CHAPTER TWO

GENERAL MATERIALS AND METHODS

2.1 Study Area

The research was based around the Southern Bluefin Tuna (SBT) farms and Silver Gull breeding colonies near Port Lincoln, however, reference Silver Gull populations were also found on the Eyre Peninsula, at Venus Bay and Lipson Island (Figure 2.1). Some research was at Outer Harbour (Port Adelaide) in 2004 and the Coorong (South-East SA) in 2003 (Harrison, 2003).

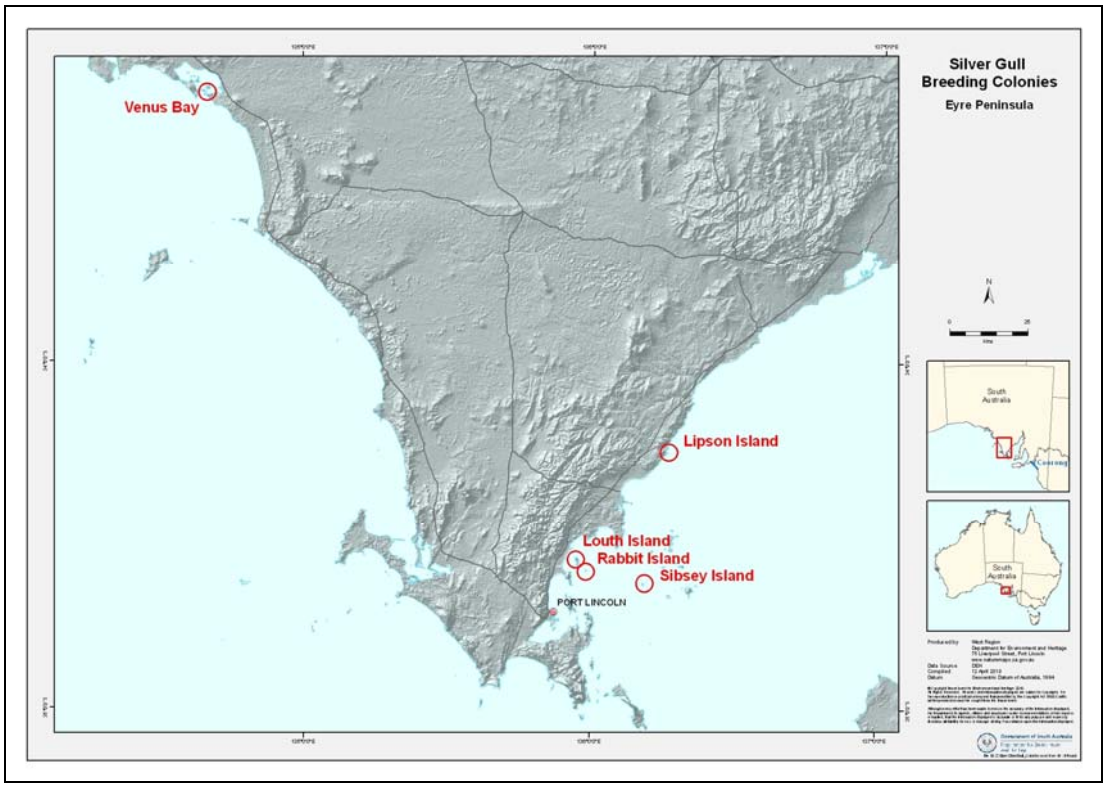

Figure 2.1: Location of Silver Gull breeding colonies used during this project.

2.2 Feed Loss at Tuna Farms and Seabird Abundance

Wild-caught Southern Bluefin Tuna are fed and grown out in ~ 130, 50m diameter pontoons in the offshore waters of Port Lincoln (Figures 2.2, 2.3, 2.4). The tuna

farming season runs from late January/early February to around September each year. The SBT are mainly fed local or imported baitfish, with 60,000 tonnes distributed per annum (Montague, 2006; Ellis pers. comm.). Formulated pellet diets have been trialled since 1994, but are not used as an alternative to baitfish at present (with one exception which is seeking to use pelleted feeds for maintaining and conditioning tuna broodstock) and thus pellets only represent about 1.5% of the total SBT feed (Montague, 2006).

