temporal change in the numbers of historic (1973-2004) SA SESSF gillnet sector bycatch, of ASL from different geographic regions. Numbers based on a hypothetical bycatch rate of 0.005 seals/km of net-lift.

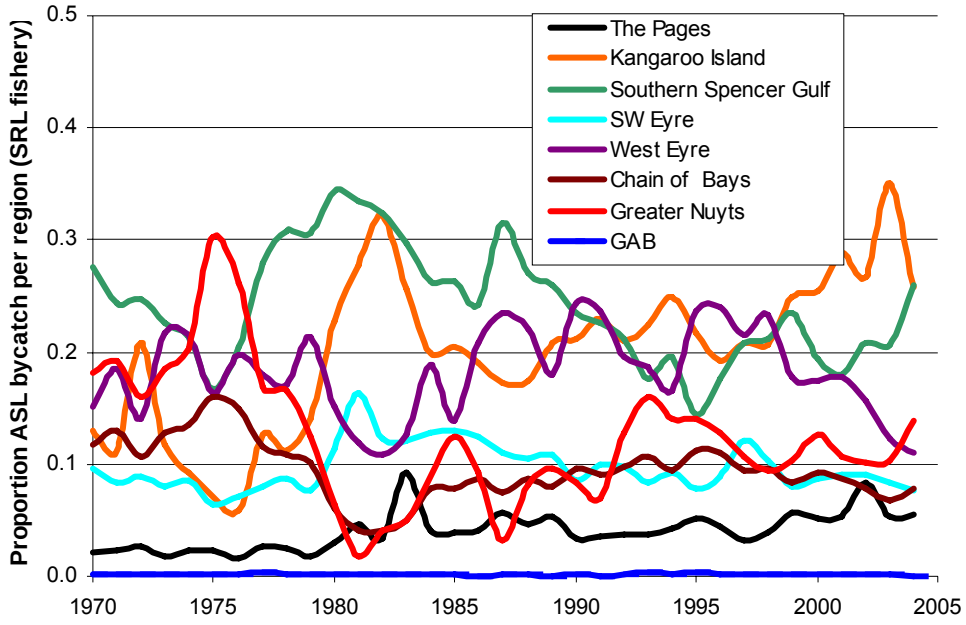


Figure 56. Estimated temporal change in the proportion of historic (1970-2004) SA RLF bycatch, of ASL from different geographic regions.

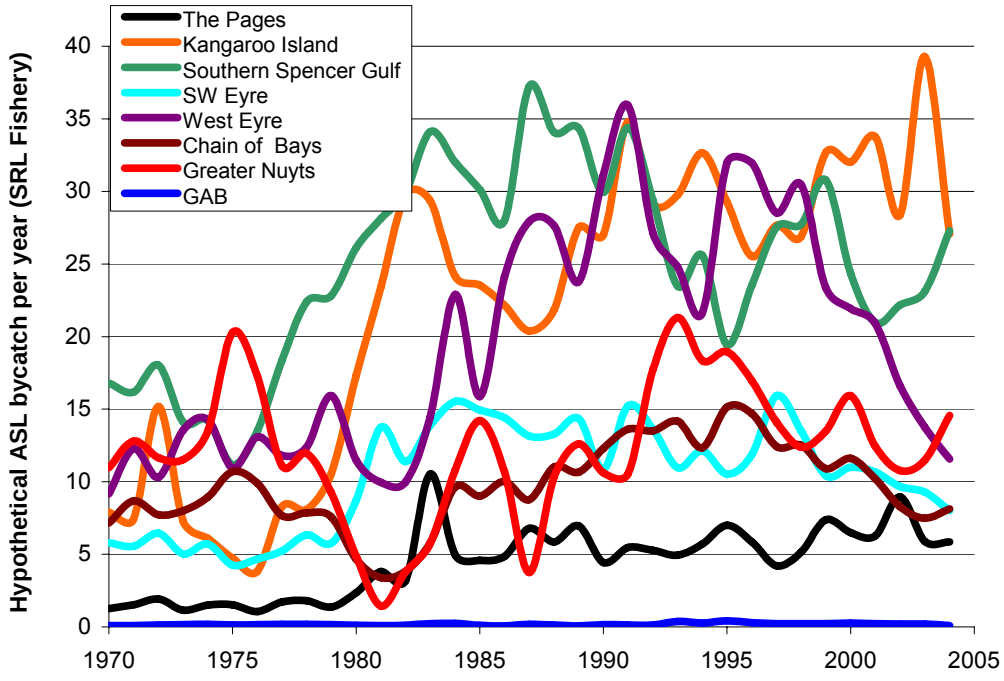


Figure 57. Hypothetical temporal change in the numbers of historic (1970-2004) SA RLF sector bycatch, of ASL from different geographic regions. Numbers based on a hypothetical bycatch rate of 0.2 seals/1,000 pot-lifts.

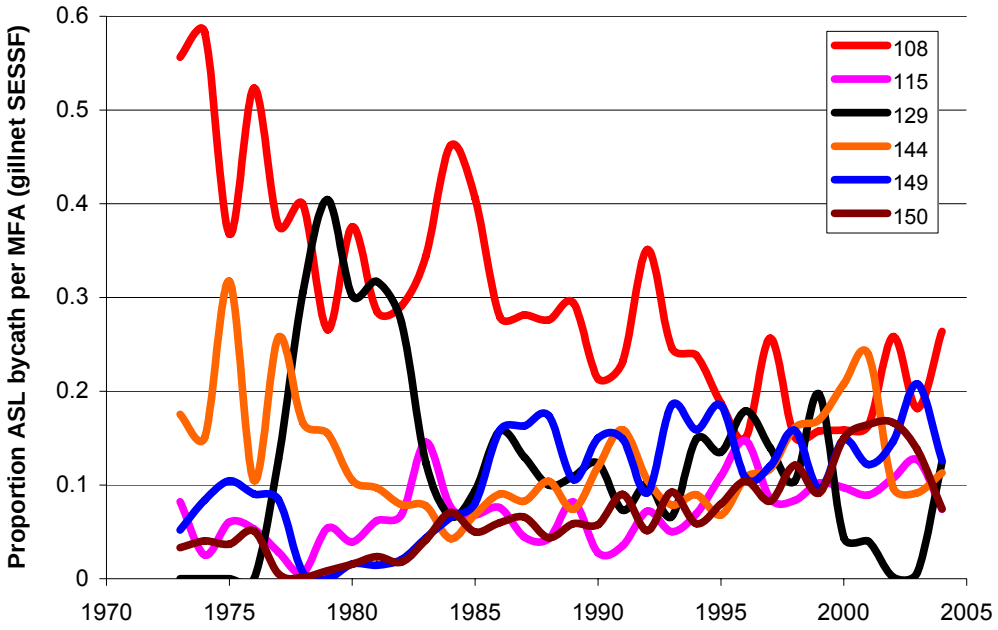


Figure 58. Estimated temporal change in the proportion of historic (1973-2004) SA SESSF gillnet sector bycatch, from the six major contributing MFAs for ASL.

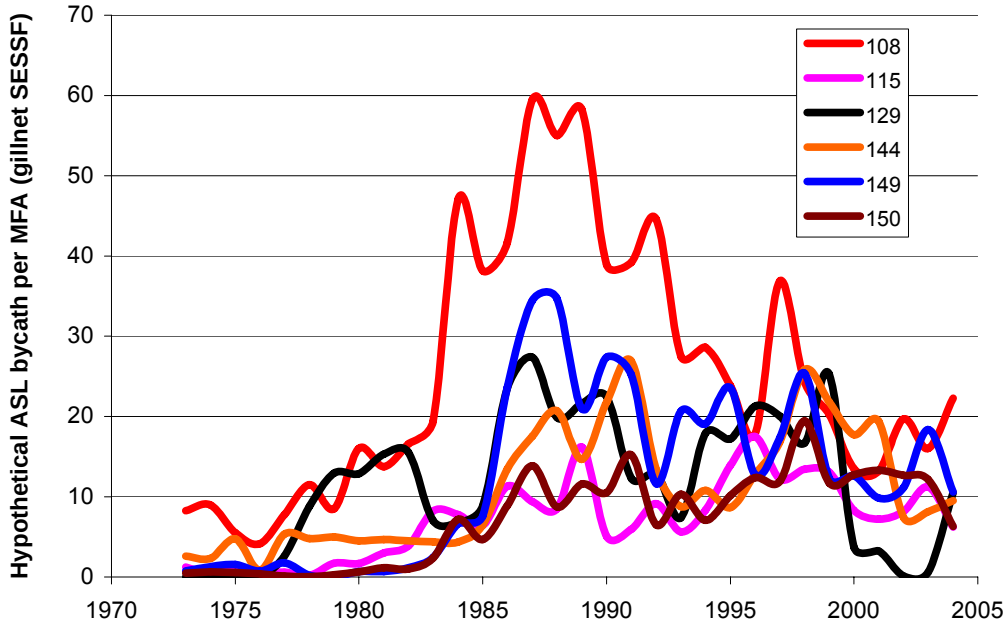


Figure 59. Hypothetical temporal change in the numbers of historic (1973-2004) SA SESSF gillnet sector bycatch, from the six major contributing MFAs for ASL . Numbers based on a hypothetical bycatch rate of 0.005 seals/km of net-lift.

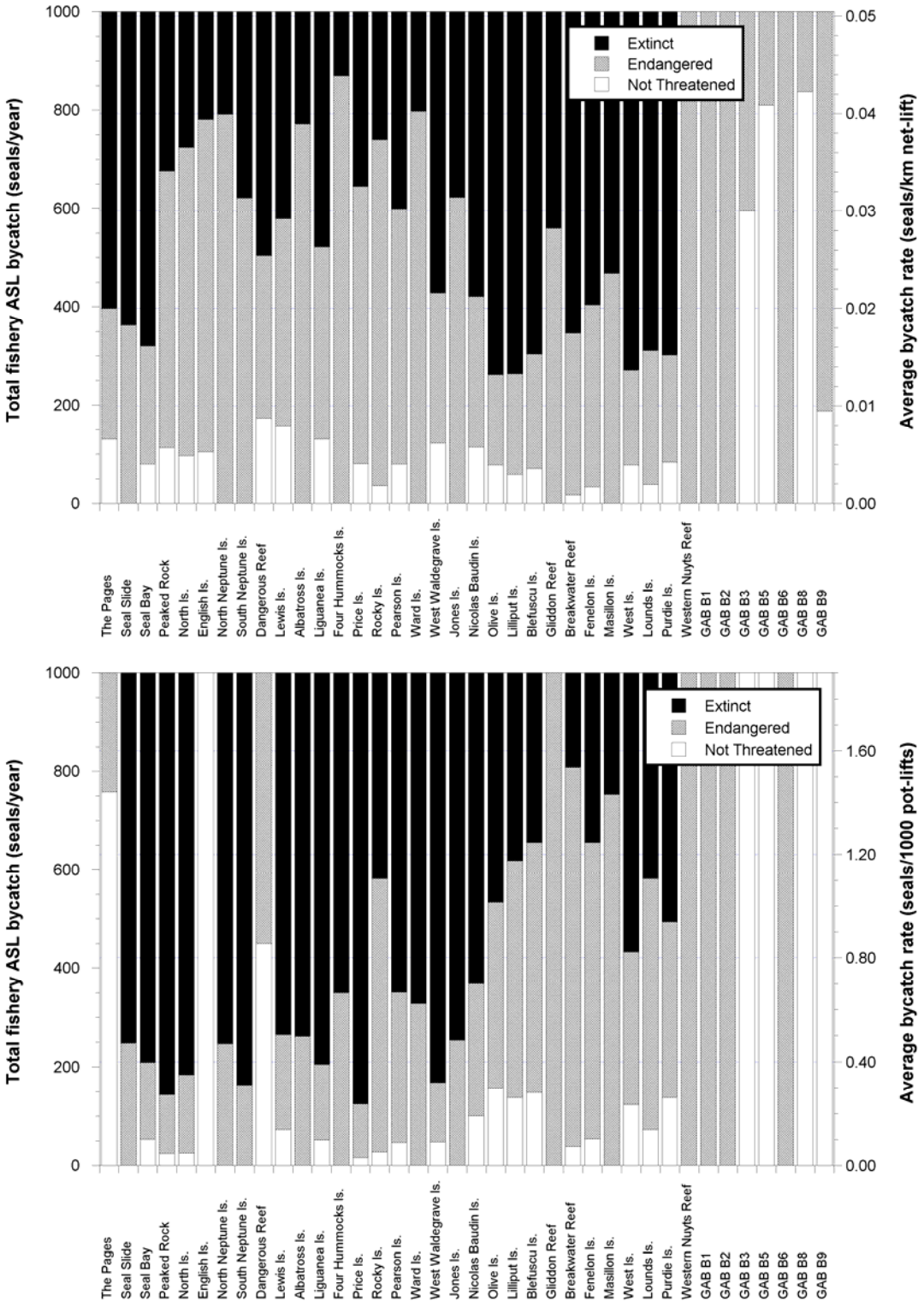


Figure 60. Estimated total number of ASL and average bycatch rate required to place different ASL subpopulations into different risk categories in SA component of (top plot) the SESSF gillnet sector (1973-2004 mean fishing effort) and (bottom plot) the SA RLF (1970-2004 mean fishing effort). The bycatch number refers to the total number seals caught per year, of which about 52% are female, which are apportioned among the 38 subpopulations based on fishery-seal interaction probabilities.

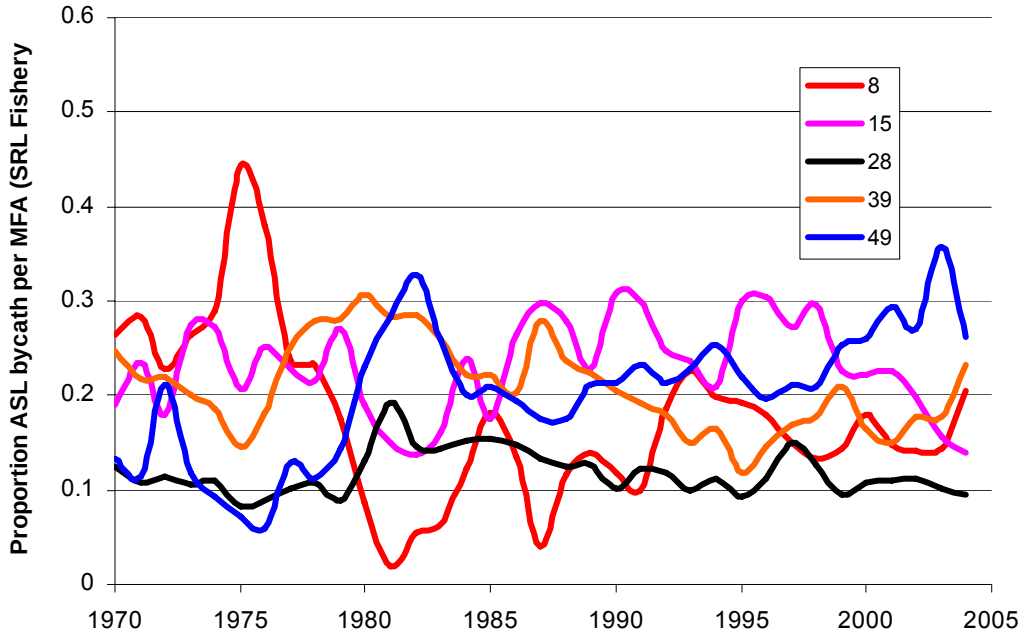


Figure 61. Estimated temporal change in the proportion of historic (1970-2004) SARLF bycatch, from the major contributing MFAs for ASL.

