

Surveying, Searching and Promoting Giant Australian Cuttlefish Spawning Activity in Northern Spencer Gulf



M.A. Steer

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Chapter Two: Giant Australian Cuttlefish Survey

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Chapter Three: Searching for Alternate Spawning Aggregations

SARDI's Alex Dobrovolskis, Ian Moody, Leonardo Mantilla and Emma Brock undertook the extensive field surveys.

Chapter Four: Using Artificial Substrate to Promote Spawning Activity

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Chapter Five: Metal Loads in Cephalopods

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Chapter Six: Quantifying Giant Australian Cuttlefish By-catch

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The awesome cover shot was taken by SARDI's Damian Matthews.

This report was reviewed by Dr Stephen Mayfield (SARDI), Dr Greg Ferguson (SARDI), Prof. Bronwyn Gillanders (UoA) and formally released by Prof. Gavin Begg (SARDI).

Abbreviations

ANOVA	Analysis of Variance
CCSA	Conservation Council of South Australia
DEWNR	Department of Environment, Water and Natural Resources
DPTI	Department of Planning, Transport and Infrastructure
EPA	Environmental Protection Authority
FRDC	Fisheries Research and Development Corporation
FSANZ	Food Standards Australia and New Zealand
GCWG	Giant Cuttlefish Working Group
ICP-AES	Inductively Coupled Plasma-atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
LOR	Limits of Reporting
MANOVA	Multivariate Analysis of Variance
MSF	Marine Scalefish Fishery
NATA	National Association of Testing Authorities
NZRLF	Northern Zone Rock Lobster Fishery
PCA	Principal Components Analysis
PERMANOVA	Permutational Multivariate Analysis of Variance
PIRSA	Primary Industries and Regions South Australia
QC	Quality Control
SARDI	South Australian Research and Development Institute
SATC	South Australian Tourism Commission
SGWCPFA	Spencer Gulf and West Coast Prawn Fishers Association
WOSBF	West of SANTOS Boundary Fence

EXECUTIVE SUMMARY

What the report is about

The size of the Giant Australian Cuttlefish (*Sepia apama*) population on the Point Lowly spawning grounds in 2014 increased for the first time since 2009, yet management is remaining cautiously optimistic as the reason for this increase is currently unknown. In addition to the annual assessment of the spawning aggregation this project, coordinated by SARDI (Aquatic Sciences), addressed a number of other key knowledge gaps as identified by the Giant Cuttlefish Working Group (GCWG) and forms part of a larger collaborative research initiative undertaken by PIRSA, SARDI (Aquatic Sciences), The University of Adelaide, South Australian Museum and the Environmental Protection Authority through combined State and Federal funding. There is a commitment by all levels of government to understand more about the biology and ecology of this species to assist in determining the future management actions required to ensure its sustainability. The specific focus of this study's research related to determining the relative significance of the Point Lowly aggregation within Northern Spencer Gulf; the impact of commercial fishing and industrial pollution (heavy metals) within the area on the population; and if spawning activity could be promoted in other areas away from historic breeding grounds through the use of artificial spawning habitat. The scope of research was diverse, involving extensive diver-based and video-based surveys; design, construction and deployment of artificial habitat; broad-scale collection of biological samples; and close collaboration with the commercial fishing industry within northern Spencer Gulf throughout 2013 and 2014.

Background

Each winter, tens of thousands of Giant Australian Cuttlefish aggregate on a discrete area of rocky reef in northern Spencer Gulf, South Australia, to spawn. This is the only known dense aggregation of spawning cuttlefish in the world, and as such, the site has been identified as an area of national significance. A recent study that collated historic survey data, as well as undertaking a structured survey in 2012 indicated that the annual spawning aggregation had declined by ~90% over 13 years (Steer et al. 2013). The nature and extent of this decline has become a concern for the general public, government and non-government agencies. A Giant Cuttlefish Working Group (GCWG), which consists of key stakeholders and whole-of-government representatives, has been established to respond to these concerns. One of the key initiatives of this group is to identify knowledge gaps and prioritise Giant Australian Cuttlefish research. Currently, five key research priorities have been identified: 1) to undertake a survey that estimates population abundance, habitat condition and water quality at the major spawning location; 2) to explore whether there are alternate pockets of spawning activity within northern Spencer Gulf; 3) to investigate whether