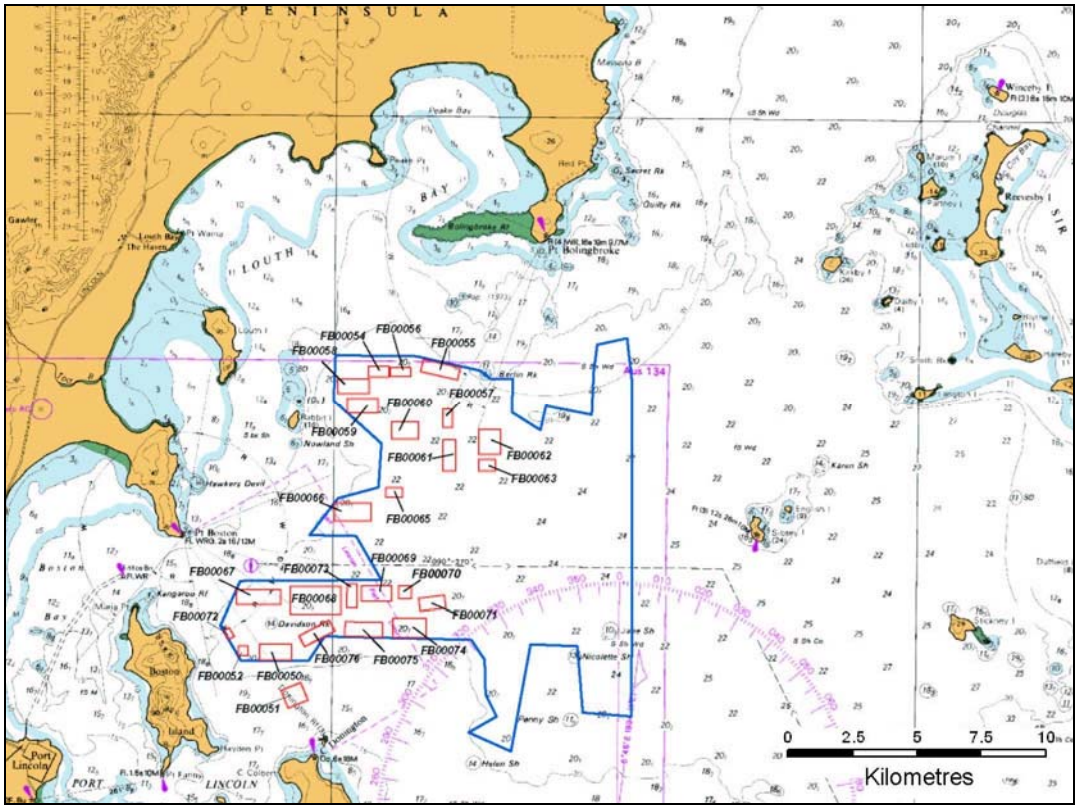

Figure 2.2: 2003 tuna lease sites (red rectangles) (PIRSA). There were approximately 120 pontoons within the tuna farming zone during 2003. The blue line is the management zone for the offshore tuna farming zone for that year.

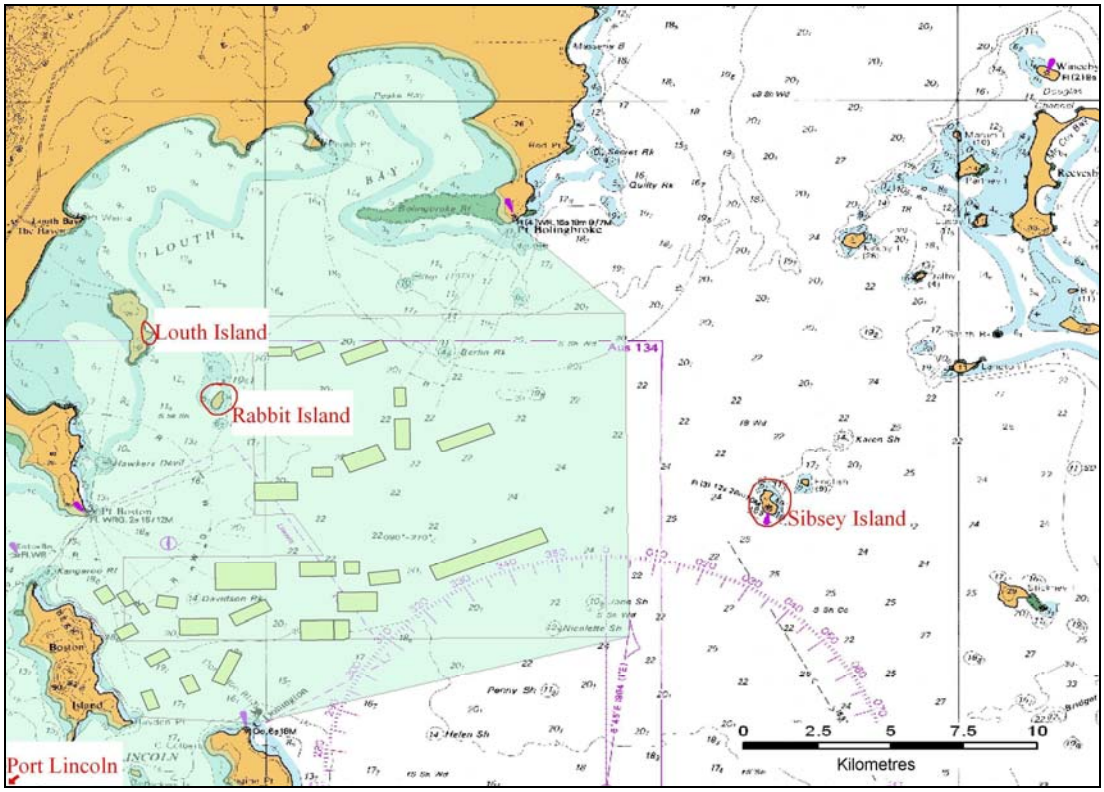

Figure 2.3: 2005 tuna lease sites, showing the location of Louth, Rabbit and Sibsey Islands (PIRSA). The rectangles are tuna leases and there were approximately 120 pontoons within the area for this year. The blue shaded area is the Lower Eyre Aquaculture Management Plan Area.