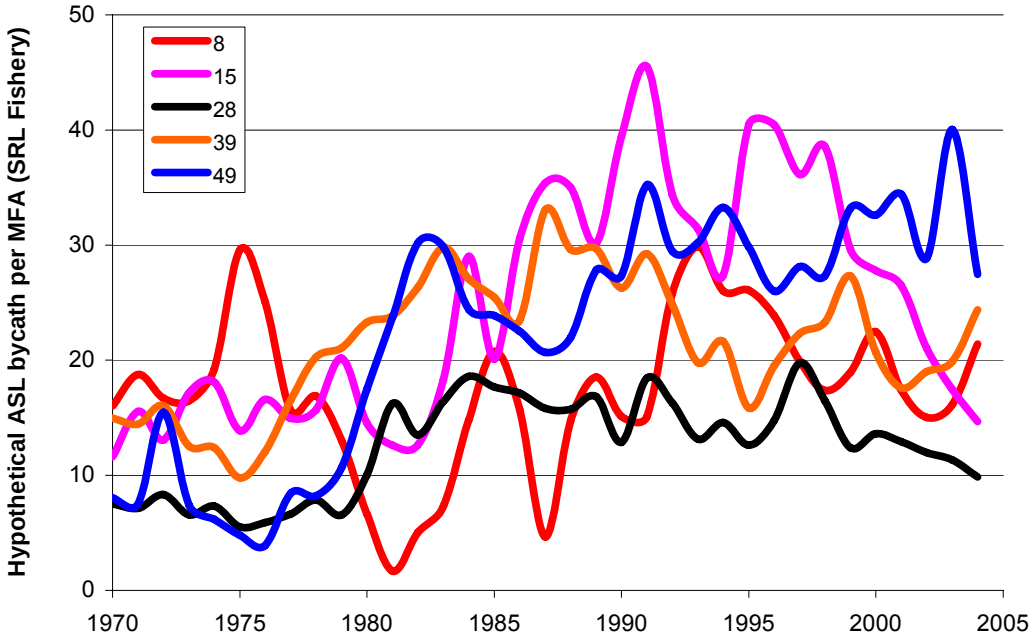


Figure 62. Hypothetical temporal change in the numbers of historic (1970-2004) SA RLF bycatch, from major contributing MFAs for ASL. Numbers based on a hypothetical bycatch rate of 0.2 seals/1,000 pot-lifts.

DEVELOPING MOLECULAR DNA TECHNIQUES TO DETERMINE THE DIET OF AUSTRALIAN SEA LIONS

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Introduction

Analysing dietary remains of marine mammals can enhance our understanding of the temporal and spatial trophodynamics of marine ecosystems. Dietary remains from digestive tracts have been used to diagnose prey in a number of pinniped species (Gales and Cheal 1992; Alonso *et al.* 2000; Yonezaki et al. 2003; McIntosh *et al.* 2006), as have regurgitates (Fea *et al*. 1999; Childerhouse *et al*. 2001; Page *et al*. 2005) and scats (Goldsworthy *et al*. 1997; Bowen 2000; Anderson et al. 2004). The collection and visual identification of indigestible hard-prey structures, such as bones, scales, exoskeleton, and eye lenses can often provide a useful means to diagnose prey to genus or species level (Boyle and Pierce, 1991), and is a successful method of retrieving large quantities of dietary information, mostly using a relatively simple, non-invasive approach (Gales and Pemberton 1994; Gales *et al.* 1993; Childerhouse *et al*. 2001). Where sagittal otoliths and cephalopod beaks are recovered and can be identified (Casaux et al. 2003; de Bruyn et al. 2003; Hume et al. 2004; Page *et al*. 2005, McIntosh et al. 2006), the number and size of prey ingested can be approximated (Bowen 2000). This is of importance when providing representative qualitative and quantitative measures of the relative biomass of each prey species consumed (Hyslop, 1980).

Studies that require prey remnants to be recovered from faecal, stomach, and regurgitate samples have some biases, which stem from the differential digestion, passage and retention rates of prey consumed (Dellinger and Trillmich 1987; Gales and Pemberton, 1990; Gales and Cheal, 1992; Cottrell *et al.* 1996). These biases are heightened in ASL, because of prolonged retention rates which may facilitate the

complete digestion of prey in the gastrointestinal tract (Gales and Cheal, 1992). Feeding trials and recent stomach analyses of ASL (Richardson and Gales,1987; Gales and Cheal, 1992; McIntosh et al. 2006) also demonstrate that complete digestion of fish otoliths and retention or regurgitation of other prey structures limits the use of morphological prey identification of diet remains.

Even though these biases confound the use of traditional diet analysis, information on the trophic interactions of ASL across their geographical range remains restricted and is based on few published diet accounts recording a range of benthic prey species including crustaceans; rock lobster (*Panulirus cygnus* and *Jasus* sp.), swimming crab (*Ovalipes australiensis*), various cephalopods; cuttlefish (*Sepia* sp.), squid (*Sepioteuthis australis* and *Nototodarus gouldi*), octopus, fish; whiting (*Sillaginodes punctata*) leather jacket (Monocanthidae), flathead (*Neoplatycephalus* sp.), swallowtail (*Centroberyx lineatus*), common bullseye (*Pempheris multiradiata*), eastern school whiting (*Sillago flindersi*), yellowtail scad (*Trachurus novaezelandiae*), Australian salmon (*Arripis truttaceus*), sharks; school shark (*Galeorhinus galeus*) and gummy shark (*Mustelus antarcticus*) and birds; little penguin (*Eudyptula minor*) (Walker and Ling (1981); Richardson and Gales (1987); Gales and Cheal (1992); Ling (1992); McIntosh et al. 2006).

The recent use of molecular genetics to determine relationships between predators and the prey they consume is gaining popularity in diet studies where stomach or faecal contents are morphologically unrecognisable. Polymerase chain reaction (PCR) based techniques using species-specific and group-specific primers have successfully detected krill as a prey species from pygmy blue whale (*Balaenoptera musculus brevicauda*), fin whale (*Balaenoptera physalus*) (Jarman *et al.* 2002), and whale shark (*Rhincodon typus*) faeces (Jarman *et al.* 2002). Fish and krill have been identified from DNA refined from the stomach contents of giant squid (*Architeuthis sp*.) (Deagle *et al.* 2005b) and scats from Adélie penguins (*Pygocelis adeliae*) (Jarman *et al.* 2002). Teleost fish and cephalopod prey DNA have been distinguished in Steller sea lion (*Eumetopias jubatus*), Antarctic and New Zealand fur seal scats collected from captive (Deagle *et al.* 2005a; Deagle et al. 2006; Deagle and Tollit 2007; Casper et al. 2007b) and wild seals (Casper et al. 2007a). This technique has shown potential to be an effective method for diet assessment for ASL.

A recent report to the Department of Environment and Heritage, which formed the basis for a Draft Recovery Plan for the species, detailed the impediments to growth in ASL

populations (McKenzie et al. 2005). The report identified the development of techniques to provide quantitative analyses of sea lion diets as a key research priority, with faecal DNA assessment appearing the most promising technique.

Captive feeding experiments are necessary to assess the suitability of using faecal DNA analysis to provide a quantitative estimate of prey composition in the diet of ASL. The development of these methods, which forms the basis of this research, could provide key information to assist in the recovery of the species, especially the identity of key prey species, which may have limited distributions or habitats. Additionally, we will gain a better understanding of the extent of trophic interactions and the level of which ASL target commercially important species.

Methods

Trial animals and feeding trial enclosures

Two long-term captive ASL (*Neophoca cinerea*) (one 6 year old male and one 26 year old adult female) were used in a captive feeding trial conducted at the Adelaide Zoological Gardens, South Australia, between January and February 2006. Sea lions were chosen on individual health but also on their ability to show consistency when consuming food on a regular basis.

The female and the male were housed separately during daylight hours (~ 8 -10 hrs/day) but overnight the female was housed with a third sea lion (~ 14 hrs / day). The male was displayed during daylight hours with a third sea lion but isolated in the evenings. Because the trials were conducted over the Australian summer, the main display enclosure contained a large salt chlorinated pool (11m x 8m x 2m) while evening enclosures contained a shallow pool with sprinkler system.

Trial duration and diet protocols

The adult male was fed experimental diets, which contained cephalopods and shark (novel diet) and fish (normal diet). The adult female was fed a control diet, which was entirely fish. Sea lion mass was recorded throughout the duration of the trial. Because some remains of food may be retained by captive ASL for >96 h (4 days) (Richardson and Gales, 1987; Gales and Cheal, 1993; Bodley et al. 1999), the feeding trial was

divided into 4 weekly periods. The study commenced with a 5 day period, during which novel prey were not fed to the male. Different experimental diets were then fed each week for 4 weeks. The trial ended after a 14-day period, when no novel prey were fed to the male, to assess retention times of novel prey (Figure 63).

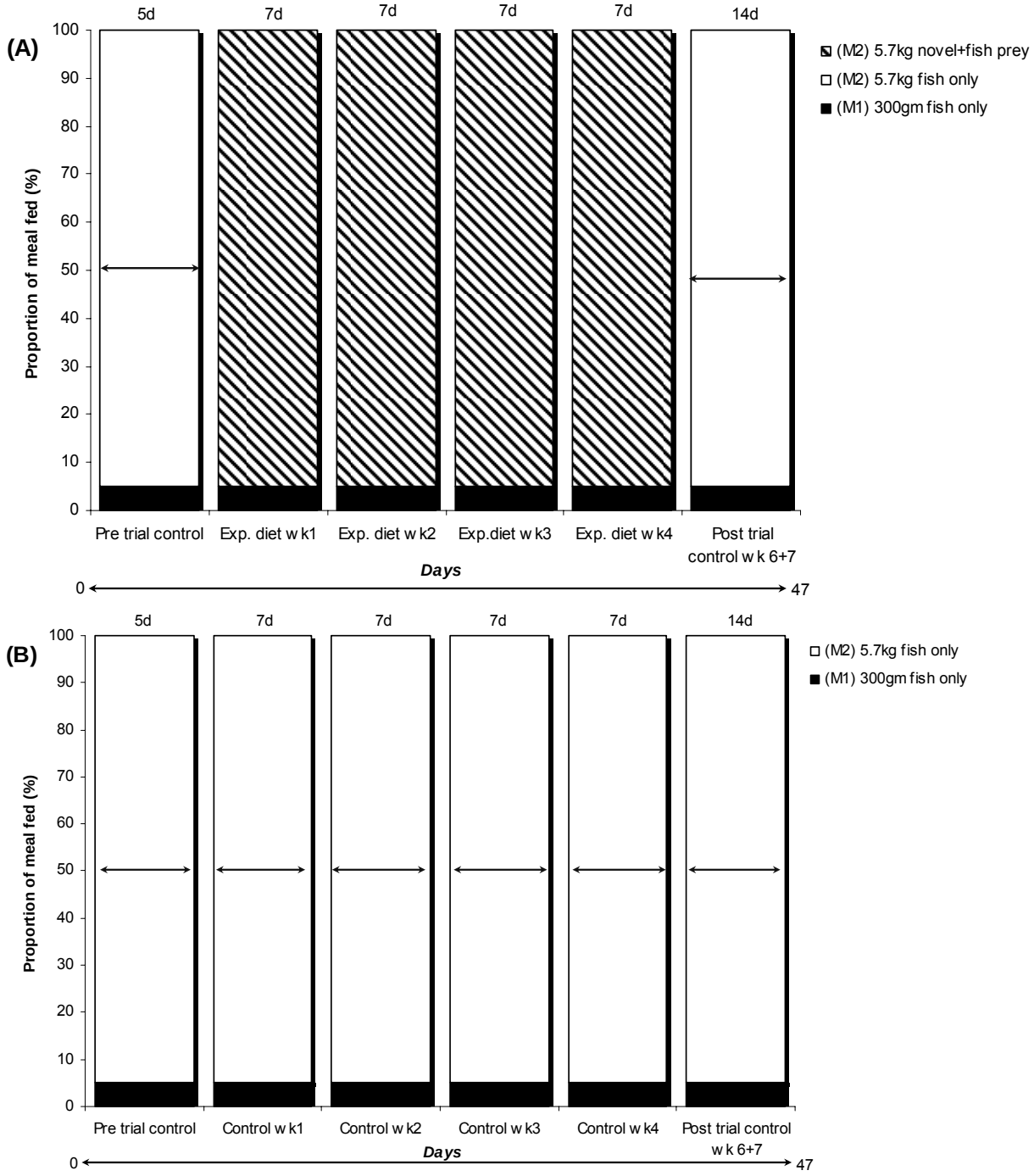

Figure 63. Feeding trial duration (days) and meals fed (%) during mornings (M1) and evenings (M2) to the male (A) and female (B) ASL at the Adelaide Zoo between January and February 2006. Arrows indicate the control periods (only fish and no novel prey). Novel prey and fish introduced into the diet (hashed columns) for 4 weeks. Values above columns are number of days that each diet was fed.

A 6 kg daily diet was fed in two allocations to each sea lion following the feeding protocols employed at the Adelaide Zoo. Meal 1(M1, ~11 am), comprised 300 g (5%) fish of the total 6 kg diet. For the adult male, meal 2 (M2, ~ 530pm) combined different proportions (by wet weight) of novel prey species and fish during the experimental diet weeks, but comprised only fish (5.7kg) during pre- and post-trial periods. The second meal fed to the female each day was fish (5.7kg) over the trial duration (Figure 64). To mark the commencement of the experimental trial diet for the male and because both sea lions interacted with a third sea lion, 6 x 0.95 ml dissolvable gelatine capsules (Surgi-Pak®) containing red (male) and green (female) corn grit diet markers (Microgrits®) were impregnated in the dietary items to ensure that scats could be assigned to the trial animals.

Tommy rough (*Arripis georgianus*) and striped perch (*Pelates octolineatus*) are commonly fed to ASL at the Adelaide Zoo and were used in the feeding trial as the normal diet. Southern calamari squid (*S. australis*: Cephalopoda) and gummy shark (*M. antarcticus*: Elasmobranchii) were used as the novel prey taxa, because they, or closely related species, occur in the diets of wild ASL (Gales and Cheal, 1992; McIntosh *et al*. 2006). Only the soft tissue of the shark was used in the experimental diets, to determine whether completely digested prey could be detected using DNA-based methodologies when diagnostic remains were absent.

Adult male experimental diet and female control diet

Each week the male was fed one of the four experimental diets; Diet A (60% squid, 30% tommy rough, 10% striped perch), Diet B (10% squid, 60% tommy rough, 30% striped perch), Diet C (10% squid, 60% tommy rough, 30% shark), Diet D (30% squid, 40% tommy rough, 30% shark) (Figure 64). Pre and post trial diet control periods contained 50% tommy rough and 50% striped perch.

The diet of the female consisted of tommy rough (50%) and striped perch (50%) for the duration of the feeding trial, which provided a means of assessing differences between DNA detection of novel prey fed to the male and quantification with different levels of diet complexity.