spawning Giant Australian Cuttlefish prefer certain den dimensions in which to lay eggs with the expectation that this information can be used to design and develop artificial spawning habitats; 4) to undertake residue testing of Giant Australian Cuttlefish tissues to determine their susceptibility to coastal contaminants; and 5) to quantify the Giant Australian Cuttlefish by-catch from commercial fishing.

Aims/objectives

1. To use the standard survey methodology described in Steer et al. (2013) to estimate Giant Australian Cuttlefish abundance and biomass of the Point Lowly spawning aggregation, characterise the spawning habitat and analyse the ambient water quality.
2. To explore and assess the potential of alternate Giant Australian Cuttlefish spawning areas in northern Spencer Gulf.
3. To characterise the natural spawning substrate during the 2013 spawning season. Use the 'natural spawning preference' information to design and develop artificial habitat with the intention of strategically deploying it in northern Spencer Gulf prior to the 2014 spawning season.
4. To assess whether there are abnormally high levels of metals accumulating in Giant Australian Cuttlefish in northern Spencer Gulf.
5. Determine the potential impact of fishing on Giant Australian Cuttlefish in northern Spencer Gulf.

Methodology

Giant Australian Cuttlefish survey

The specific methods are provided in Steer et al. (2013). In summary, the survey used replicated 50 x 2 m belt-transects to determine the relative density and size of Giant Australian Cuttlefish across key spawning sites along the Point Lowly Peninsula in northern Spencer Gulf throughout the 2013 and 2014 peak spawning season. The habitat was also characterised at each site through analysis of replicated under-water photo-quadrats with water samples taken to assess the relative concentration of inorganic and total nutrients.

Exploring alternate spawning sites

Sites within northern Spencer Gulf that share similar characteristics to known Giant Australian Cuttlefish spawning grounds (e.g., Point Lowly, Backy Point, Fitzgerald Bay) were identified from archived habitat maps and aerial photographs. A towed waterproof video camera secured within a protective cage was used to search for Giant Australian Cuttlefish at each site in June 2013, coinciding with the peak in spawning activity. All digital footage

was replayed through a computer monitor and GPS position, depth and time was recorded for each encountered Giant Australian Cuttlefish.

Designing and deploying artificial spawning substrate

Divers assessed the dimensions (maximum width, height, depth and orientation of the entrance) of 41 natural dens that contained Giant Australian Cuttlefish eggs on the main spawning ground fringing Point Lowly in July 2013. The dimensions of the various dens were statistically compared to determine whether cuttlefish had any den preferences. These results subsequently informed the design and construction of the artificial substrate. Three replicate artificial reefs were deployed at five sites within northern Spencer Gulf; Black Point, North Backy Point, Point Douglas, OneSteel Wall and Point Riley, in late March 2014. These sites were selected as they were known to have either supported spawning Giant Australian Cuttlefish in the past or shared similar coastal geography and exposure to prevailing oceanographic conditions to known spawning areas. Each artificial reef was positioned on sand and orientated towards the incoming swell. All three reefs were placed within 50 m of each other to ensure they can be easily monitored during a single dive. Five Reef Watch volunteer divers inspected all artificial reefs on 21-22 September 2014. Each construction was examined for any evidence of Giant Australian Cuttlefish spawning activity (i.e. presence of either developing eggs or hatched egg casings); presence of any other species; and integrity and condition of the structure.