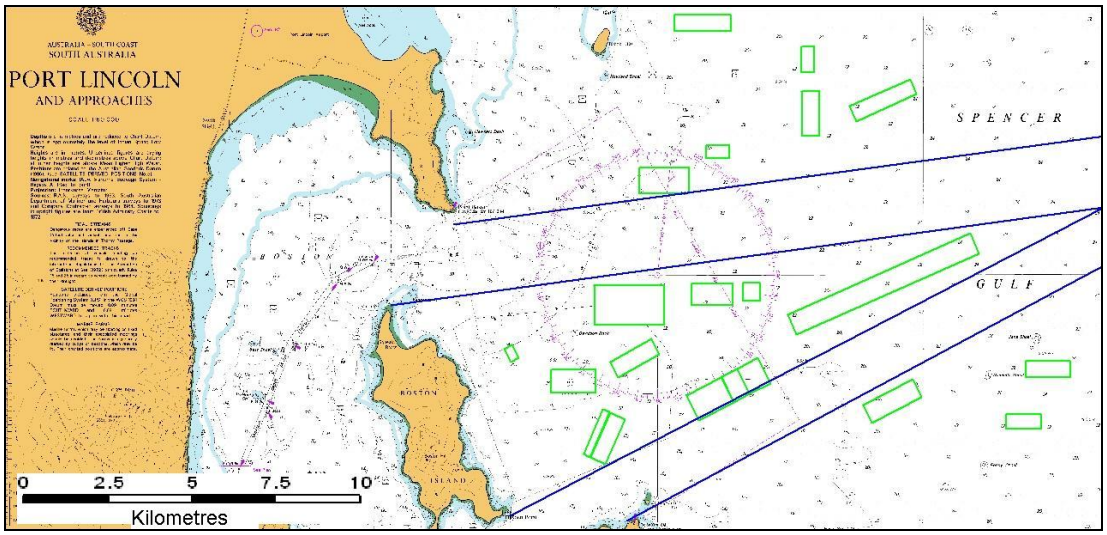

Figure 2.4: 2006 tuna lease sites (green rectangles) (PIRSA). There were 141 pontoons within the tuna farming zone in 2006. The blue lines are shipping channels.

2.2.1 Feeding Methods

The two main methods used to distribute baitfish feed on the SBT farms are shovel feeding and frozen block feeding, with most companies using a combination of both, although siphon feeding was used occasionally. In 2003/2004 approximately half of the feed was distributed using the shovel method, and the other half using the frozen block method, however, this had changed to 25:75 by 2005/2006. The tuna are fed twice a day on most days of the week (weather dependent).

2.2.1.1 Shovel Feeding

Fresh local baitfish or thawed local and/or imported species were shovelled from a metal container on the feed boat and distributed across a small part of the cage adjacent to where the boat tied up to the pontoon, which varied depending on prevailing wind (Figure 2.5).

Figure 2.5: The shovel feeding method.

2.2.1.2 Frozen Block Feeding

This method uses frozen ~25kg blocks of mainly imported baitfish which are placed into an enclosed floating feed cage within the pontoon and left to thaw slowly. The frozen blocks are pre-stacked onto a pallet until they weigh approximately a tonne, before being trucked to the boat and loaded onto the deck (Figure 2.6). Most feed boats use a HIAB (crane) to place the pallet into the feed cage at the farm site. If there was no crane on the boat, the blocks are thrown into the feed cage by hand. The lid of the feed cage is then shut and the enclosed cage is moved to the middle of the pontoon (Figure 2.7). These blocks slowly thaw over several hours, releasing the baitfish (Figure 2.8).

Figure 2.6: Frozen blocks of baitfish on pallets on the deck of a feed boat.

Figure 2.7: Frozen blocks of baitfish immediately after deposition in the feed cage. The lid will be pulled down and the feed cage will be moved to the centre of the pontoon. Note that some baitfish have separated from the blocks, floating on the water surface.

Figure 2.8: The feed cage being moved to the middle of the pontoon, from where the frozen blocks will thaw slowly.

2.2.1.3 Siphoning

The siphoning method involves gravity feeding seawater to convey the baitfish from a metal bin on the deck of the feed boat, through a PVC pipe which distributes the feed just above the surface of the water in the pontoon. The pipe is moved laterally across the cage using ropes on either side of the feeding device (Figure 2.9).

Figure 2.9: The siphoning feed method. Note the elevated bin and the yellow deck hose filling the bin with seawater.

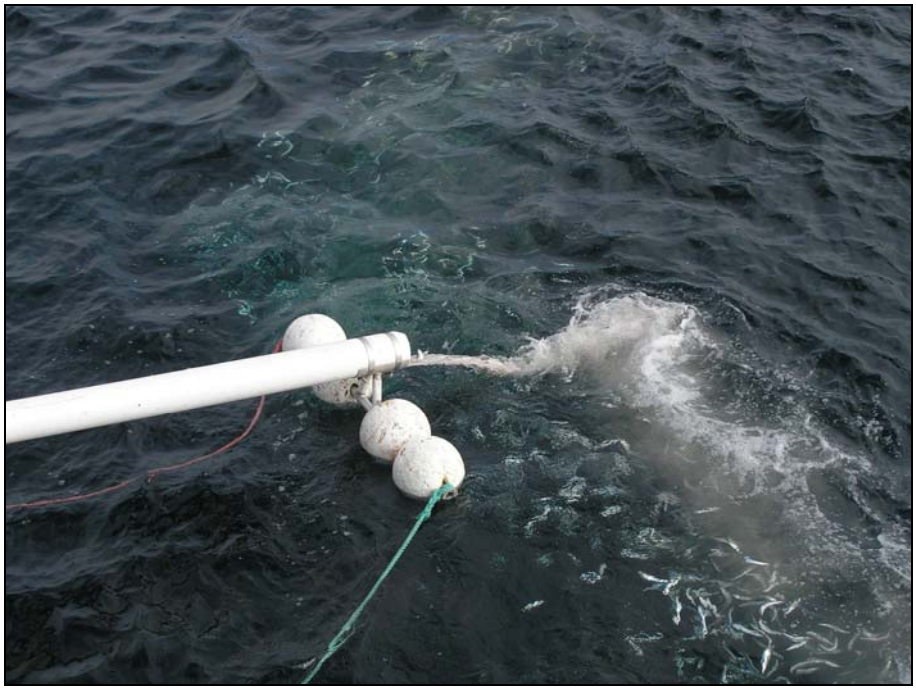

Figure 2.10: The baitfish being distributed from the PVC pipe. Note the rope attached to the white float, which is used to move the feeding device laterally.

2.2.1.4 Pellet Feeding

Pellet feeding was observed during 2004 only, although it was scarcely used throughout the project. Pellets were packaged in ~25kg bags that were opened on the deck and the pellets shovelled into the cage.