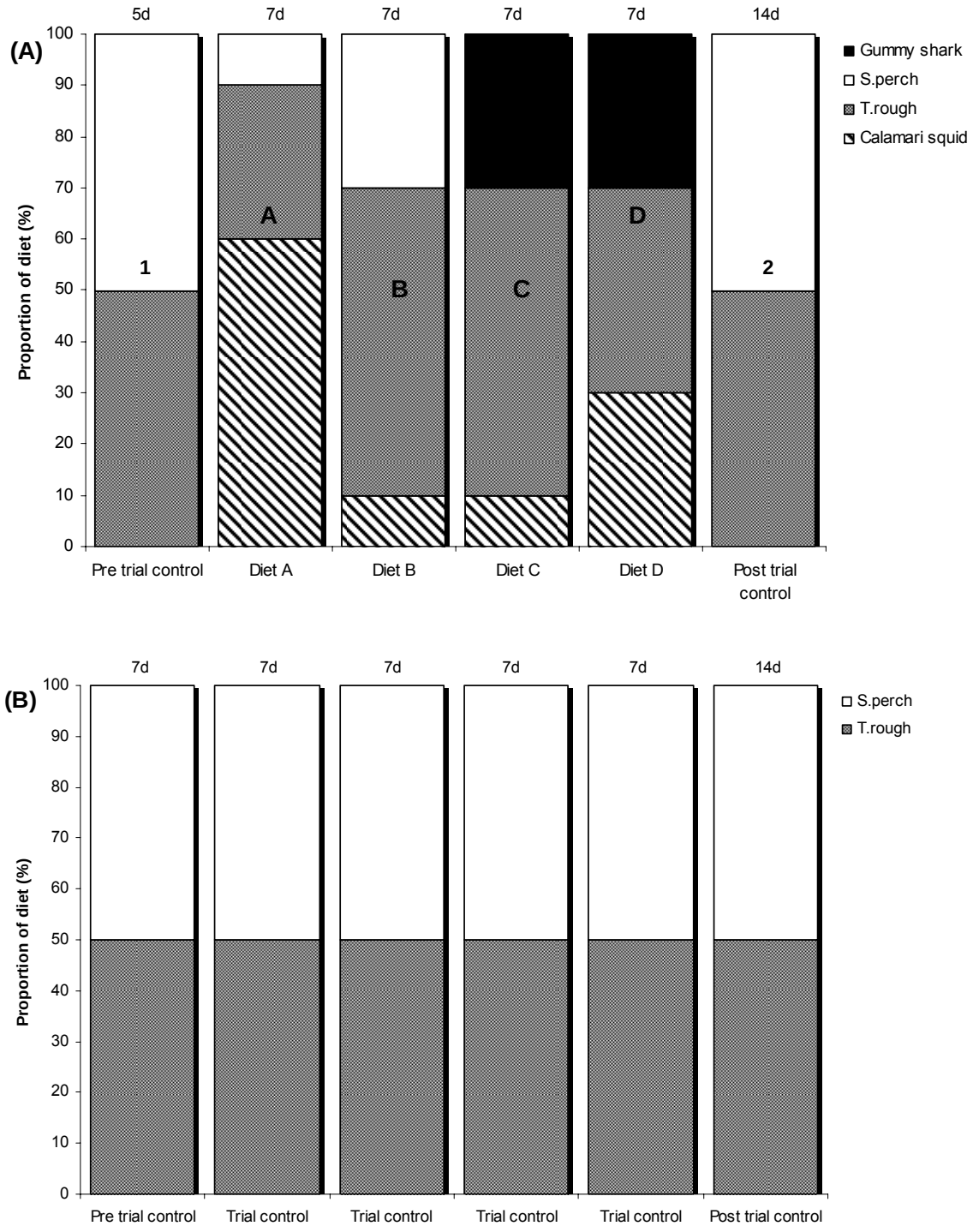

Figure 64. Prey species contributions (%) in the 6kg diet fed to the ASL male (A) and female (B). A, B, C, and D are experimental diets consisting of novel prey; squid and shark, combined with 2 fish species, including tommy rough and striped perch. The female diet (B) was tommy rough (3kg) and striped perch (3kg) in equal proportions. Columns (1, 2) are normal diets (2 fish species and no novel prey) to compare DNA detection signals of novel prey fed during experimental diets A, B, C, and D. Values above columns are number of days fed for each diet.

Collection and storage of faecal samples

Faecal samples were collected for the duration of the trial (48 days). Each morning the enclosures were checked for faecal samples and then hosed clean. Enclosures were checked for samples at every hour from 7am until 12pm and every 2 h until 530 pm. All pools were fitted with 100µm fine drain outlet filters to retain scats in pools, before the pools were cleaned each day. Scats were stored in sterile 500ml Nasco Twirl® double sealed bags, which contained a 1:4 ratio of scat to 99.7% ethanol (Murphy et. al. 2002, Vink et al. 2005). The ethanol and the scat was homogenised to preserve the DNA and stored at –20C.

For long term DNA preservation, each faecal sample was freeze-dried using a Cuddon™ Freeze-Dryer (model GP 1015FD) with shelf temperature of 40 C and final pressure of 2 Torr. This approach can significantly increase DNA amplification success rates during PCR assays (Murphy et al. 2000). The ethanol/scat slurry was homogenised, a sub sample was removed then placed into a collection cylinder to settle. With supernatant removed, samples were freeze-dried. Once dry, each sample was finely ground and the resultant scat dust homogenised dry through inversion.

Prey morphological analysis

Prey remains were isolated from ethanol/scat slurry by washing through a series of 3 nested sieves of decreasing size (smallest 100µm). Sagittal otoliths and squid beaks were stored dry and other remains were stored in plastic vials with 90% ethanol. The faecal sample collected from the male and the 3 from the female in the pre-trial period were excluded from the hard-prey analysis to ensure that prey consumed prior to the feeding trial was excluded from all analyses.

All otoliths were photographed and measured (Image pro 5.1) using a Leica digital microscope, which was calibrated at magnification of x 0.71. Where possible, otoliths were identified as being from the left or right side, identified to species and categorised according to the level of erosion (adapted from Hull et al. 1999). Categories were: 1) unrecognisable to species; heavily worn, no distinguishable features, no rostrum, no ventral crenulations, very slight or no sulcus, featureless disk, 2) identifiable to species; with slightly visible sulcus, mostly unrecognisable features, no rostrum, few visible ventral crenulations, exterior worn; 3) identifiable to species; visible and definite sulcus,

slight rostrum or not present, slight ventral crenulations but worn, 4) identifiable to species; intact sulcus, partial rostrum, ventral crenulations marginal and slightly worn, and 5) identifiable to species; pristine otolith with undamaged sulcus and rostrum, ventral crenulations pronounced. Frequency of occurrence and numerical abundance estimates were determined. Reconstructed fish mass (wet weight/kg) was calculated based on the minimum number of individuals identified in each scat, using weight data collected from fresh fish.

Faecal DNA extraction

Two DNA extraction methods were compared to isolate DNA from scats. Five faecal samples were randomly chosen and DNA from 500mg of the re-homogenised freeze-dried sample was extracted using 1) QiAmp® DNA Mini Stool Kit (Qiagen, 2007) and 2) an in-house extraction facility (SARDI, 2007). DNA extracted from faecal samples contained 2 extraction blanks (reagents only) as an internal control to screen for cross contamination. DNA yield was quantified by fluorescence of 2µl x 5 replicates of each sample using Picogreen dsDNA Quantitation reagent (Molecular Probes-Invitrogen®) on a Wallac1420 multilabel fluorometer with fluorescence set to read at 485nm/535nm for 0.1sec. Because DNA from scats is typically of poor quality (Murphy et al. 2000; Deagle et al. 2005; Deagle et al. 2006) SARDI in-house facility was used because commercial extraction kits consistently yielded 1000pg less DNA. Replicate PCR assays containing DNA from both extraction methods were tested using PCR and gel electrophoresis. DNA from 58 faecal samples (male = 28; female = 30) was extracted. Each sample was randomly given a reference number (1-58) to eliminate bias when scoring PCR products.

Prey tissue DNA extractions

DNA taken from the tissue of sharks and squid was removed from internal tissues, which could not have been exposed to foreign DNA. Samples were washed in dilute 1:20 Tween® and RNA free water and 15mg was removed for DNA purification using Qiagen DNeasy Tissue kits. A final elution of 100µl was used to increase the final DNA concentration of each sample. Sample contamination was monitored using extraction blanks while aerosol resistant filter pipette tips were used to avoid cross contamination between samples.

Primer development

Short, species-specific primer sets of <100 base pairs were constructed to amplify and quantify DNA for each dietary species in the feeding trials (Table 17). Mitochondrial DNA was chosen because it is often in higher concentration than nuclear DNA within faeces (Birky et al. 1989) and preserves well during the freeze-drying process (Murphy et. al. 2002). Sequences were aligned using the Bioedit®: Biological sequence alignment editor (Ibis Biosciences, 2007) and primer pairs constructed using ABI Primer Express V 2.0® (Applied Biosystems) with amplicon parameters set to distinguish between 50 and 100bp. The BLAST algorithm (Altschul et al.1997) was used to screen primer sets for specificity and assess the level of similarity against sequences in Genbank. Primer sequences were tested against aligned sequences in Bioedit and assessed for sequence specificity.

Primer optimisation and Qualitative Standard PCR amplification

Because faecal DNA is a combination of digested prey and the defecator's gut bacteria and exfoliated gastrointestinal epithelial cells (Albaugh et al. 1992), primer sets screened DNA for the target species and all non-target trial prey and sea lion DNA. Initial PCR assays were optimised using temperature and $MgCl_2$ concentration gradients which varied between 55 ^oC and 65 ^oC and 1.0mM and 2.5mM $MgCl_2$ (final concentration) generated on either PTC-200 or PTC-225 Peltier Gradient Block Thermal Cyclers (MJ Research).

Optimised primer sets for amplifying shark DNA, consisted of 25µl PCR reactions containing 2µl DNA template, 0.5µl 2.0mM $MgCl_2$, 2.5µl 10 x Qiagen PCR buffer, 0.2µl 1 x BSA (Bovine serum albumin), 0.5µl 0.2µM DNTP, 0.5µl 0.4µM forward and reverse primer, and 1 x Hotstar Taq polymerase (Qiagen). Thermal cycling conditions were 95 ^oC for 15 min, then 94 ^oC for 2 min, followed by 32 cycles of 94 ^oC for 10 s, 64.1 ^oC for 30 s, 72 ^oC for 30 s with final extension of 72 ^oC for 10 min. A 20µl volume for squid PCR reactions contained the same concentration and volumes of all reagents as shark PCR, except 2.5µl 15mM $MgCl_2$ (contained within PCR Buffer) was used. Thermal cycling conditions were 95 ^oC for 15 min, then 94 ^oC for 2 min, followed by 32 cycles of 94 ^oC for 10 s, 60.0^oC for 30 s, 72 ^oC for 30 s with final extension of 72 ^oC for 10 min.

Detection of PCR products was determined by electrophoresis on ethidium-bromide stained 1.5% agarose gel. Product bands were visualised using Gel-DOC UV illuminator (Bio-Rad®) supported by Quantity One-Quantitation analysis software (Bio-Rad®).

Replicates of successful PCR amplifications were performed to confirm that positive results were not due to false-positives from the first PCR attempt. All PCRs were blind scored to remove scoring bias.

Quantitative PCR amplification - Real time PCR

Quantitative estimates for the relative amount of DNA of each prey species were achieved using real-time quantitative qPCR reagent PowerSybr Green (Applied BioSystems) on an ABI prism® Real–time 7900HT sequence detection system (Applied BioSystems).

DNA reference standards (8 lambda) with known concentrations of DNA (between 0 ng/µl to 12ng/µl) were developed in triplicate and fitted to a standard curve (Figure 65). The concentration of DNA from squid and shark tissue was estimated against λ standard values and the exact DNA concentration (pg/µl) was determined (shark 5845 pg/µl, squid 33017 pg/µl) (Figure 65). The cycle threshold (Ct) values for each prey species standard curve plots were set at 0.1 determined PCR assay sensitivity for detecting DNA of shark and squid at 2fg/µl and 20fg/µl respectively.

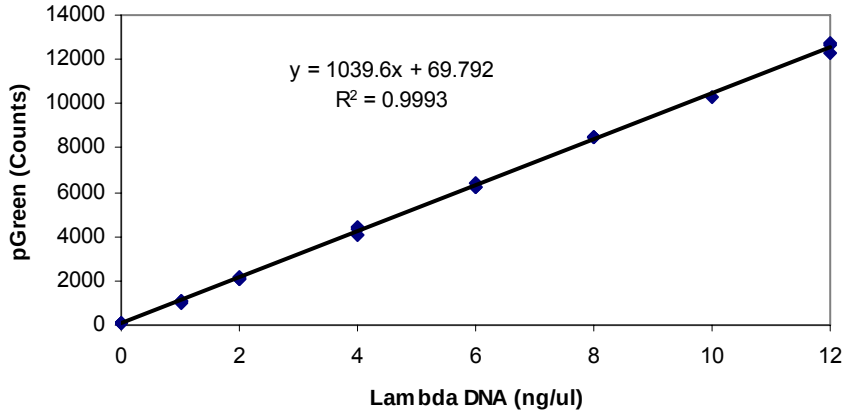

Figure 65. Standard curve from 8 Lambda (λ) DNA diluted standards (0 ng/µl to12 ng/µl) and PicoGreen counts. Series dilutions (0-200000 fg/µl) of DNA (extracted from shark and squid tissue) are quantified against the known (λ) DNA. Relative amounts of gummy shark and squid dsDNA within faecal samples are determined against these values.

Quantitative estimates of prey DNA from scats were determined using primers and real-time PCR. Power SYBR Green® contains the fluorescent marker SYBR green that binds to all double-stranded DNA (dsDNA) during PCR. Real-time PCR reactions contained 4µl faecal DNA template, and 6µl Power SYBR Green master mix (final concentration 0.4µM and 1X ABI PCR mastermix). Cycling conditions for each PCR reaction followed default Power SYBR Green protocols with activation time of polymerase at 95 ^{o}C 10 min,

followed by 40 cycles of 95 ^{o}C 15 s, and 60 ^{o}C 1 min, and a dissociation stage (95 ^{o}C 15 s, 60 ^{o}C 15 s, 95 ^{o}C 15 s) for both squid and shark primers.

Cloning and sequencing

PCR products that produced visible bands using gel electrophoresis were excised and purified using a Nucleospin® Extract II PCR cleanup gel extraction kit (Macherey-Nagel). Samples were cloned into a pGEM®-T easy cloning vector (Promega) for DNA sequencing. For each sample, 2 transformation cultures containing transformed *Escherichia coli* cells were plated and grown to generate transformant colonies. Eight white colonies per sample provided the DNA template for the SP6/T7 (vector primers) for standard (25µl) PCRs, which were designed for positive identification of the insert. Thermal-cycling conditions were 95 ^{o}C 15 min, 94 ^{o}C 1 min, followed by 29 cycles of 48 ^{o}C 45 s, 72 ^{o}C 45 s, then 72 ^{o}C 8 min, 4 ^{o}C 5 min. Cultivated bacterial cells (3 ml) were centrifuged and the remaining supernatant was removed. Plasmid DNA from the bacterial cells was purified using a NucleoSpin® Plasmid DNA purification kit (Machery-Nagel). Genetic sequences were generated at the Australian Genomic Research Facility using 5.5µl plasmid DNA combined with 2.6µl (20µM) Sp6/T7 primer.

Results

Mean body mass of male and female captive ASL fluctuated during the 47 day trial between (± 3.5 kg) and (± 5.75 kg) from the pre-trial mass of 200kg and 100kg respectively.

Prey remains from sample collections

In total, 58 faecal samples were collected and assigned to an individual seal (male = 28, female = 30).Two scats were collected intact from the main display pool and identified from diet markers as having been produced by the male. Scats were not collected from night enclosure pools. Twelve (43%) scats were collected from the male over control diets, while 3 (11%), 3 (11%), 2 (7%), and 8 (28%) scats were collected during Diet A, B, C and D respectively. Squid was increased to 30% in the final 2 days of Diet C because only one scat was collected 6 days after the initial consumption of shark. Multiple scats (2 and 3) were collected on 3 mornings during Diet D. One regurgitate containing unidentifiable tissue was collected. Highly eroded vertebral processes, eye lenses and

scales of fish were present in 23 (82%) of male scats, but could not be identified to species and were not used in the analysis.

Only one fish otolith (<1%) was recovered from 6674 otoliths consumed by the female. Other hard remains were limited to 2 unidentifiable vertebrae, scales and fragments of eye lenses. Although 61% (17/28) of scats from the male contained otoliths, only 2% of all otoliths consumed (110 out of 5170) were recovered. Thirteen otoliths were identified as striped perch, 70 were tommy rough and 27 (25%) were heavily eroded and could not be assigned to species (Table 16). There was no relationship between the number, and type of fish species fed, to the number of otoliths recovered (r^2 =.001, n = 7, *P* = 0.941), while otolith recovery rates were not related to the size of otoliths ingested (χ^2 = 0.077, *df* = 1, *P* = 0.782). Reconstructed mass recovery estimates (% wet weight/kg) for the fish component of the diet were low for both species (~1% recovered of mass ingested), except diet A (4%) (Table 18). Squid beaks were not recovered from scats from the 116 ingested.