Metal loads in cephalopods

In May 2013, five adult Giant Australian Cuttlefish were sampled from commercial prawn trawling surveys from the waters off Wallaroo approximately 100 km south of Point Lowly. In July 2013, 18 adult Giant Australian Cuttlefish from five sites and 21 adult Southern Calamary (*Sepioteuthis australis*) from three sites along the Point Lowly peninsula were captured by SARDI staff. Southern Calamary were collected as a comparative species as they occupy the same habitat and share a similar life-history to Giant Australian Cuttlefish, and their abundance have moderately increased in recent years. All animals collected from Point Lowly were dissected, separating the digestive gland, mantle and viscera for metal analysis to determine whether there was any variation in metal concentration between different organs. Only the digestive glands were removed from Giant Australian Cuttlefish collected from Wallaroo. Metal levels were analysed in triplicate using inductively coupled plasma mass spectrometry (ICP-MS).

Quantifying Giant Australian Cuttlefish by-catch

Fishery-independent and dependent programs were conducted to quantify all cuttlefish by-catch in the Spencer Gulf Prawn Fishery. The fishery independent program relied on

scientific observers to count, weigh and sub-sample cuttlefish that were incidentally caught during routine stock assessment surveys. They coincided with the dark lunar phase in November, February (or March) and April and consisted of approximately 180 fixed, 30 minute, trawl shots that are distributed throughout the Gulf. Onboard scientific observers counted and weighed all cuttlefish by-catch from each survey shot. From every second shot, all cuttlefish caught on one side of the trawl net were retained for biological analysis. The fishery-dependent program relied on commercial fishers to assess all cuttlefish catch during their regular fishing activity, extending from May 2013 to June 2014. This involved 5-12 representative vessels out of the entire fishing fleet. All cuttlefish caught on one side of the trawl net were counted and recorded after each shot (including zeros). Initially, all cuttlefish from one shot (the 4th of the night) were retained for biological analysis (see Section 4.2.1), however, this was increased to two shots per night (3rd and 7th) from November 2013 to improve the resolution of the analysis. Quantification of total cuttlefish by-catch in the Spencer Gulf Prawn Fishery was calculated at the finest spatial and temporal resolution possible (i.e. at the level of fishing block, region, or zone). Cuttlefish catch rates (number per hour) were calculated for each survey shot. Cuttlefish by-catch data were also retained from historic surveys carried out in the Northern Zone Rock Lobster and Spencer Gulf Blue Crab Fisheries.

Results/key findings

The Giant Australian Cuttlefish spawning population increased by 325% (abundance) and 589% (biomass) in 2014 compared with the 2013 estimates. This increase occurred after a steady period of decline and coupled with the return of larger animals, indicated that 2013/2014 was a relatively favourable year for Giant Australian Cuttlefish reproduction, growth and survival. An investigation of the daily average temperature over an estimated 120 day embryo development period, has, so far, provided the strongest signal for explaining the recent inter-annual variation in both abundance and biomass of the Point Lowly spawning population. Although important, the temperature regime during the early life history is not the exclusive determinate of favourable conditions. Other factors such as predatory/prey abundance and water quality are likely to contribute in shaping the population.

Ambient water chemistry properties appeared relatively consistent throughout the surveys, however, changes in the density of the opportunistic alga *Hinckesia sordida* increased from sparse coverage (<20%) in 2013 to a maximum of 70% in 2014 at key spawning sites. Drawing a definitive link between this increased coverage and nutrient input is difficult as the alga also proliferates with increasing temperature. Given its sparse coverage in 2013, *H. sordida* appeared to have a negligible effect on embryonic development as the subsequent

2014 recruiting population was relatively successful. A significant regional difference in metal burden was detected in Giant Australian Cuttlefish, with the relative concentration of many metals (i.e. Cd, Zn, Pb, Au, Cu) being more pronounced (ranging from 2.4 to 16.1 times greater) in animals collected from the Point Lowly spawning grounds compared to those collected further south (Wallaroo). This finding was not surprising given the long history of metal contamination in northern Spencer Gulf (Gaylard 2014). Cephalopods typically detoxify metals through the digestive gland (Bustamante et al. 2002a), and this study confirmed this organ constituted >90% of the animal's total metal burden, whereas the metal concentration in the mantle (edible portion) was well within food safety standards. The limits of physical tolerance of the Giant Australian Cuttlefish are not known, but given no clear association was found between the recent decline in the population and reported levels of anthropogenic discharges of heavy metals from 1994 to 2012 (Steer et al. 2013), they do not appear to be adversely affected by the modern levels of metal contamination within northern Spencer Gulf.