2.2.2 Estimating Tuna Feed Loss to Seabirds

The estimation of the amount of feed consumed by seabirds was established using a similar method to that developed in my Honours project, Harrison (2003) and outlined below.

2.2.2.1 Shovelled baitfish

For shovelled baitfish, the amount scavenged by seabirds was estimated by recording the number of individual baitfish consumed by each seabird species for randomly selected shovelling events. Seabirds could take the shovelled baitfish whilst it was in

the air, on the water surface or within the top ~20-30cm of the water column. The number of shovel loads required to feed out the total mass of baitfish to each pontoon was recorded, as was the total weight of baitfish distributed to each pontoon (data acquired from the skipper). A sample of each baitfish species fed out per day was collected (~10 fish) and taken back to the lab for measuring. Body weight (g), total length (mm) and width (mm) were recorded to provide averages for baitfish used on given days. These data were used to calculate the average number of baitfish per shovel load. The number of random shovelling events observed per feeding event differed, with a range of 10-250 shovel loads. The number observed was proportional to the total mass of feed distributed. The larger the amount of feed distributed, the more shovel loads required to distribute the feed, which meant that a larger number of random shovel events could be observed.

2.2.2.2 Frozen Block Baitfish

For the frozen block method, the amount of feed scavenged by birds was estimated by observing the number of baitfish consumed by each bird species during timed, one minute intervals after the feed cage lid was shut. The weight of frozen baitfish put into each pontoon was also recorded. As the feed boat usually left the pontoon 1-10 minutes after the frozen blocks were placed into the feed cage, it was not possible to observe the cage after this, with only a few (1-6) one minute observations. The results calculated assume scavenging rates and thawing rates were constant throughout the thawing of the block, which was probably unrealistic, but the observations were the best guide available. Thawing times of different weights of baitfish blocks were estimated from discussions with several skippers and range from 60 minutes for <500kg to ~300 minutes for 3000-3500kg. A set thawing time was

allocated for each weight range and this was used as a guide for all frozen block observations (Table 2.1). Wind speed, wave height and water temperature were not taken into account, although they would undoubtedly affect thawing time.

A sample of each baitfish species was taken back to the lab for weighing and measured to obtain an average for that day, as described for the shovel method.

Table 2.1: Thawing times for frozen block feed.

Weight Range	Thawing Time (mins)
<500kg	60
500-1000kg	90-120
1000-1500kg	120-160
1500-2000kg	140-180
2000-2500kg	180-200
2500-3000kg	200-240
3000-3500kg	240-300

2.2.2.3 Siphoning

For the siphoning feed method, the amount of feed scavenged by birds was estimated using the number of baitfish consumed by each seabird species during 11-19 random one minute intervals. The total time taken to feed out the baitfish was recorded, as was the weight of baitfish distributed. As described for the shovel method, a sample of each baitfish species was used to obtain an average size for that day.

2.2.2.4 Method Calibration

The accuracy of the method used to estimate feed loss to scavenging birds described above was tested by comparing real time data to video taped data. For an explanation of the methods used see Chapter 3.2.1.4, for results see 3.3.1.

2.2.3 Seabird Abundance at the Tuna Farms

Seabird numbers were recorded for two locations at the tuna farms.

1. Inside and above the pontoon: Birds that were either floating inside the pontoon, flying above it or sitting on the net or ring. They were generally feeding or scavenging birds.
2. Outside the pontoon: Birds rafting outside the pontoon or within sight (naked eye) of the pontoon (within the lease).

2.3 Seabird Numbers

Seabird abundance was measured on many occasions throughout this research, including at the tuna farms, around Port Lincoln and at breeding colonies. Seabird numbers were either directly counted or estimated. These methods were derived from Komdeur *et al.,* (1992), which explains how to estimate bird abundance for large flocks of birds.

2.3.1 Direct Counting

When there were few seabirds or large groups of stationary birds they were directly counted with a hand held counter. This was usually not possible with Silver Gulls because their flocks were too large.

2.3.2 Estimation Method

When there were many seabirds (such as at the refuse depot) or they were very mobile (such as during feeding events at tuna pontoons) their numbers were estimated. The number of birds in a manageable proportion of the flock was counted

directly and this number was then extrapolated to the whole flock to obtain a good estimate of total number (Komdeur *et al.*, 1992). I first used this method in my Honours project (Harrison, 2003), where the estimated number was calibrated with digital photographs and abundance was found to be within 5% of the actual numbers in the flock. During the three seasons of field work in this project, photographs were occasionally taken to calibrate the estimated count data.

2.4 Analysis of the Reproductive Output of Silver Gulls

2.4.1 Study Area

The Silver Gull breeding sites included those in the vicinity of the tuna farms (Port Lincoln breeding sites) and those of three reference sites away from Port Lincoln (Figure 2.1, Table 2.2). The main breeding colonies in the Port Lincoln area included Rabbit, Sibsey and Louth Islands (Figures 2.11-2.13). The smaller breeding colonies on Winceby, Donington and Boston Islands (Fanny Point) were not used. The reference islands used in this study were Lipson Island, Venus Bay Island C and Pelican Island (Outer Harbour, Adelaide) (Figures 2.14-2.16). Lipson Island had the potential to be included in the Port Lincoln breeding sites due to its close proximity to the area and tuna farms (Table 2.2), however, results from the diet analysis (Chapter 4) indicated that it was a reference site. The population of Silver Gulls at Venus Bay do utilise anthropogenic food sources (tourists food and fishing scraps), however, results from the diet analysis (Chapter 4) indicated that a lot of the diet during the breeding season at least was naturally obtained food.

Historically, Rabbit Island was the main breeding colony for Silver Gulls in the Port Lincoln area, with only a small colony on Sibsey Island (Robinson *et al.*, 1996).