Table 16. Frequency of occurrence (FOO) and numerical abundance (NA) of diagnostic hard remains (otoliths) identified from scats (*n* = 28) collected from the male ASL during feeding trials. Diagnostic remains from squid were not recovered.

		FOO		NA	
Common name	**Species**	***n***	**%**	***n***	**%**
Tommy rough	*Arripis georgianus*	13	46.4	70	63.6
Striped perch	*Pelates octolineatus*	5	17.9	13	11.8
Unknown otoliths	Not identified	10	35.7	27	24.6
Calamari squid	***Sepioteuthis australis***	**0**	**0**	**0**	**0**
Gummy shark	***Mustelus antarcticus***	**-**	**-**	**-**	**-**

Primer assessment

Primer amplicon size affected amplification success rates for detecting gummy shark and squid DNA from scats. Six primer sets (>100bp) were optimised for detecting gummy shark and squid DNA but no products were amplified. Small primer sets (<100bp) designed to detect shark and squid DNA were successful (Table 17).

Table 17. PCR primers tested in standard and real-time quantitative PCR for detecting squid and shark in sea lion scats. Successful primer sets (+) in bold. All other primers amplified target taxon DNA but not prey DNA from sea lion faeces (-).Shark refers to *M. antarcticus* and squid refers to *S. australis*. [a] Unpub. data current study, [b] Jarman et al. (2006), [c] Deagle et al. (2005), [d] Casper et al. (2007), [e] Jarman et al. unpub. data.

Primer name	Target species	Product size (base pairs)	Gene region	PCR amplification
Sepio71cf[a]	*S. australis*	71	mtDNA	+
Sepio71cr	*S. australis*	71	mtDNA	+
Gummy71cf[a]	*M. antarcticus*	71	mtDNA	+
Gummy71cr	*M. antarcticus*	71	mtDNA	+
CephMLSf1[b]	Squid	212-244	mtDNA:LS	-
CephMLSr1	Squid	212-244	mtDNA:LS	-
Squid 28Sf[c]	Squid	180	nDNA:28S	-
Squid 28Sf	Squid	180	nDNA:28S	-
Squid 28Sf[d]	Squid	100	nDNA:28S	-
Squid 28Sr	Squid	100	nDNA:28S	-
Gummy207f[a]	*M. antarcticus*	207	mtDNA:C01	-
Gummy207r	*M. antarcticus*	207	mtDNA:C01	-
Gummy207fb[a]	*M. antarcticus*	207	mtDNA:C01	-
Gummy207rb	*M. antarcticus*	207	mtDNA:C01	-
Actinopterygii f[e]	Fish (ray finned)	-	mtDNA:SS	-
Actinopterygii f	Fish (ray finned)	-	mtDNA:SS	-

Identification of novel prey- PCR amplification success rates

Standard and quantitative PCR were successful in detecting and quantifying the presence of squid and gummy shark DNA in faeces that were collected from the male. Of the 16 scat samples collected over the experimental diet period (Diet A, B, C, D), 16 (100%) successfully amplified squid DNA during periods where squid was present in the diet, while 9/9 (100%) scats amplified shark DNA when shark was fed (Table 19). Differences in amplification success were apparent between PCR type; standard PCR produced lower detection rates for gummy shark (8/9 (89%)) and squid (14/16 (88%)) (Figure 66), while real-time quantitative PCR (qPCR) amplification were 100% successful in detecting DNA from each novel prey species from scats during periods where novel prey was fed (Table 19).

A low quantity of squid DNA (52 fg/µl) was detected by qPCR in a scat 48 hrs after the final ingestion, while gummy shark DNA occurred in 2 scats collected concurrently 24 hrs after its final consumption. Squid qPCR amplifications detected low amounts (45 -117 fg/µl) of squid DNA from three scats collected during the last 4 days of the post trial control (fish only) period, but 6 scats collected prior to these did not produce positive amplification for squid. Sequenced products for all successful shark and squid PCR amplifications complemented target DNA of both prey taxa. Negative controls (reagents only) from PCRs did not produce positive PCR products.

Quantitative estimates and diet comparison

Quantitative PCR performed on scats provided estimates of the amount of mitochondrial DNA content, but these results were highly variable relative to the amounts of novel prey fed. For squid, DNA estimates ranged from 380 to 9276 fg/µl (Table 19), with means of 3215 ± 1562, 861 ± 423.24,1027 and 5208 ± 2762.44 (Diet A, B, C and D respectively). Scats collected during periods when squid was fed at 60% (Diet A) and 30% (Diet C) yielded higher estimates of squid DNA than scats from diets containing only 10% squid and the difference either approached significance or was significant (Diet A: one-way ANOVA; $F_{1,5}$ =6.187, P = 0.055) (Diet C; one-way ANOVA; $F_{1,10}$ = 6.923, P = 0.025). There was a positive correlation (0.01 level) in squid DNA yield when the mass of squid increased in the diet (Spearman two-tailed correlation; r^2=0.86, P = 0.003), but the quantity of DNA detected when contributions of squid in the diet were 30% and 60% were not significantly different (one-way ANOVA; $F_{1,10}$ = 1.190, P = 0.001).

Quantitative estimates from scats for gummy shark DNA were highly variable (range 106-3411 fg/µl; 1258 ± 1079.07 (mean + s.d) diet C and D respectively) but comparisons of shark DNA quantity were not assessed for different percentage contributions because it was fed at 30% for only diets C and D. Two samples collected consecutively on day 26 and 27 produced significantly higher amounts of gummy shark DNA (U = 0, *df* = 1, 8, P = < 0.001) than all other quantitative estimates for gummy shark detected in scats (Table 19). Shark DNA estimates were correlated to the ingestion of shark (Spearman two-tailed correlation; r^2 = 0.59, n = 7, P = 0.043).
DNA yield was significantly higher for squid (5208 ± 2762.44) than gummy shark (1258 ± 1079.07) when squid and gummy shark were fed as equal proportions (30%) during Diet D (one-way ANOVA, $F_{1, 16}$ = 15.963, P = < 0.001).

Table 18. Total mass ingested (TMI), mass recovered (MR) (wet weight/kg) and percent (%) of mass recovery reconstructed for the total number of fish recovered (total otoliths recovered divided by 2) from (n) scats for Diet A, B, C, D and controls. Post trial controls were pooled. Mass estimates were reconstructed from weight length data collected from 100 fresh specimens of each fish species.

	Pre control (*n* =2)		**Diet A** (*n* =3)		**Diet B** (*n*= 3)		**Diet C** (n=*2)*		**Diet D** (*n* =8)		**Post control** (*n* =10)	
Species	t.rough	s.perch	t.rough	s.perch	t.rough	s.perch	t.rough	s.perch	t.rough	s.perch	t.rough	s.perch
TMI (kg)	12.6	4.2	12.6	4.2	25.2	12.6	18	0	21.6	0	42	42
MR (kg)	0	0	0.46	0.18	0	0	0.17	0	0.23	0	0.75	0.45
% Recovery	**0**	**0**	**3.7**	**4**	**0**	**0**	**<1.0**	**NA**	**1.1**	**NA**	**1.8**	**1**

* Tommy rough (t.rough)

* Striped perch (s.perch)

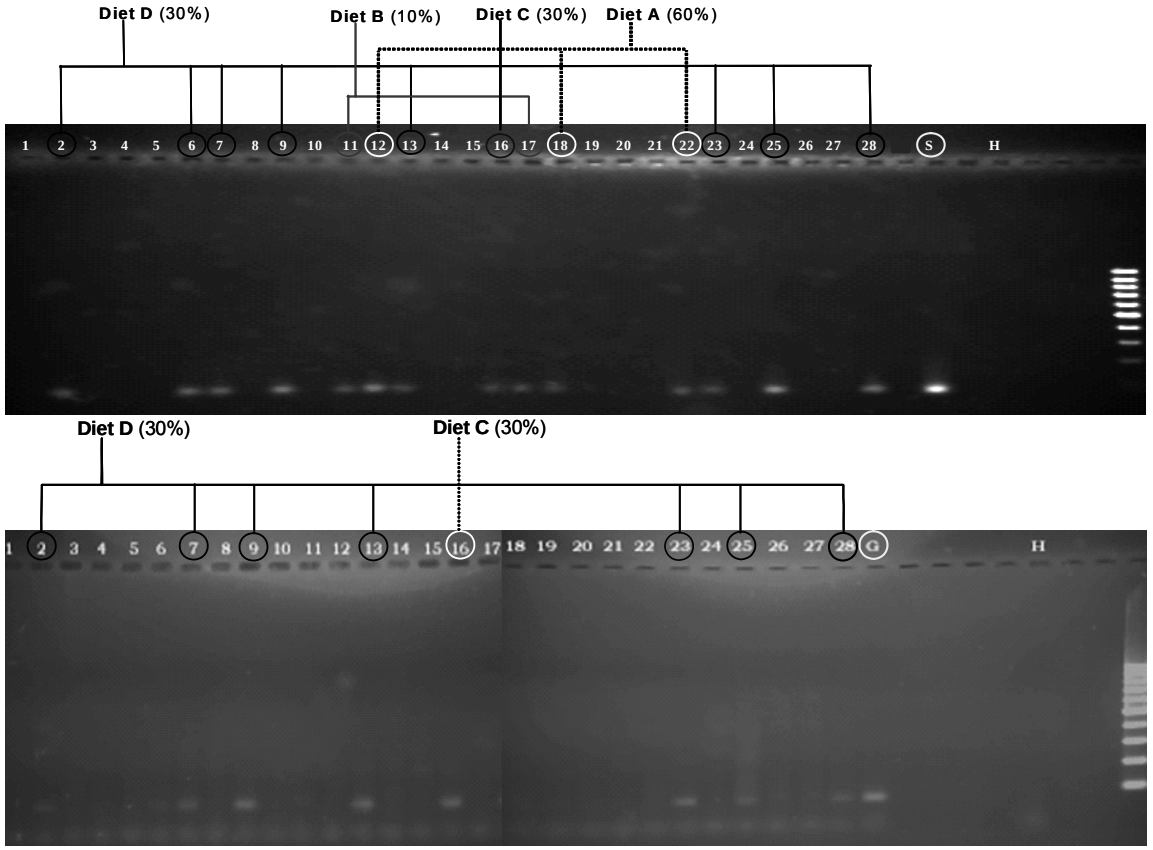

Figure 66. Agarose gel of PCR amplification products showing detection of mtDNA by primers Sepio71c and Gummy71c from DNA recovered from 28 male faeces. Scats represented by numbers 1-28 were not tested in chronological order of collection, to remove scoring bias. Circled numbers denote scats collected during each diet week (A = 12, 18 and 22, B = 11 and 17, C = 16, and D = 2, 6, 7, 9, 13, 23, 25, 28) and numbers in parenthesis are the contribution (% wet weight/kg) of novel prey fed during the 6kg daily diet. Positive controls of target species are represented by circled S (top plot) and circled G (bottom plot). H indicates negative controls for PCR reaction testing for cross contamination.

Table 19. PCR amplification results (+) presence (-) absence for standard and real-time quantitative PCR (qPCR) values (fg/µl) for the detection of squid and gummy shark mtDNA from scats collected from a captive male ASL during feeding trials at the Adelaide Zoo. Scat samples represented in chronological order of collection while trial day (n) indicates actual day of collection for duration of 48 days.

		Gummy shark		***Squid***	
Diet period	**Scat No. (trial day)**	**Std. PCR**	**qPCR (fg/µl)**	**Std. PCR**	**qPCR (fg/µl)**
Pre control	1 (5)	-	0	-	0
(0% squid, 0% shark)					
Diet A	2 (8)	-	1	+	4959
(60% squid, 0% shark)	3 (11)	-	0	+	3732
	4 (12)	-	0	+	1232
Diet B	5 (14)	-	8	+	2938
(10% squid, 0% shark)	6 (16)	-	3	+	380
	7 (18)	-	3	+	1177
Diet C	8 (20)	-	2	-	1027
(10% squid, 30% shark)	9 (26)	+	3411	+	1129
Diet D	10 (27)	+	2072	+	1746
(30% squid, 30% shark)	11 (29)	+	409	+	9276
	12 (29)	+	1062	+	4079
	13 (29)	+	1532	+	4254
	14 (30)	+	360	+	5474
	15 (30)	+	466	+	7965
	16 (33)	-	106	+	5289
	17 (33)	+	1907	+	7659
Post control	18 (35)	-	3	-	52
(0% squid, 0% shark)	19 (36)	-	1	-	0
	20 (37)	-	1	-	0
	21 (38)	-	1	-	0
	22 (39)	-	2	-	0
	23 (40)	-	1	-	0
	24 (41)	-	11	-	6
	25 (43)	-	1	-	45
	26 (46)	-	0	-	117
	27 (47)	-	0	-	57
	28 (48)	-	0	-	0

Discussion

Morphological assessment of hard-prey remains

Morphological identification of undigested prey remains recovered from dietary samples is a widely used and effective method to assess the diet of many marine predators, including fish (Cortés, 1997; Cortés 2000; Simpendorfer et al. 2001), seabirds (Gales and Pemberton, 1990; Cullen et al. 1992; Clausen and Putz, 2002) and seals (Gales et al.1993; Gales and Pemberton, 1994; Goldsworthy et al. 1997; Page et al. 2005). Most studies based on this methodology are constrained by the probability that the recovery of the prey remains is biased toward prey species with robust skeletal structures. This method may underestimate the consumption of soft bodied species and species with reduced digestive resistance (Harvey, 1989; Fea and Harcourt, 1997; Lalas, 1997; Needham, 1997; Orr and Harvey, 2001; Staniland, 2002; de Bruyn et al. 2003; Deagle et al. 2005b). Studies that identify prey remains from faeces incorporate these inherent biases, but the method remains a simple non-intrusive means of obtaining large quantities of dietary information.

Morphological analysis of captive ASL scats identified very few of the prey ingested and was limited to the robust diagnostic remains of the fish. Otolith recovery in scats collected from the experimental male was highly variable (1-22 per scat), and because identifiable fish remains were limited, their relative importance in the captive diet was highly underestimated. Given that otolith recovery rates were not related to the number of fish per species fed or otolith size ingested, the higher occurrence of tommy rough otoliths in scats collected from the male may be explained by inter-specific differences in the digestibility of the fish which can result in under or overestimates of the prey consumed (Jobling, 1987; Cotrell et al.1996; Tollit et al. 2003). Hard-prey analysis did not however, produce quantitative information of the fish ingested by the control female sea lion, even though a mixed diet of 3337 fish were fed over the duration of the trial. Low otolith recovery rates in captive male and female ASL fed integrated diets were noted by Gales and Cheal (1992), who also highlighted the potential for individual and/or sex-specific biases in the recovery of cephalopod remains from scats. Similar to their study, otolith recovery rates from this study were lower for the female (0.0001%) than the male (2%) but a disparity was shown between the recovery rates of cephalopod remains in scats from male (9%) and female (98%) sea lions. This contrasts our study, as morphological analysis did not recover squid beaks even when they were the

predominant prey species by mass in the diet of the male. Even though outputs of cephalopod remains are highly variable between captive otariids (Tollit et al. 1997; Orr and Harvey, 2001), this study is the only otariid captive diet study we are aware of that has not recovered cephalopod beaks from a feeding trial. Similar extremes in low recovery of prey remains observed in captive otariids have also been documented by Casper (2007b) for *A. forsteri* and *A. tropicalis* and Gales and Cheal (1992) for *N. cinerea*.