Estimates of total annual cuttlefish (all species) catch from the Blue Crab Fishery were negligible, with fishers recording a maximum catch of 2,483 cuttlefish in 2004 at a rate of approximately 0.02 per potlift. Estimated catches from the prawn fishery were greater (up to 73,176 in May 2014), however, Giant Australian Cuttlefish rarely constituted more than 20% of the total cuttlefish by-catch. The prawn fishery was estimated to harvest up to 9.6% (2013) of the spawning population. The estimated harvest fraction declined to 6.5% in 2014. Given the 2014 spawning population was 325% larger than the previous year, the inverse trend in the prawn fishery's estimated annual harvest fraction suggested that the recent dynamics of the trawl fleet has not adversely affected the Giant Australian Cuttlefish population in northern Spencer Gulf.

The relative importance of the Point Lowly spawning population is currently unknown, but is likely to be significant. This was supported by an exploratory survey that found no evidence of spawning activity outside of the spawning grounds, and the absence of spawning on artificial habitats strategically placed in areas where Giant Australian Cuttlefish are known to occur. The lack of optimal spawning habitat throughout northern Spencer Gulf (north of Wallaroo) was clearly apparent in this study. The deployment of artificial spawning habitat is unlikely to significantly promote the recovery of the population to the levels that were observed in the late 1990s. The effectiveness and relative ecological value of the artificial dens used in this study in mitigating habitat loss is unknown as none of the structures supported spawning animals during the 2014 spawning season.

Implications for relevant stakeholders

Given the uncertainties regarding the processes that contribute to shaping the population dynamics of Giant Australian Cuttlefish, protecting the known spawning aggregation is the most appropriate precautionary approach to ensure the maximum supply of eggs is attained to buffer against the unpredictability of the environment. Although fishing has not been identified to adversely affect the Giant Australian Cuttlefish population, the broader-scale protection of the northern Spencer Gulf sub-population from targeted fishing remains a practical strategy, particularly when the population is at a low level. Continued collection of cuttlefish by-catch data through the established fishery-independent program in the Spencer Gulf Prawn fishery would also add value in the on-going assessment of Giant Australian Cuttlefish. Relying on fishery-independent programs within the prawn fishery would streamline the process as this study indicated that it was a relatively accurate representation of the fishery-dependent data. An on-going monitoring program that assesses the spawning population would be beneficial, particularly in relation to the future expansion of coastal industries and planned infrastructure within the area, as well as providing an indication of the population's capacity to fluctuate over short and long-term time scales.

Keywords

Giant Australian Cuttlefish, spawning, aggregation, population dynamics, by-catch, heavy metals, Spencer Gulf.

1 GENERAL INTRODUCTION

1.1 Background

Each winter tens of thousands of Giant Australian Cuttlefish (*Sepia apama*) aggregate on a discrete area of rocky reef in northern Spencer Gulf, South Australia, to spawn. The size of this population has been formally quantified since 1998 (Hall and Fowler 2003) and is the only known dense aggregation of spawning cuttlefish in the world, and as such, the site has been identified as an area of national significance. A recent study that collated historic survey data, as well as undertaking a structured survey in 2012 indicated that the annual spawning aggregation had declined by ~90% over 13 years (Steer et al. 2013). The nature and extent of this decline raised significant concerns about the long-term viability of the population amongst the general public, government and non-government agencies. This issue has continued to gain considerable national attention as it is widely recognised that northern Spencer Gulf is a prospering region for South Australian resource-based industries and its future development needs to be optimised in close consideration with the gulf's unique biodiversity.