However, when Rabbit and Sibsey Island were surveyed in 2003, Sibsey Island was the larger breeding colony (7,330 nesting pairs), with a smaller colony on Rabbit Island (2,000 nesting pairs) (determined by counting occupied and new nests within quadrats (extrapolation) or by counting pairs from a boat) (Harrison, 2003). In 2004, Rabbit Island was once again the main breeding colony, although, the population on Sibsey Island remained.

Table 2.2: Silver Gull breeding island information. Some of the information obtained from Robinson *et al.,* (1996).

	Rabbit Island	Sibsey Island	Louth Island	Lipson Island	Venus Bay Island C	Pelican Island
Site	Port Lincoln	Port Lincoln	Port Lincoln	Reference	Reference	Reference (2004 only)
Conservation Status/location	Part of the Port Lincoln National Park. "Biologically disturbed" from guano miners and rabbits	Sir Josephs Banks Group Conservation Park	Privately owned near Louth Bay. Almost completely altered by agriculture	Conservation Park, between Tumby Bay and Port Neill	Venus Bay Conservation Park. Degraded by past agriculture.	Outer Harbour (Port Adelaide). Directly opposite container ship terminal and SA Yacht Squadron
Size	20ha	30ha	182ha	1ha	6ha	-
Maximum Elevation	10m	25m	23m	8m	~5-8m	-
Rock Type	Granite	Granite	Lincoln Complex Granite	Lincoln Complex rock with large deposit of sand	Calcarenite platform	Sand
Distance from Port Lincoln	16km NNE	30km NE	16km NNE	62km NNE	250km	~280km (straight line – 646 by road)
Distance from closest tuna farm	3km	9km	6km	43km	~265km	~280km
Dominant Vegetation	Introduced species. Ankle to waist height	Low shrubland (Ap), mainly Marsh Saltbush	Very little on rocky outcrop	Low lying Nitre Bush	Low lying shrubs and grasses	Low lying shrubland to tall open shrubland
Other bird species breeding during Silver Gull breeding season	Black-faced & Pied Cormorant, Australian Pelican, Rock Parrot, Little Penguin, Cape Barren Goose, others	Cape Barren Goose, Rock Parrot, Little Penguin, White-bellied Sea Eagle	Cape Barren Goose.	Black-faced Cormorant, Little Penguin, feral pigeon, Pacific Gull.	Pacific Gull, Caspian Tern, Sooty & Pied Oystercatcher	Australian Pelican, several ibis and spoonbill species
Other species present, or breeding at other times of year.	Sooty & Pied Oystercatcher, White-bellied Sea Eagle, Pacific Gull, White-faced Storm Petrel	Black-faced & Pied Cormorant, Pacific Gull	Pacific Gull, Black-faced & Pied Cormorant	Crested Tern, Sooty & Pied Oystercatcher, Caspian Tern, Fairy Tern, starling	?	?
Snakes	No	No	Yes	No	No	?
Relevant Figure	Figure 2.11	Figure 2.12	Figure 2.13	Figure 2.14	Figure 2.15	Figure 2.16