The low recovery of squid beaks in scats may be explained as a function of gastric retention whereby larger cephalopod beaks are retained within the stomach through an inability to pass through the narrow pyloric region, into the small intestine and lower colon (Richardson and Gales, 1987) or alternatively, remains are destroyed in the stomach by the presence of gastric stones (Needham, 1997). Stomach and regurgitate diet analysis studies on ASL support this theory as they have shown that cephalopod hard parts, among other larger hard prey remains are retained within the stomach (McIntosh et al. 2006b, Gales and Cheal 1992). Gales and Cheal (1992) indicated that the probability of recovering cephalopod beaks from the scats of captive ASL was related to their size; larger beaks were absent in female scats and few (9-16%) were present in scats of the male. Consistent with this trend observed in many wild otariid seals and highlighted in free living ASL by McIntosh et al. (2006), it is reasonable to suggest that the absence of cephalopod beaks in scats collected from this study were likely retained within the stomach of the adult male, as we found no evidence over the duration of the trial of any egested beaks in the single regurgitate or scats.

The marked differences of hard prey recovery of fish remains between male and female ASL in our study may be related to the differences observed in the activity levels of the two animals. Tollit *et al.* (2003) observed periods of inactivity in captive fed Steller sea lions (*Eumetopias jubatus*) resulted in long retention rates of fish prey remains, while physical activity induced faster excretion. Similarly, Helm (1984) found that captive fed pinnipeds defecated during higher levels of activity such as swimming, while Dellinger and Trillmich (1987) recovered fewer experimental beads fed to sea lions prior to events of swimming than periods of confinement. This study observed a high level of activity by the male; each day consisted of continual swimming in a large deep pool, while the female was primarily inactive. Even though the number of scats collected from male and female was similar, elevated activity by the male may have generated higher otolith recovery rates by stimulating the movement of digesta through the gastrointestinal tract that facilitated the faster voiding of prey remains. Because prey digestion occurs predominantly in the stomach of pinnipeds (Frost and Lowry, 1980) inactivity of the

female possibly induced a higher level of digestion whereby skeletal structures were exposed to gastric acids for longer periods than the male accounting for their absence in the scat samples.

If our morphological analysis provided the primary source of evidence to infer diet about ASL from this captive study, it would be interpreted that captive sea lions from these trials consume limited number of tommy rough and striped perch and do not consume squid or gummy shark. Even though discrepancies were found in our morphological analysis, this method does provide some qualitative data of the prey species consumed. However, because prey consumption by free foraging seals does not guarantee that identifiable remains are consumed, care must be taken when applying this dietary assessment technique to wild ASL scats as the biases exhibited in this study can impose significant implications when interpreting prey frequency and abundance from dietary remains.

DNA assessment of diet – Standard PCR and quantitative PCR

This is the first study to assess the suitability of molecular based DNA analysis on scats collected from ASL. This study has shown that DNA analysis is the most reliable of the two methods tested, in detecting the presence of specific prey species that would otherwise not have been detected using morphological analysis.

Our DNA analysis was based on the mitochondrial DNA detection of squid and shark novel prey species consumed in the diet by captive adult male ASL. Molecular DNA-based analysis detected and quantified the relative proportions of each novel prey species from scats, when hard remains were completely absent.

The results from our PCR experiments suggest that DNA extracted from ASL scats was highly degraded because we could not detect DNA from prey unless short DNA sequences were targeted of less than 100 base pairs in size. We tested a number of target sequences >100bp in length for each novel prey and fish prey taxa on scats, and although we found no flexibility in the robustness of the DNA quality or DNA fragment target size, we believe that detection is reliable as even prey biomass at 10% of the diet could be detected. Casper et al. (2007) noted similar results to our study, but used nested PCR DNA-based detection to identify prey in scats from captive sub-Antarctic and New Zealand fur seals fed integrated diets. They surmised that by targeting smaller fragments of prey DNA (100bp) in a matrix of low quality DNA template may have

contributed to their higher detection rates of squid DNA from scats, while larger target DNA fragments of 200 bp were less successful. Frantzen et al. (1998) has also shown from their DNA-based faecal diet study on primates that success of PCR amplification is likely to increase with a decrease in target product size using short DNA sequences (100-200bp) because longer DNA fragments are less likely to survive the digestive process. Other studies using molecular DNA-based assessments of diet have utilised small product sizes to target prey DNA in faeces (Kohn et al. 1995; Farrell et al. 2000; Jarman et al. 2002; Purcell et al. 2004; Deagle et al. 2005b; Casper et al. 2007b), but in contrast to these, our study and one other DNA diet assessment of wild Antarctic fur seals scats by Casper et al. (2007a) have targeted prey sequences less than 100 bp. Using larger DNA target sequences for faecal diet assessment could lead to substantial differences in the frequency of detection of important prey species DNA using these methods given that DNA template quantity and quality is likely to be compromised (Taberlet et al. 1999).

Qualitative DNA assessments using standard or quantitative PCR to determine the initial detection time or day of origin of the novel prey DNA being detected was unclear for each prey species because scats were produced sporadically and our sample size was relatively small. Initial detection time of shark DNA was constrained by the absence of scats during the first week after its introduction into the diet while squid was only detected ~24-36hrs after its initial ingestion because samples were not produced for the first two days following its introduction. These estimates of initial detection time were also proximal because of the limited access to scats overnight. Given that both our PCR assays were highly sensitive and could detect extremely low quantities of gummy shark (2 fg/µl) and squid (20 fg/µl) DNA, the final detection times could not determine the day of origin for the prey DNA being detected but could determine how long prey persist in the gut.

We did find that detection success rates for squid and shark decreased rapidly after the final ingestion of either species which supports the rapid passage rates documented for ASL (Richardson and Gales, 1987; Bodley et al. 1997). Our results demonstrate that successful DNA detection is limited to a maximum of 48hrs after the final ingestion after which the quantity and potential quality of prey DNA become significantly reduced or absent. No scats were collected the day following the final ingestion of either novel prey, so estimates of the rate which larger amounts of prey DNA persist in the digestive tract of ASL remains unclear. Our final DNA detection times of squid were similar to other otariid faecal DNA studies using these techniques. Casper et al. (2007a) found that DNA

detection of squid was limited to a maximum of 32hrs after the final prey ingestion by captive sub-Antarctic (*A. tropicalis*) and New Zealand (*A. forsteri*) fur seals, while Deagle et al. (2005) used quantitative PCR and detected prey DNA 48hrs after prey was fed to captive Steller sea lions. Two possible explanations for these results are that 1) prey DNA is voided rapidly in active ASL, or 2) DNA within the gut degrades rapidly so that DNA detection after defecation is highly reduced. This has significant implications when samples are collected from wild ASL colonies, as DNA extracted from scats may only represent the most recent days of prey ingestion rather than prey consumed over foraging trips distant from the colony.

The detection of small proportions of squid DNA (45-117 fg/µl) in 3 scats on conclusion of our trial may lend support to the elevated stomach retention rates of prey observed in pinnipeds. Even if all remains were voided from the small intestine from the adult male, remnant DNA from remains trapped and broken down in the stomach have the potential to be detected weeks later. This hypothesis is plausible, given that ASL have shown high retention of larger prey remains within their stomachs and squid beaks from our morphological analysis from this study were absent.

We compared the application of standard and real-time quantitative PCR to determine whether detection success of prey DNA from scats would be significantly different. Because small amounts of target DNA from scats cannot always be detected using standard PCR methodologies, fluorescent markers such as SYBR green can increase the detection rate, and generate a quantitative value for the relative amounts of DNA present in each sample. Quantitative PCR assessment of DNA from scats was a more reliable DNA detection method than standard PCR because it produced a higher DNA detection success rate in all scats containing the novel prey, and could detect some prey DNA 48hrs after its final ingestion where standard PCR could not. Standard PCR success rates were 88% for shark and 89% for squid, with at least one PCR assay for each primer not detecting either prey species within a scat collected during the experimental diets. Detection was limited by poor visualisation of the PCR products or low estimates of dsDNA, most likely as a result of the surprisingly low levels of amplified DNA in each scat even after PCR amplification. This may suggest that our initial DNA template prior to PCR was in extremely poor condition which could be attributable to the digestive processes of ASL.

We assessed quantifying novel prey DNA to determine if the relative amounts of prey recovered in faeces were proportional to their mass contribution in the diet. Quantitative

PCR is useful for determining prey contributions in pinniped diet studies when identification of hard-parts is ambiguous (Deagle et al. 2005; Deagle et al. 2007). In our study, all scats containing DNA from novel prey were quantifiable, but the relative amount of DNA varied markedly for each prey species between all scats. Scats that were collected within the same experimental diet also showed significant differences, but these results are not surprising given that digestion and subsequent voiding of prey is not uniform. Nevertheless, if the amount of novel prey DNA detected in scats could be directly attributable to each diet, when scat qPCR measurements were pooled for each diet, larger contributions by mass of squid (60%, 30%) consistently produced higher DNA estimates, which were statistically different from lower DNA estimates produced from scats when the diet contained small mass contributions of squid. We did observe that DNA content increased in scats when the consumption of the novel prey species increased, but the relationship for shark was weaker than squid primarily because shark was only fed at one mass contribution throughout the trial and the number of scats collected after its initial ingestion became highly infrequent.

Our qPCR values did not indicate a difference between the two higher consumption rates of squid in the diet, but this may have also resulted from the low number of replicates of scat samples collected when squid was fed at it highest mass contribution at the beginning of the trial. Our DNA estimates are consistent with the results from DNA recovered and quantified from captive Steller sea lion scats as noted by Deagle and Tollit (2007). The quantification of prey DNA from faeces collected from their captive study also showed massive variation in DNA copy number between 3 fish species fed, even when prey contributions remained constant throughout their feeding trial. They suggested that prey specific DNA-biases associated with DNA survival were a contributing factor affecting DNA recovery, which may overestimate or underestimate actual importance of prey taxa in the diet.

Our quantitative estimates did show significant higher amounts of DNA recovered for squid than shark when fed in equal proportions by mass in the diet. This difference was not likely a result of our primers targeting different prey DNA fragment size as we removed this bias by designing primers to detect identical sequence lengths for each novel species. Alternatively, even though increased PCR amplification success rates can sometimes remain unexplained, the elevated yield of squid DNA may not be explained by the PCR efficiency rates, as qPCR efficiency for detecting and quantifying gummy shark DNA was 10% higher than squid. These differences may be explained in relation to the concentration of DNA in the different tissue types represented by each of the novel

prey species, the ability of each tissue's cells and DNA to survive the digestive process or a combination of both. Cephalopods generally exhibit hyperplasic exponential tissue growth over a short life span compared to elasmobranchs that demonstrate slow growth rates and size plateaus at maturity (Moltschaniwskyj, 1994; Cortes, 2000; Frisk et al. 2001,). Given that whole squid containing a diverse range of tissue was fed compared to only the muscle tissue of mature shark could increase the likelihood of detecting larger amounts of DNA if more DNA is present. Our prey tissue DNA extractions used for positive controls did yield significantly higher concentrations of squid DNA than shark DNA from the initial tissue samples. These differences are noteworthy given that Deagle and Tollit (2007) also found considerable differences in the relative proportions of DNA in prey tissues when compared to mass proportions. These potential biases require further investigation.

Conclusions

The application of molecular DNA assessment of scats, collected from captive ASL is an effective method to identify ingested prey taxa, even when hard remains are not present in scats. For wild ASL colonies, this will be useful technology as preliminary morphological analysis of scats from some colonies has revealed limited representation of diagnostic skeletal structures which has hindered the taxonomic identification of the important prey in the diet. The sensitivity of quantitative PCR assessment has the advantage of detecting minimal quantities of target DNA in faecal samples that otherwise may not be detected by standard PCR, but these outcomes are highly dependant on the quality of DNA template and the size of the target DNA sequences.

Furthermore, prior knowledge of key taxonomic groups and species such as those data from traditional diet analysis techniques will provide greater resolution for the potential prey targets of DNA analysis, which otherwise would be difficult to target and time costly if DNA-based methodologies are used alone. Molecular DNA techniques provide further insight into information on dietary preferences and trophic relationships. For ASL its application deserves further investigation, because the consumption of the major prey species can be determined using this non-invasive approach.

RECOMMENDATIONS FOR FURTHER RESEARCH

As detailed in previous sections, results from the risk assessment clearly demonstrate that the potential risk to ASL from bycatch in the gillnet sector of the SESSF and the SARLF are significant and needs to be mitigated.

Spatial management options could be investigated to assess how effort and catch could be reallocated to reduce the impact of bycatch on high-risk ASL subpopulations. Enhanced spatial tools for risk assessment will be required if spatial management of fishing effort is to become a management strategy for mitigating ASL bycatch in the demersal gillnet fishery. Further development of such tools are required, because current models are limited by the absence of data on the foraging movements of sea lions in some high-risk regions, as well as the absence of accurate fishing effort data. Further satellite tracking of ASL at subpopulations identified as high-risk should be undertaken to improve the accuracy of spatial foraging models.

In 2006, demersal gillnet fishers were required to record the latitude/longitude positions of each net-set, and from July 2007, all vessels will be fitted with satellite-linked vessel monitoring systems that will significantly improve the resolution of fishing effort. Following improvements of these data sets, it is recommended that bycatch probabilities be re-estimated and used to model the benefits of different spatial-management scenarios that could include area-closures, and reductions or redistributions of fishing effort. Improved fishery observer coverage will be essential to provide real estimates of bycatch rates and support for spatial-closures in regions of high-risk. They will also be essential to provide ongoing performance measures to managers about the effectiveness of spatial management approaches adopted in reducing bycatch to sustainable levels, and ensuring it does not threaten the viability of any subpopulation. Ongoing abundance estimates of ASL subpopulations will be the critical performance measure of spatial management in the fishery.

This study demonstrates the feasibility of quantifying prey DNA in the faeces of ASL and provides an assessment of using this data to obtain quantitative diet composition data.

BENEFITS AND ADOPTION

The benefits of adopting the recommendations detailed in this report and in supporting future research will be:

- the development of, and industry and management adoption of mitigation options to reduce seal bycatch in both the SARLF and gillnet sector of the SESSF
- addressing the outstanding ESD recommendations detailed in fishery ESD assessments
- mitigation of the key threatening process identified in the ASL Draft Recovery Plan
- recovery of the ASL, and potential future delisting of the species as *Threatened*.

The major beneficiaries will be the gillnet sector SESSF and SARLF, natural resources managers (PIRSA Fisheries, AFMA, Commonwealth DEH, SA DEH), fisheries and marine mammal biologists and the Australian community. All will benefit from the knowledge of the significance and role of fishery bycatch on seal populations. Furthermore, future development of the recommendations will assist in:

- implementing ESD objectives in the southern rock lobster and southern shark fisheries
- environmental accreditation to enhance market opportunities for the fisheries,
- the recovery of the ASL and
- achieving benchmarks in ecosystem-based fisheries management.

The use of genetic analysis of faeces for studying the diet of ASL allows assessment of potential biases from traditional approaches. DNA analyses can also provide improved taxonomic identification of prey and can be used to address questions beyond the scope of traditional methods of diet analysis. Such information is required to understand their key prey species, habitats and trophic interactions with fisheries.

FURTHER DEVELOPMENT

An FRDC proposal developed from the findings of the pilot study has recently been approved for funding (2007/041). It will form a comprehensive research and development program to develop mitigation options to manage seal bycatch issues in the SARLF and gillnet SESSF.

The proposal was developed with extensive stakeholder consultation. Findings of the pilot study, a report to FRDC (Goldsworthy and Page 2007) and the objectives and details of the aims of the FRDC project being developed were presented to both the southern and northern zone FMCs of the SARLF, and to the GHATMAC. Ongoing consultation is progressing with PIRSA Fisheries, AFMA, Commonwealth and SA DEH and SharkRAG.