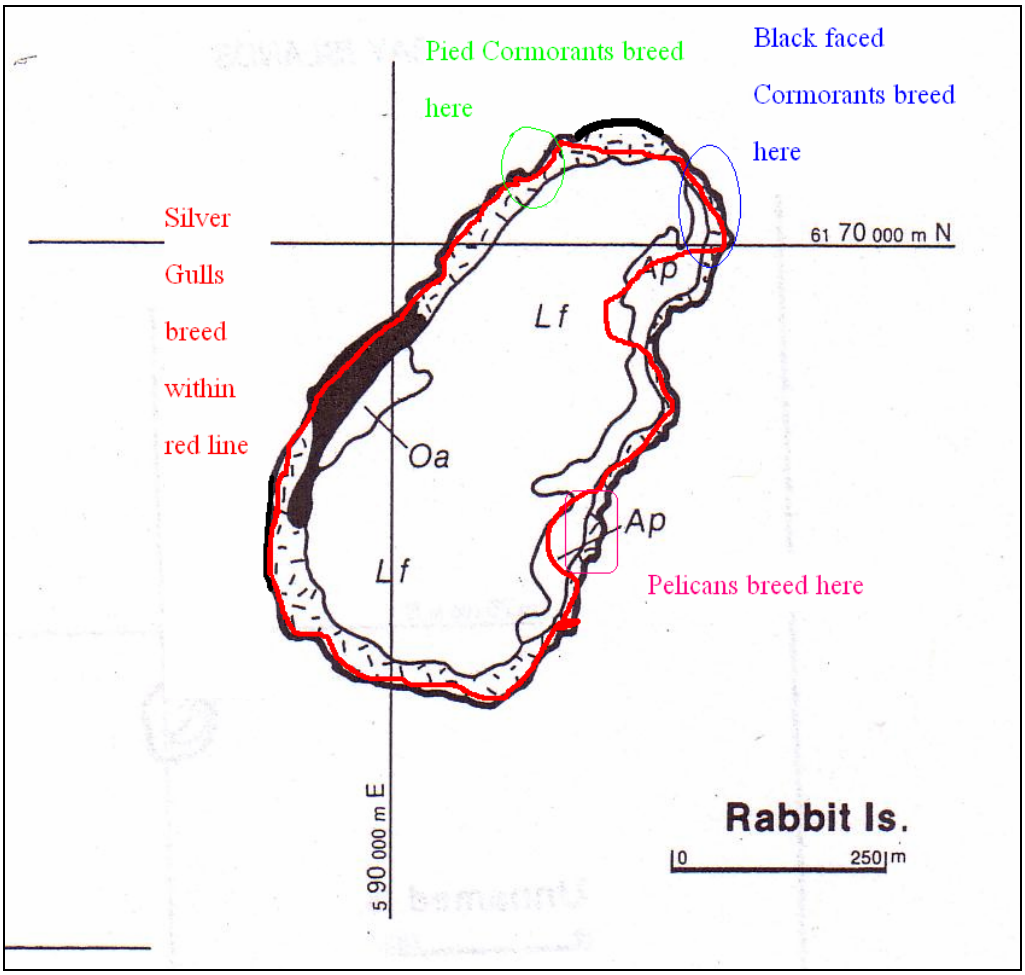

Figure 2.11: Rabbit Island, showing the approximate breeding areas for the Silver Gulls (the entire area within the red line). The pelican breeding colony (pink rectangle), the Pied Cormorant (green oval) and the Black-faced Cormorant (blue oval) breeding colonies are also shown. The dark area is bare sand, the stippled area is bare rock, and the letters depict vegetation type (Table 2.3). Map obtained from Robinson *et al.*, (1996).

Table 2.3: Vegetation guide for maps obtained from Robinson *et al.*, (1996).

Vegetation Guide
Ap: *Atriplex paludosa* chenopodioid shrubland
Ed: *Eucalyptus diversifolia* open scrub
G: Introduced Grassland
Lf: *Lycium ferocissimum* tall open shrubland
Nb: Nitre Bush *Nitraria billardieri* low shrubland
Oa: *Olearia axillaris* tall open shrubland

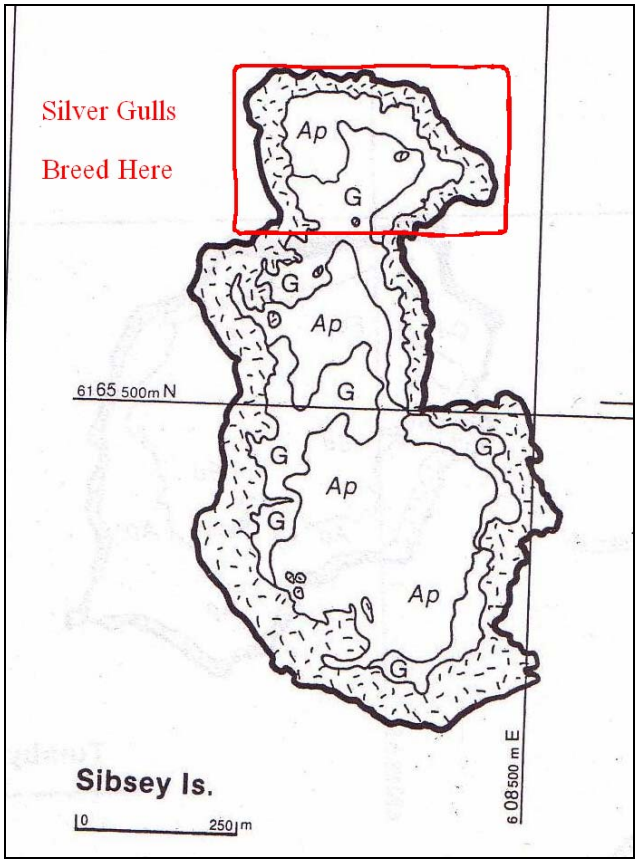

Figure 2.12: Sibsey Island Silver Gull breeding colony (within the red rectangle). The stippled area is bare rock, and the letters depict vegetation type (Table 2.3). Map obtained from Robinson *et al.*, (1996).

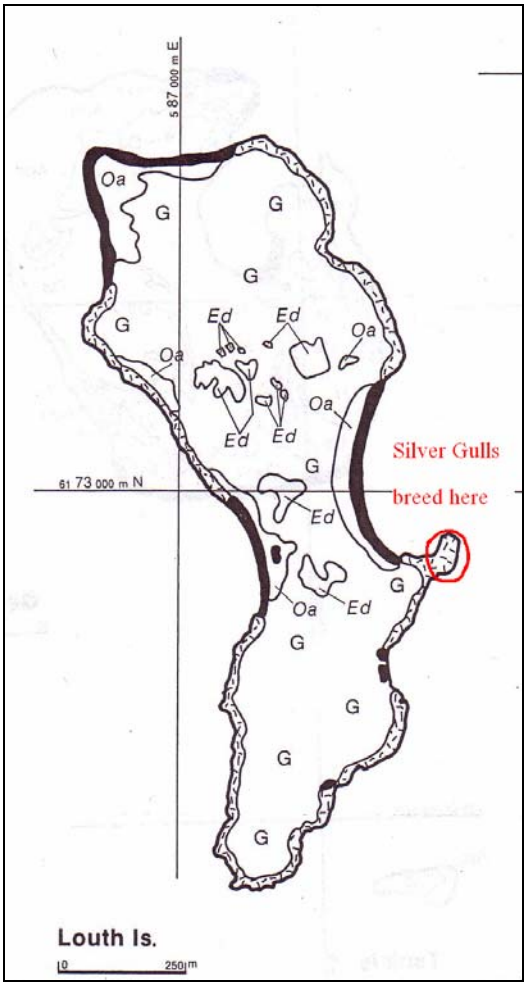

Figure 2.13: The small Louth Island Silver Gull breeding colony (within the red oval). The dark area is bare sand, the stippled area is bare rock, and the letters depict vegetation type (Table 2.3). Map obtained from Robinson *et al.*, (1996).

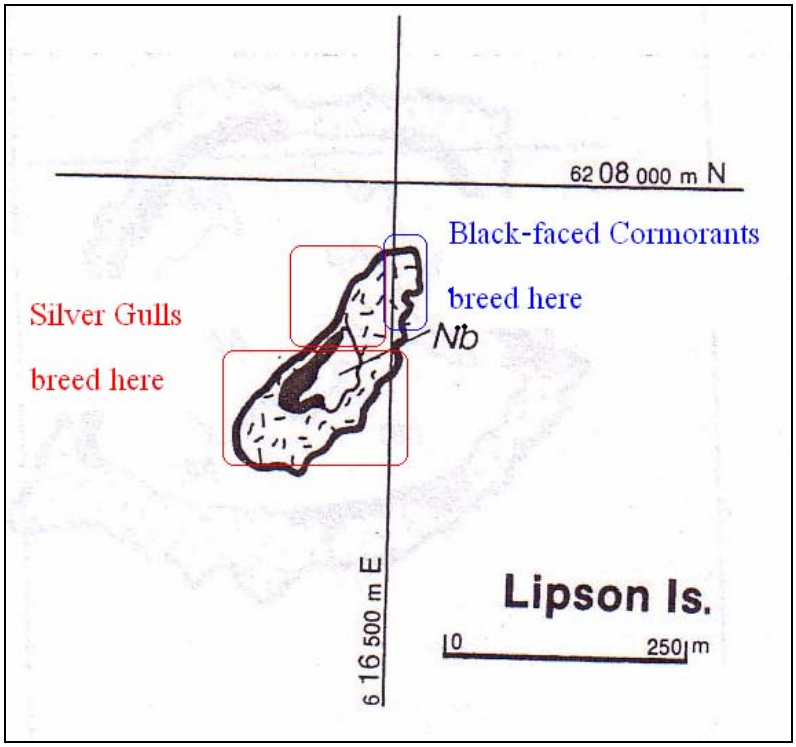

Figure 2.14: The Silver Gull breeding colony on Lipson Island (red rectangles). The Black-faced Cormorant breeding colony is also shown (blue rectangle). The dark area is bare sand, the stippled area is bare rock, and the letters depict vegetation type (Table 2.3). Map obtained from Robinson *et al.*, (1996).

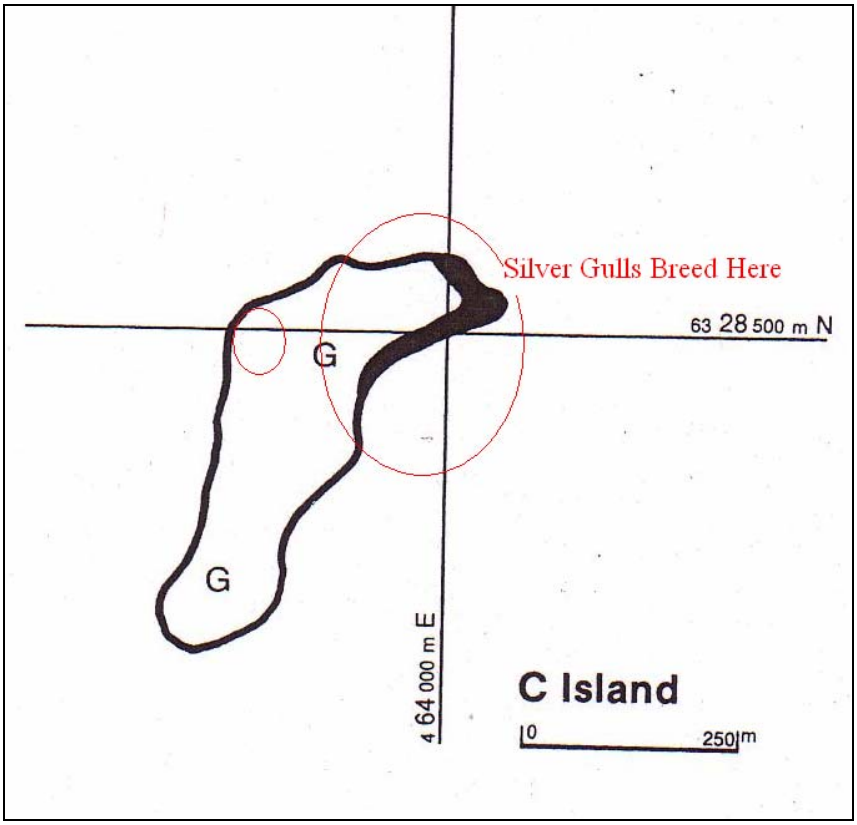

Figure 2.15: The Silver Gull breeding colony on Island C (red ovals). The dark area is bare sand, and the letters depict vegetation type (Table 2.3). Map obtained from Robinson *et al.*, (1996).

Figure 2.16: Pelican Island at Outer Harbour. Silver Gulls nest wherever terrestrial vegetation (Nitre Bush with some boxthorn) is visible.

2.4.2 Assessing Reproductive Output

2.4.2.1 Nest Selection

Each year nests were selected in small groups (over different sections of each island), so that they were easy to relocate and monitor (see Table 6.1 for dates visited). The initial groups were randomly selected, however for subsequent years, some new groups were chosen, whilst some similar sections were also used. Ten to twenty nests were selected per group with several sites chosen over different parts of the island. Over the whole season approximately 1-20% of the nests of each population were marked and monitored (depending on population size). Selected nests were identified with 30cm tall plastic nest markers on which the nest number and date were recorded in black, permanent ink (Figure 2.17). Nests with pipping eggs or hatched chicks were rarely used.

Islands were visited approximately once a month during the breeding season, unless nests were marked, and then the colony was visited approximately weekly until the fate of the nests, eggs or chicks were known. This included observing any unsuccessful eggs until two weeks after estimated hatching date or with successful eggs, until the large chicks could no longer be found. Nests were visited often enough to determine the fate of the eggs within the nest. Additional nests were selected at regular intervals throughout the season, both in similar areas, and in new areas to gain reproductive output data over the majority of the breeding season. It was not possible to measure the same nest over the season or consecutive seasons as we could not visit the colony often enough to do this.

Figure 2.17: A marked Silver Gull nest.