Assessment of the presence/absence and importance of several commercially-fished species in the diets of wild ASL has commenced using faecal/enema samples obtained from a range of colonies and South and Western Australia.

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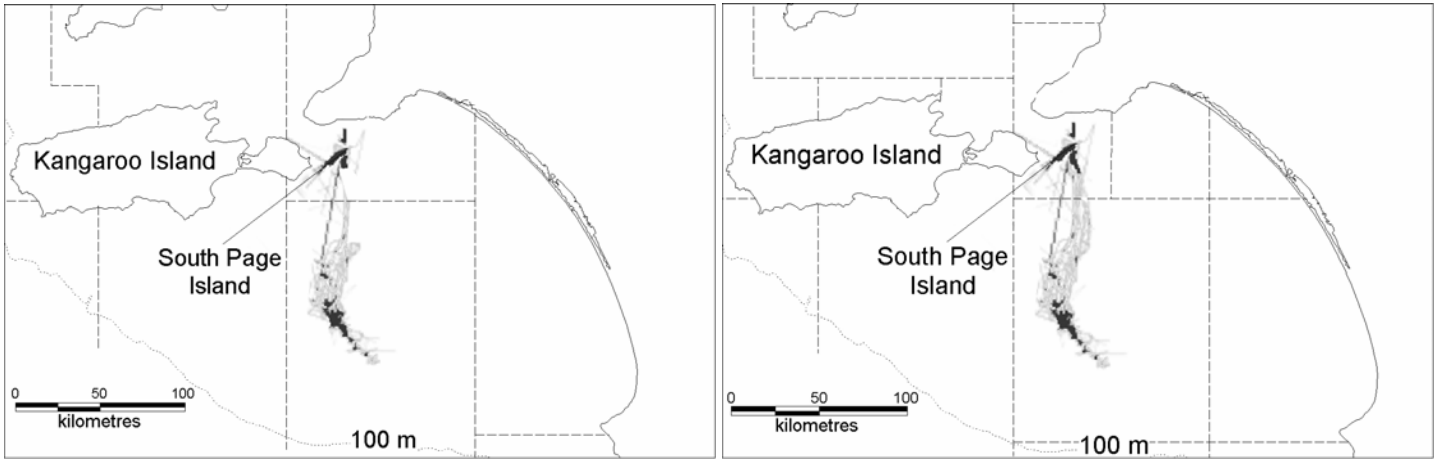
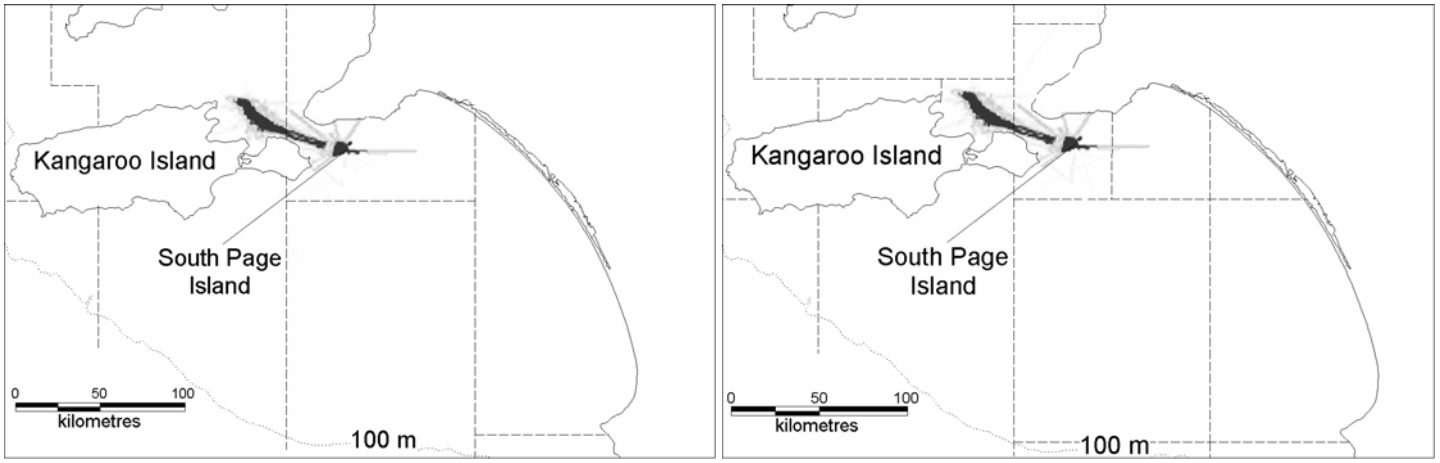
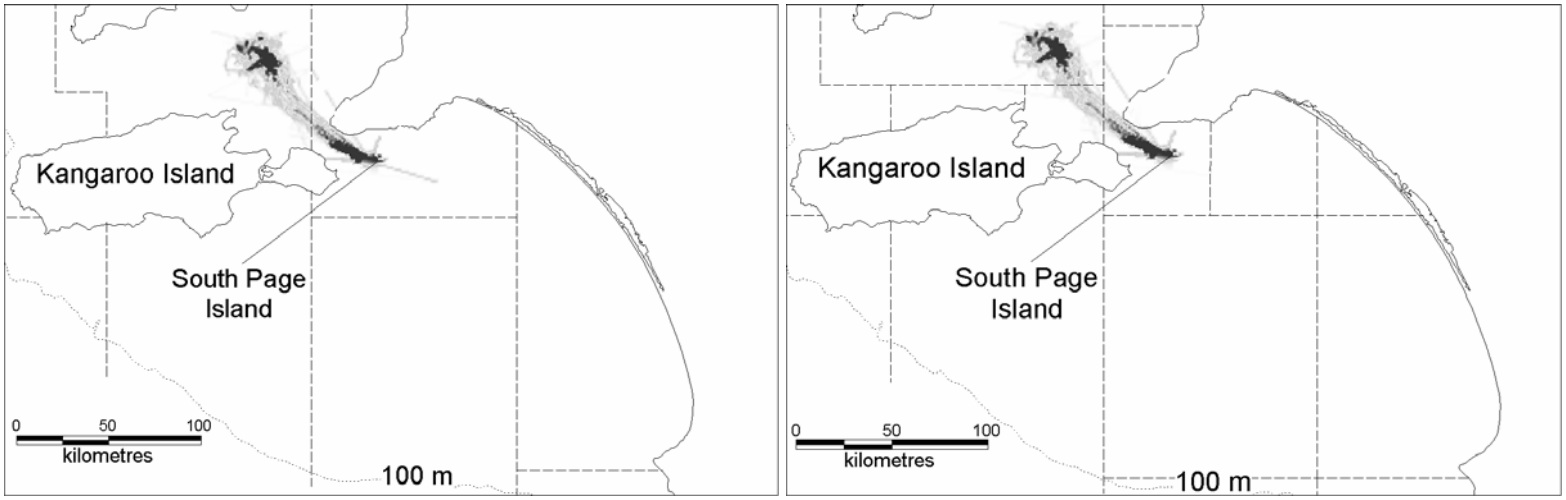
APPENDIX



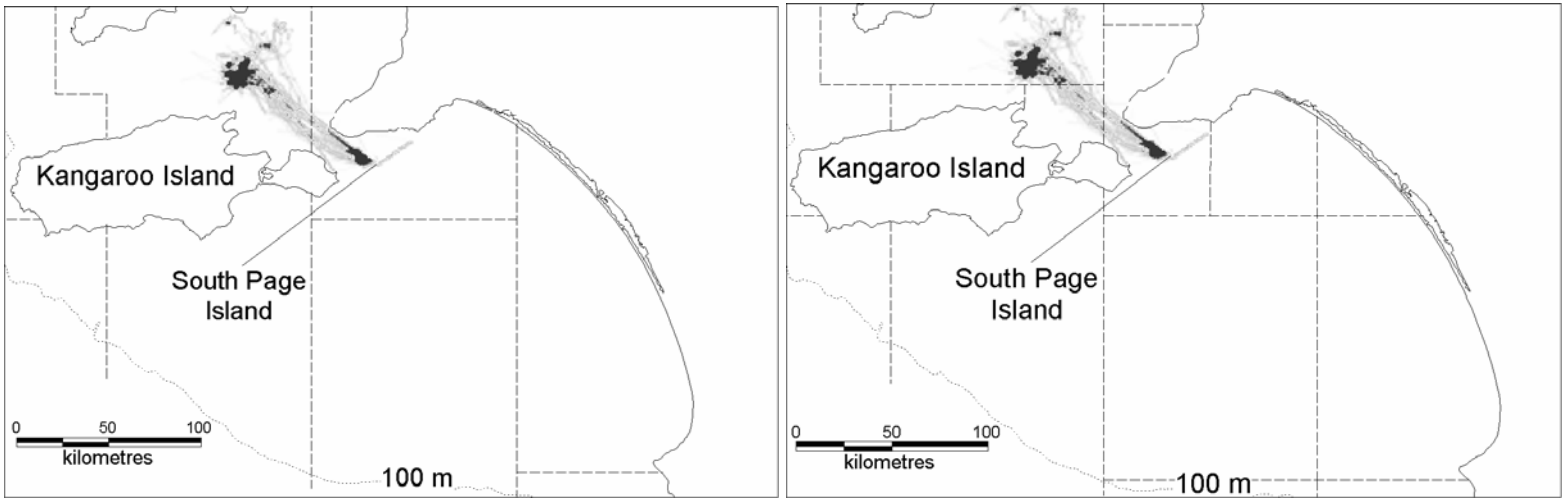
Adult female 956 time spent in 1 x 1 km areas from South Page Island. SESSF (left) and SARLF (right) MFAs are shown.



Adult female 961 time spent in 1 x 1 km areas from South Page Island. SESSF (left) and SARLF (right) MFAs are shown.



Adult female 958 time spent in 1 x 1 km areas from South Page Island. SESSF (left) and SARLF (right) MFAs are shown.



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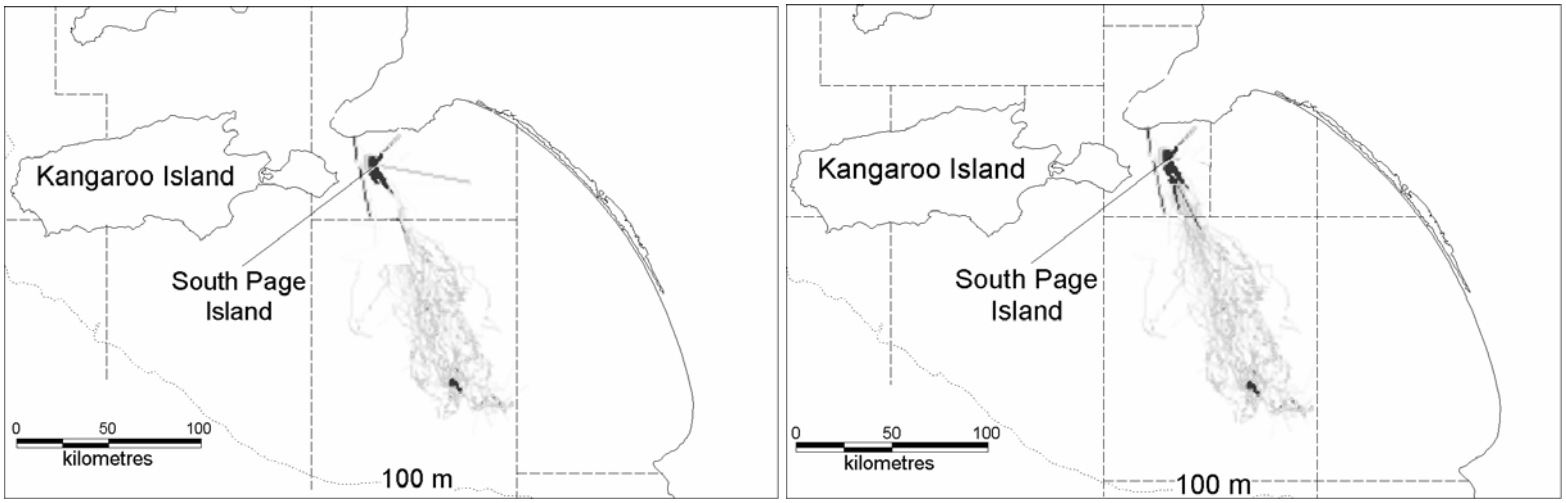

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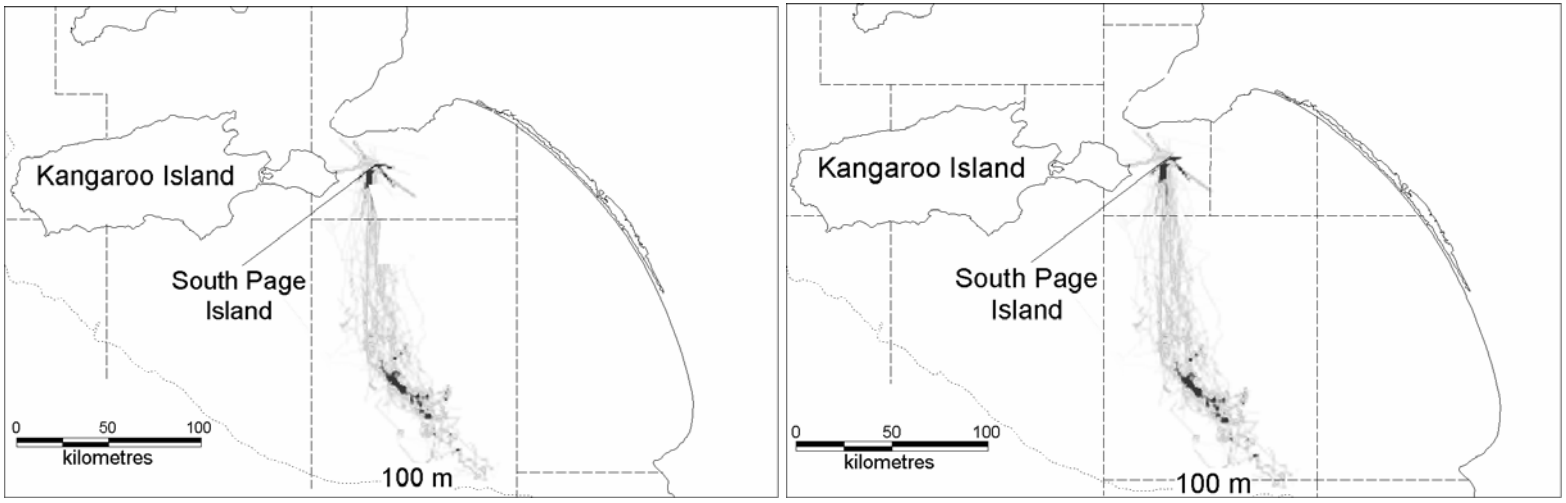