2.4.2.2 Clutch Size, Egg Weight and Egg Volume

For each selected nest, clutch size was recorded and each egg was weighed and maximum length (L) and breadth (B) measured to calculate egg volume using the formula $0.496LB^2$ that is accurate to within 2% (Wooller & Dunlop, 1979). Eggs were weighed to the nearest 0.1g with a portable balance and measured at the widest points (length and breadth) with vernier callipers to the nearest 0.1mm. After each egg was measured, it was numbered on the shell either in order of weighing, or order of laying (if known) with black, permanent (non-toxic) ink and nest number written on the bottom of the egg.

Unlike egg volume which remains constant over time, like most bird eggs, Silver Gull eggs lose weight (mainly due to water loss) at a constant rate during incubation (Wooller & Dunlop, 1980), which means that egg weight would vary at different times of incubation. As we could not gain regular access to the breeding islands, the

eggs were not weighed consistently over a period of time. Therefore, egg weight was not used as a reproductive output parameter in the results of the relevant Chapters, but the results are included in the Appendix.

2.4.2.3 Hatching Success

Each nest was checked weekly and monitored for hatching success or fate of the egg. Pipping eggs were weighed and hatching success was determined as whether a chick successfully hatched from the egg. Chicks successfully emerging from eggs were scored as a hatching success and dead chicks found within or close to the nest were also a hatching success. If the chick died while hatching or while still attached to the egg, it was deemed to not have successfully hatched. Unhatched eggs were monitored until two weeks after estimated hatching date but were classed as unsuccessful. Eggs that disappeared well after estimated hatching date were assumed to be unsuccessful. If no chicks were present but eggs were last seen pipping, or crumpled, hatched eggs with blood membranes were found, chicks were assumed to have hatched. Hatched chicks were weighed and if known, egg number recorded. They were then banded so that they could be individually identified later (See 2.5 of this chapter).

2.4.2.4 Chick Survival to Fledging

Fledging success was difficult to measure precisely because the chicks left the nest within a week of hatching and because parents usually direct their chicks away from the colony at about 3.5-4 weeks of age (Wheeler & Watson, 1963). The patches of dense vegetation present on all of the islands also made them hard to find. Thus Silver Gulls were assumed to have reached fledging if they survived to pin feather

stage (Class 2) or 21 days or more (Smith & Carlile, 1992).

Chicks were searched for each time the colony was visited. They were caught on foot where possible and identified using the unique number on their stainless steel band. Any dead chicks found inside or around the nest and older banded chicks that were found dead within the colony were also recorded. It can be assumed that a proportion of chicks died between four weeks of age and fledging (5-7 weeks of age) (Smith, 1995), however, due to their mobility, it was not possible to gauge this.

The mark-recapture data obtained was modelled and analysed using Program Mark (White & Burnham, 1999) and the survival probabilities of chicks (post hatching to fledging) from both the reference and Port Lincoln populations were obtained.

2.4.2.5 Estimate of Overall Reproductive Output

The estimated overall reproductive output of the Port Lincoln and reference gulls was calculated using the reproductive output data obtained (clutch size, hatching success and chick survival probability). However, as this was only used in Chapter 6 the methods are described in 6.2.3.1.

2.5 Banding Birds

Silver Gulls chicks were banded to individually identify them to assess survival rate. Whilst every effort was made to locate and catch banded chicks, the dense vegetation and the infrequent weekly visits made this very difficult. Subsequently, the majority of banded chicks were not located after 3-4 weeks of age. Adult gulls were also banded after stomach flushing to avoid re-sampling from the same bird (Refer to

4.2.2). A size 8 stainless steel bird band was placed on the left leg using banding pliers and the unique number recorded. A plastic wrap around, colour band was placed onto the right leg with the colour of the band representing the natal island. Initial banding was undertaken under the supervision of Dr Jeremy Robertson, a licensed bander (ABBBS permit number 2257) until I became competent.

Table 2.4: Natal colony colour band guide.

Rabbit Island	Dark Green
Sibsey Island	Bright Pink or Yellow
Louth Island	Dark Blue
Lipson Island	Purple or White
Venus Bay	Orange

Figure 2.18: Using banding pliers to place a stainless steel band on a Silver Gull chick's leg on Lipson Island.

Figure 2.19: A recently hatched and banded chick. Note the stainless steel band on its left leg and the green colour band on its right leg. This chick was hatched on Rabbit Island.

2.6 Statistical Analysis

All statistical analyses were performed in consultation with Kylie Lange, Statistics Consultant, Information Services Division, Flinders University except for the diet data analysis which was performed under guidance from Dr Brad Page (SARDI Aquatic Sciences) and the chick survival rate data analysis which was performed under guidance from Professor Corey Bradshaw (Adelaide University). All analyses were undertaken using either SPSS version 14 or 15, Microsoft Excel, PRIMER statistical program or Program MARK. Data were examined for homogeneity and normality, and transformed if necessary (Pallant, 2005). If the data could not be suitably transformed, an appropriate non-parametric test was used. Significance was measured at the 95% significance level ($p < 0.05$). Occasionally datasets were split up and compared and when this occurred a Bonferroni adjustment was applied to the p-value.

Graphs Used

Many of the graphs used to present results within this thesis are boxplots, where the data are represented by a box and protruding lines (whiskers). The length of the box is the variable's interquartile range which contains 50% of cases, and the horizontal line across the inside of the box is the median value. The whiskers represent the range of the data and 25% of the data below and above the box. Outliers are represented as a circle or an asterisk and are cases with scores that are either much higher or lower than the remainder of the sample. Outliers represented by a circle are 1.5 boxlengths from the end of the box and those with an asterisk are extreme outliers and are more than 3 boxlengths from the edge of the box (Pallant, 2005).