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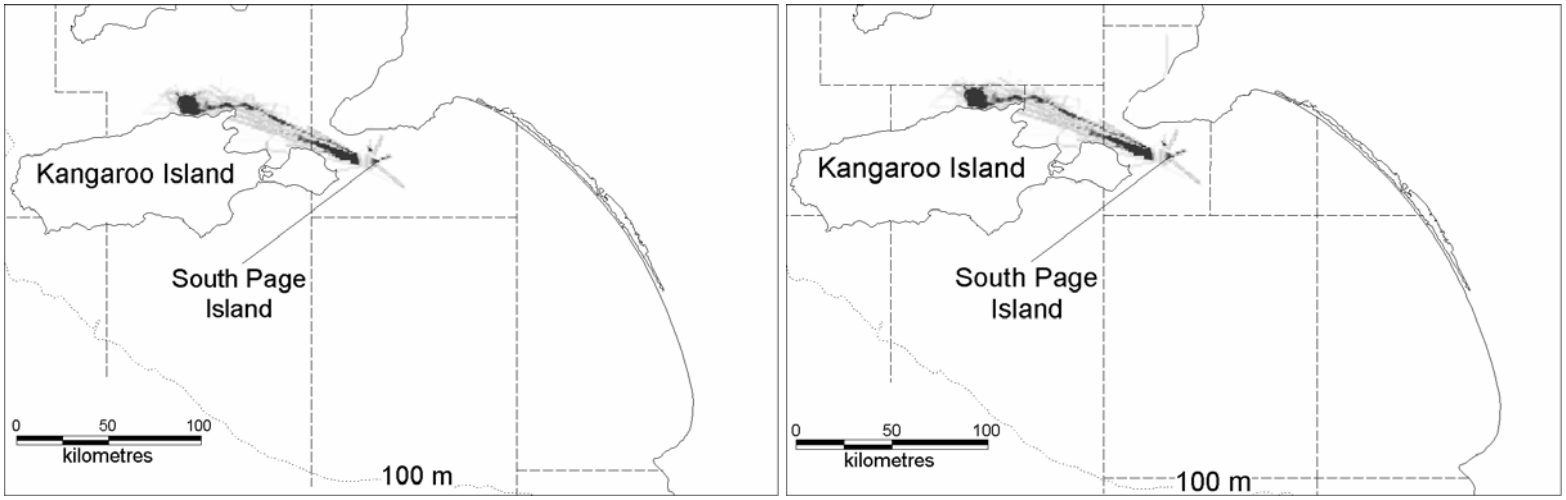

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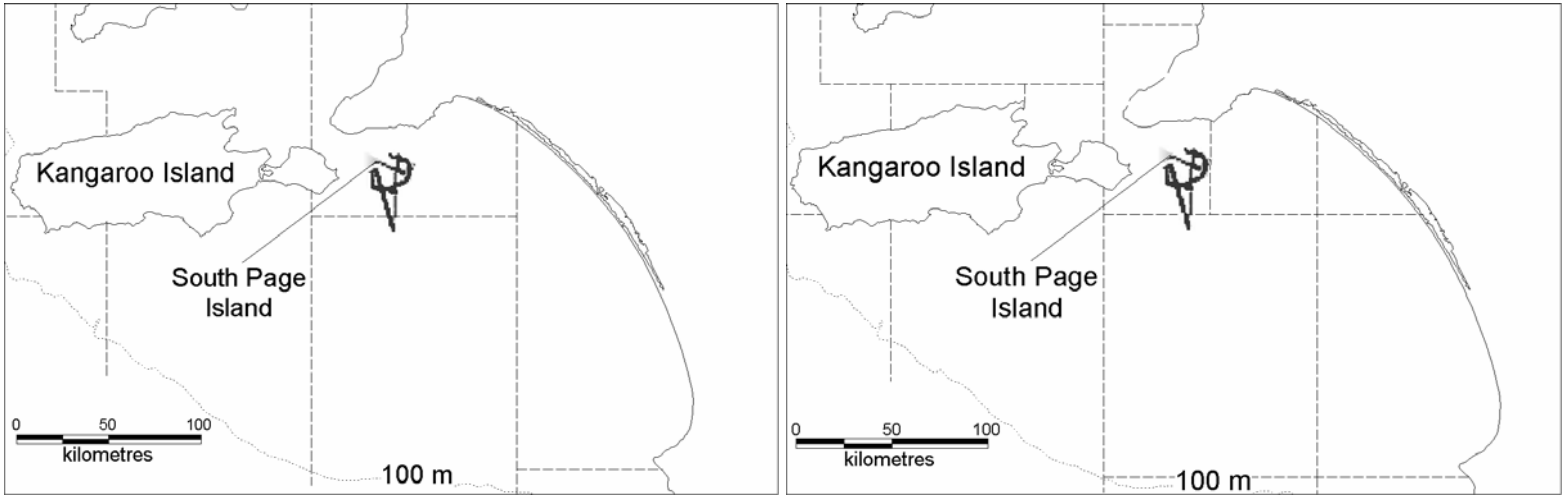

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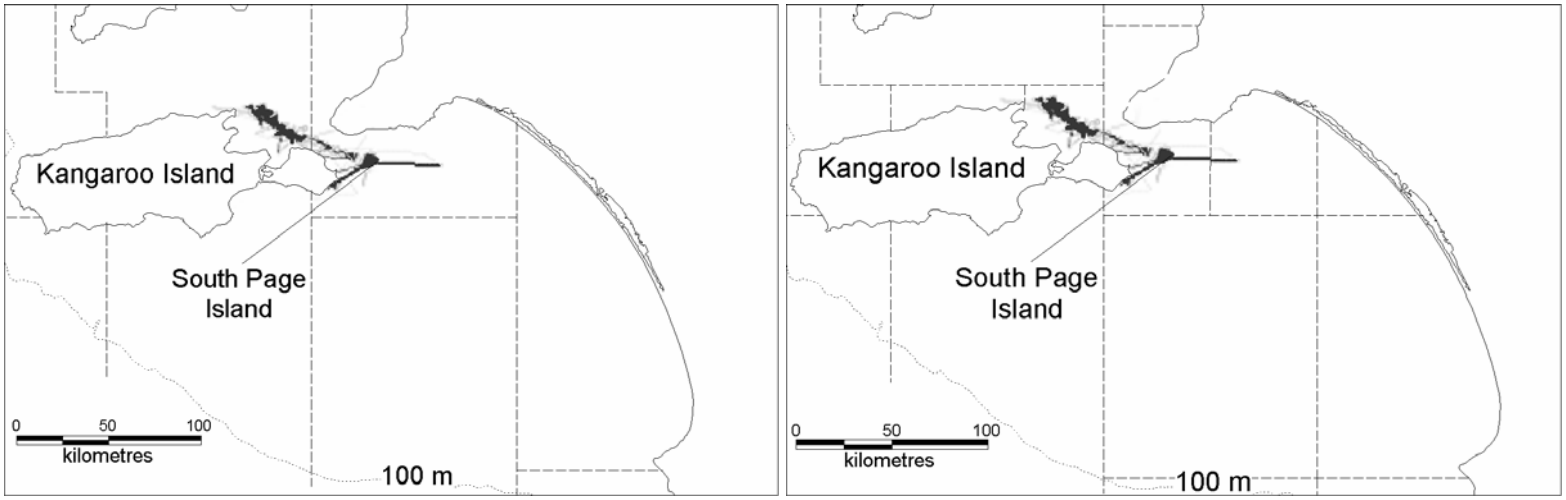

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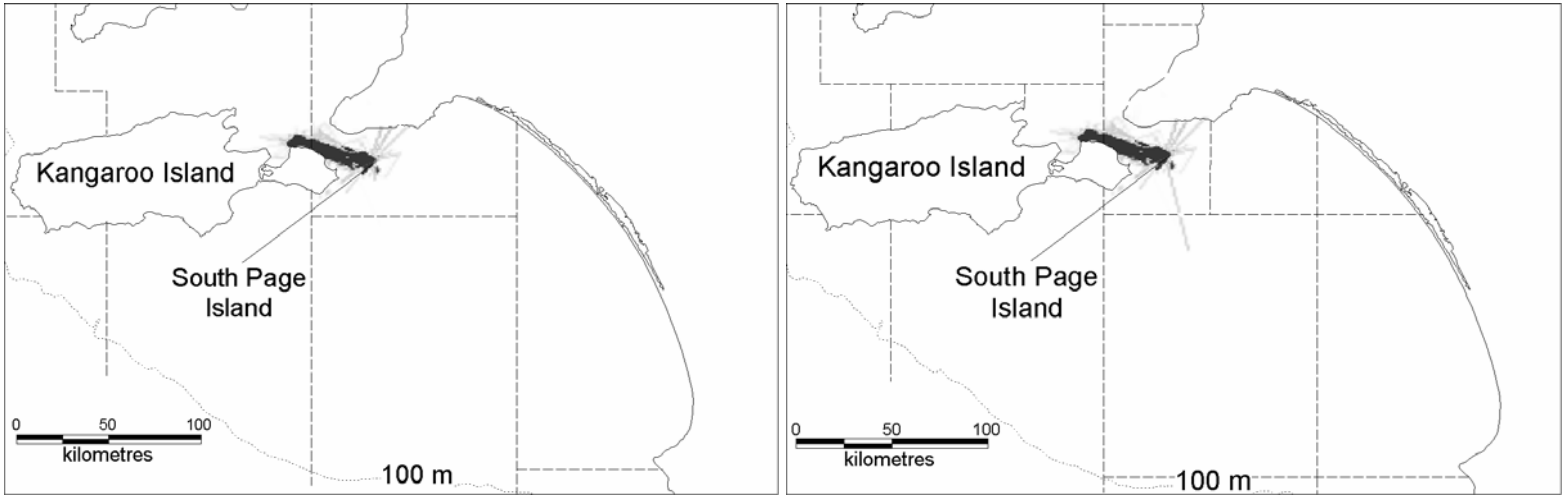
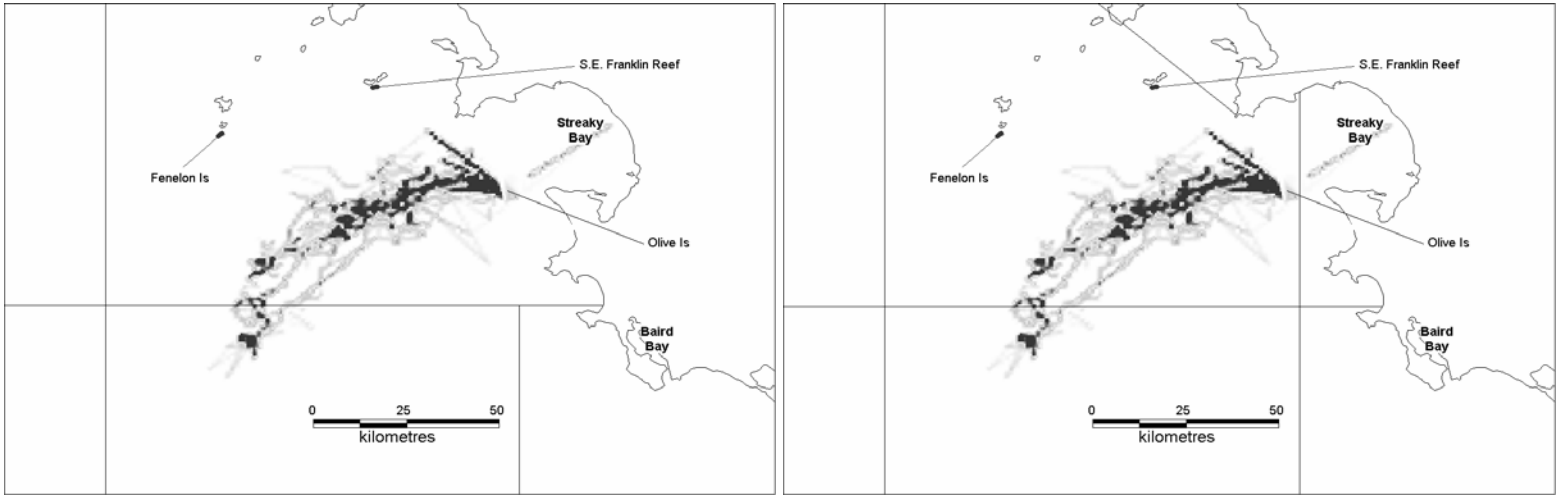


Adult female 982 time spent in 1 x 1 km areas from South Page Island. SESSF (left) and SARLF (right) MFAs are shown.

Adult female 1 time spent in 1 x 1 km areas from Olive Island. SESSF (left) and SARLF (right) MFAs are shown.



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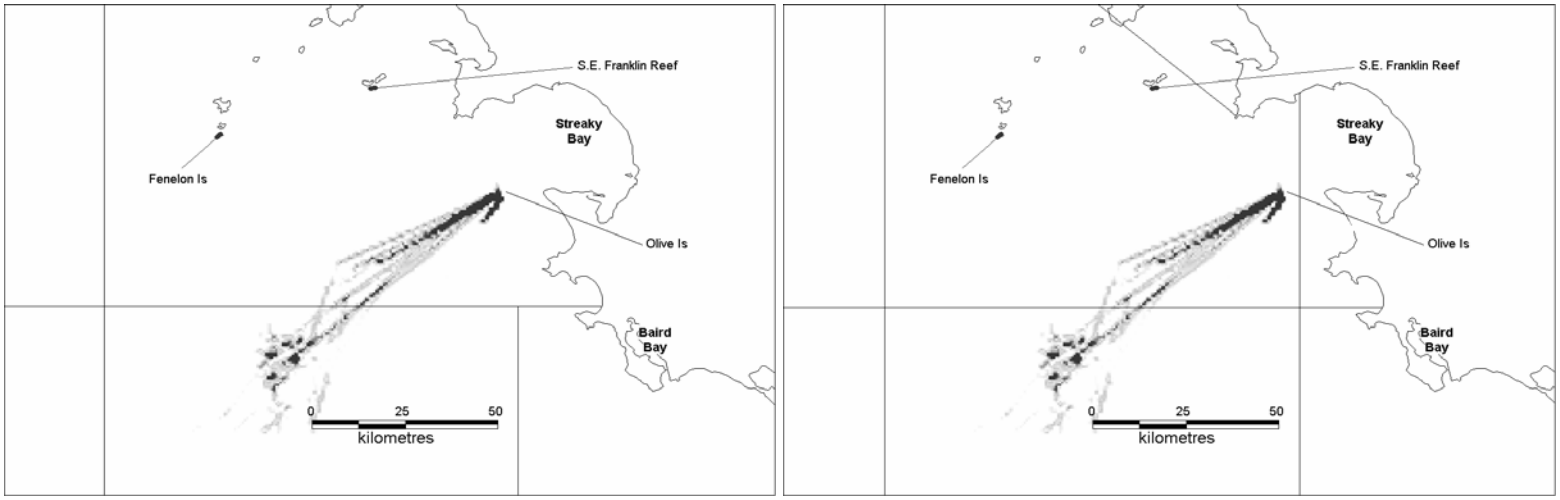
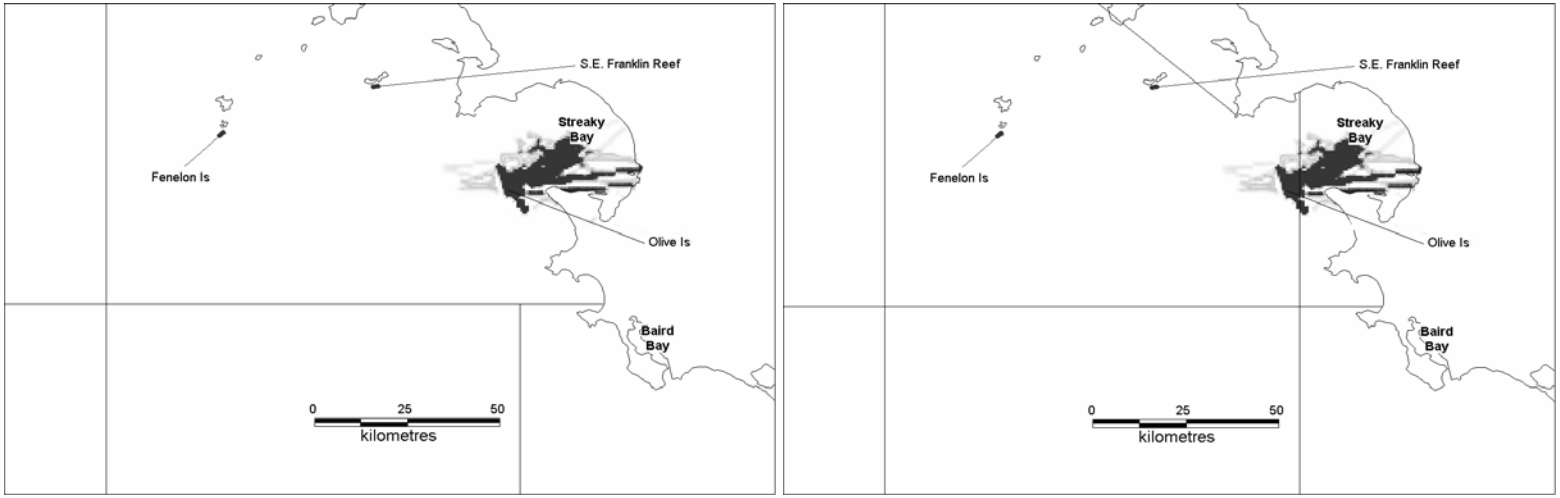


Adult female 923 time spent in 1 x 1 km areas from Olive Island. SESSF (left) and SARLF (right) MFAs are shown.

Adult female 942 time spent in 1 x 1 km areas from Olive Island. SESSF (left) and SARLF (right) MFAs are shown.

Adult female 945 time spent in 1 x 1 km areas from Olive Island. SESSF (left) and SARLF (right) MFAs are shown.



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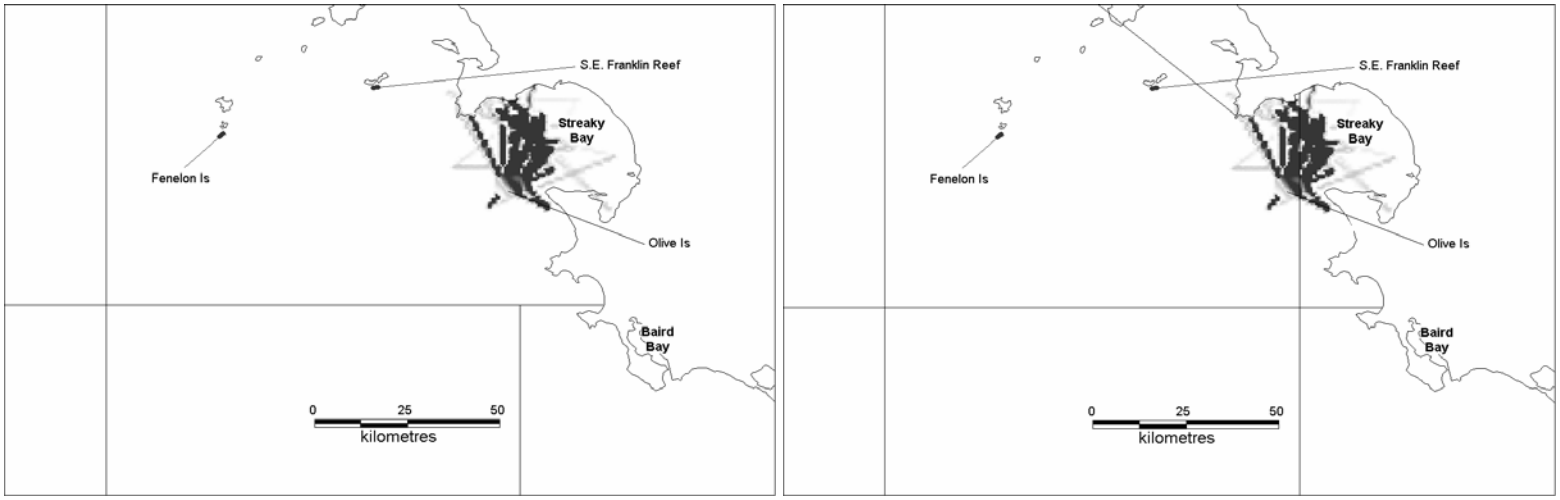

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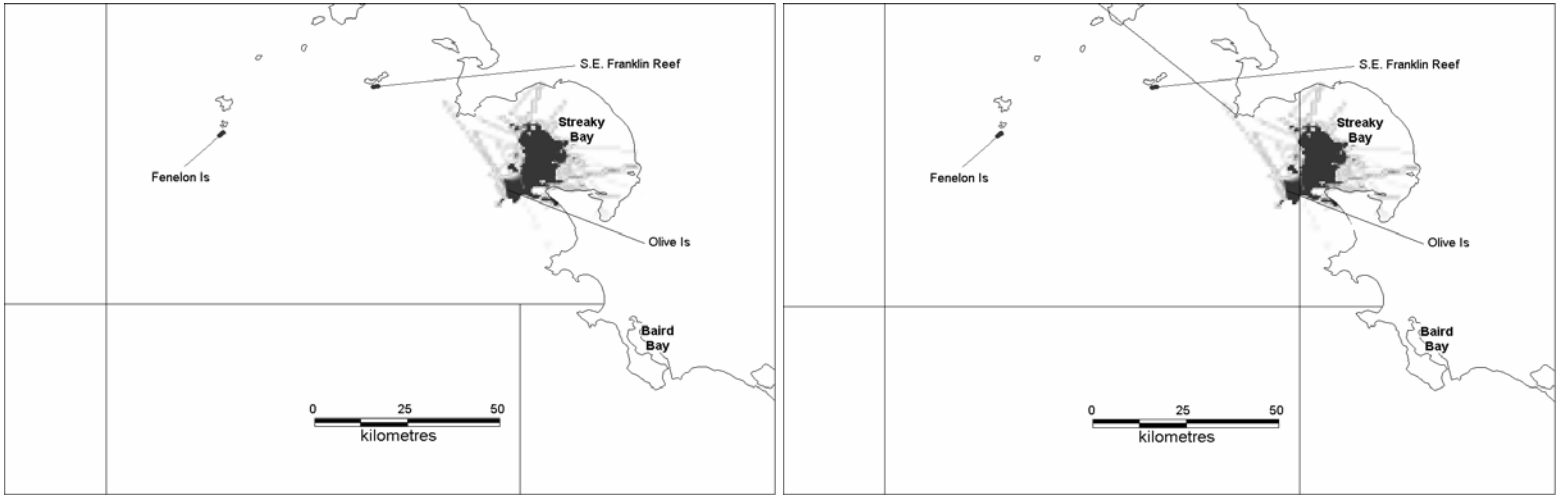

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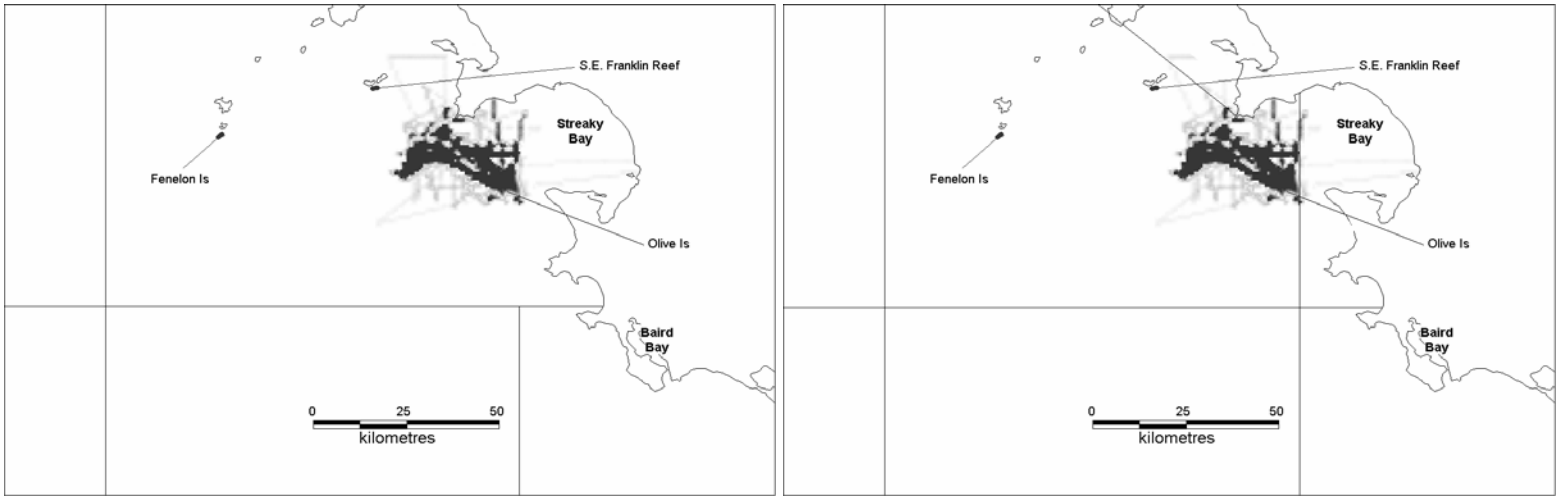

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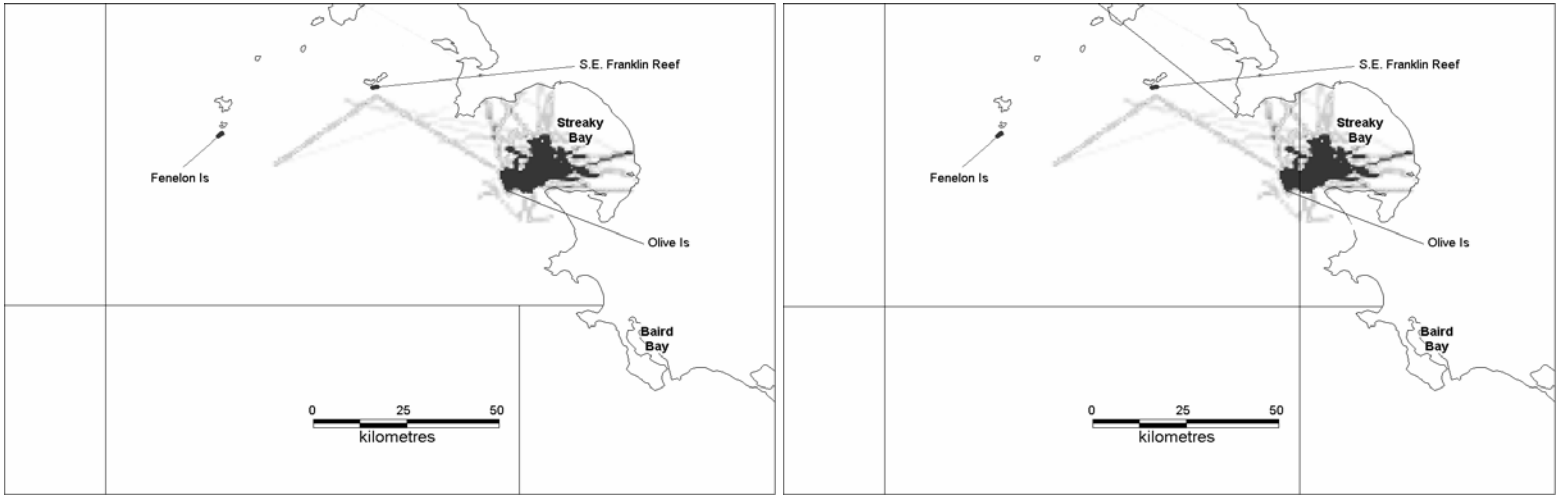
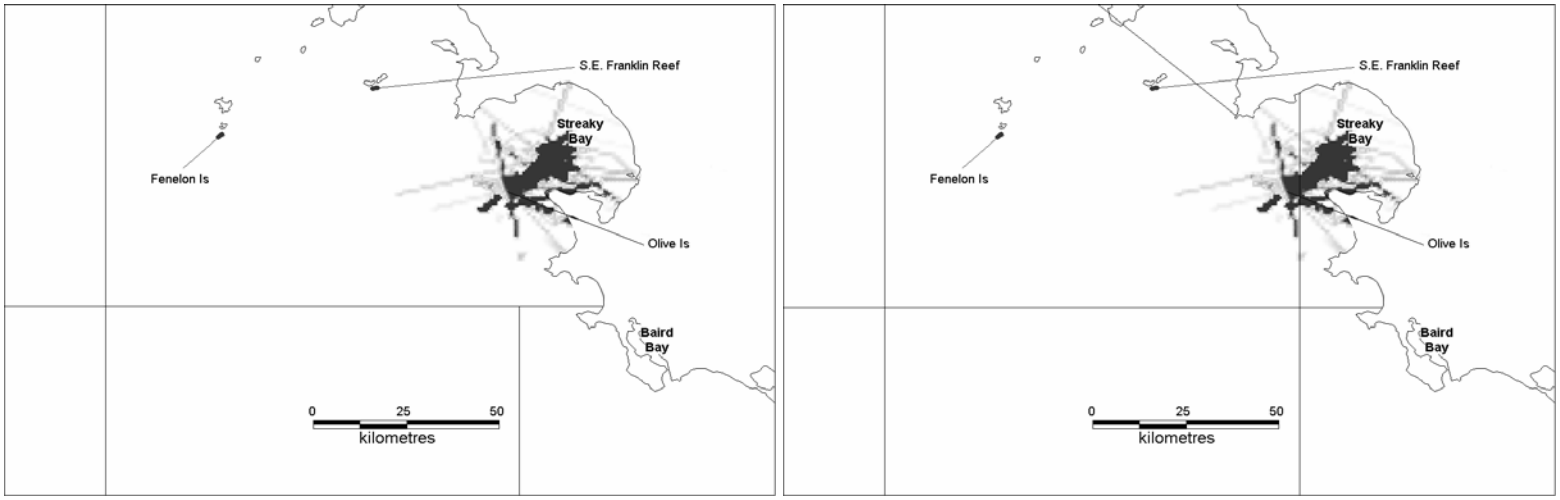


Adult female 944 time spent in 1 x 1 km areas from Olive Island. SESSF (left) and SARLF (right) MFAs are shown.



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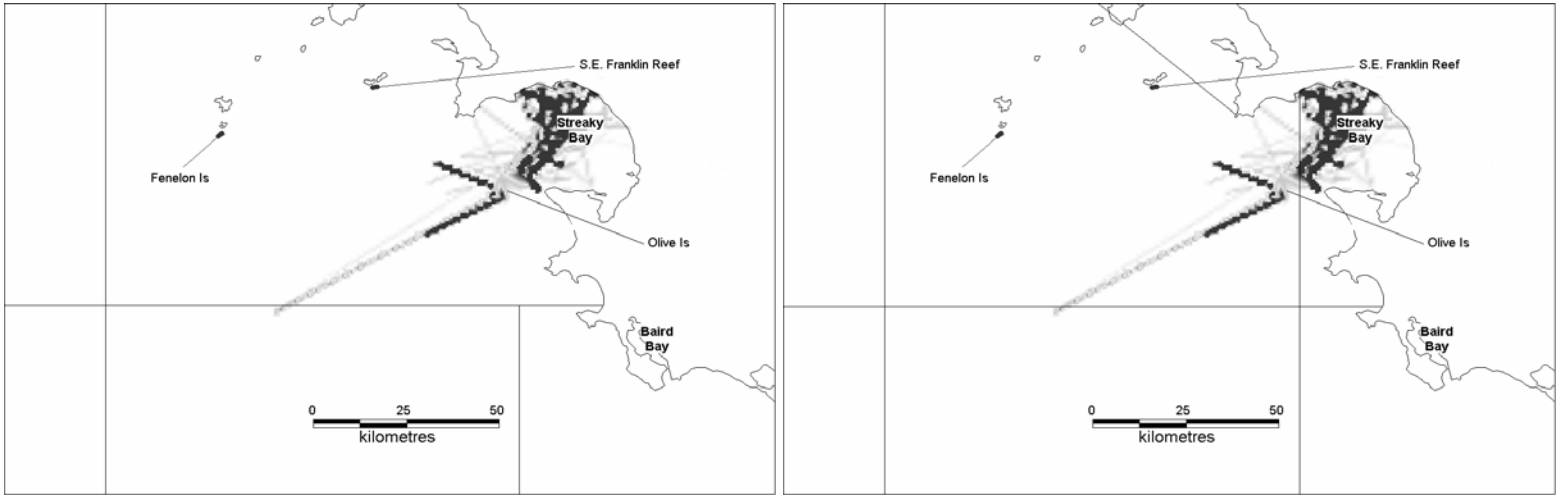

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