The known key Giant Australian cuttlefish spawning area spans approximately 10 km of semi-continuous reef that fringes the Point Lowly Peninsula (Figure 2.1). Within this area there are numerous coastal industries that have either recently operated, or continue to operate, such as sea-cage aquaculture ventures that have been in varying states of operation over the past decade and the Santos Ltd hydrocarbon facility established in the 1980s and currently exports petrochemicals from Port Bonython. Furthermore, there are extensive plans for industrial expansion within the area, such as the proposed development of a new bulk commodities export facility (Arup 2013) and desalination plant (BHP Billiton 2009) to support the expansion of South Australia's mining industry. Adjacent regional cities such as Whyalla, Port Pirie and Port Augusta have also recently expanded as a result of successful manufacturing and mining industries. Similarly, the gulf supports a number of significant South Australian fisheries, which have grown substantially over the past 25 years to account for approximately 80% of the State's commercial fishery production (Steer et al. 2014). Through these diverse industries Spencer Gulf has established itself as the most significant economic growth area in South Australia (Arup, 2013).

In July 2012, the South Australian Government established the Giant Cuttlefish Working Group (GCWG) to coordinate a whole-of-government response to concerns regarding the Giant Australian Cuttlefish in northern Spencer Gulf. This group consists of representatives from Primary Industries and Regions SA (PIRSA), South Australian Research and Development Institute (SARDI), Department of Environment, Water and Natural Resources

(DEWNR), Environmental Protection Authority (EPA), Department of Planning, Transport and Infrastructure (DPTI), South Australian Tourism Commission (SATC), Whyalla City Council (WCC) and the Conservation Council of SA (CCSA). The principle objective of this group is to consider the relevant existing information surrounding Giant Australian Cuttlefish; identify gaps in knowledge and prioritise research initiatives; frame management responses; establish an on-going monitoring program; engage with community groups and key non-government stakeholders; and provide up-to-date advice to relevant ministers.

The initial intention of this group was to coordinate an investigation into the cause of the decline of the annual spawning population. A desktop study was undertaken that considered an extensive range of potential factors (i.e. environmental irregularities, increased predation pressure, industrial pollution, fishing pressure) and assessed their relative likelihood in contributing to the Giant Australian Cuttlefish decline (Steer et al. 2013). The current lack of knowledge of Giant Australian Cuttlefish population dynamics and their proximate cues for spawning in northern Spencer Gulf, however, limited the study's ability to identify a definitive cause for the decline. Future research was subsequently directed towards gaining more information about the movement and migration of Giant Australian Cuttlefish on and off the spawning grounds and the structure of the Spencer Gulf population to ascertain the ecological significance of the Point Lowly aggregation and its conservation value. A companion FRDC funded project entitled 'Giant Australian Cuttlefish in South Australian waters' (Gillanders et al. FRDC 2013/010) is currently addressing these knowledge gaps. Within these overarching objectives a number of more focussed research priorities were identified and constitute the basis of this study. They are: (1) to undertake a survey that estimates population abundance, habitat condition and water quality at the major spawning location; (2) to explore whether there are alternate pockets of spawning activity within northern Spencer Gulf; (3) to investigate whether spawning Giant Australian Cuttlefish prefer certain den dimensions in which to lay eggs with the expectation that this information can be used to design and develop artificial spawning habitats; (4) to undertake residue testing of Giant Australian Cuttlefish tissues to determine their susceptibility to coastal contaminants; and (5) to quantify the Giant Australian Cuttlefish by-catch from commercial fishing within Spencer Gulf.

1.2 Need

Given the significance of the Point Lowly Giant Australian Cuttlefish population there is a need to provide a robust assessment of its annual status to inform management and the general public. Currently, management has initiated a spatial closure for upper Spencer Gulf (north of Wallaroo) as a precautionary measure to ensure that the Giant Australian Cuttlefish population is not unnecessarily compromised by commercial and recreational fishing.

Although, fishing has not been specifically identified to detrimentally affect the population it was the most amenable factor to control. It is therefore important to assess the relative status of the Point Lowly Giant Australian Cuttlefish population to inform management and assist in the development of the most appropriate management strategies. Quantifying Giant Australian Cuttlefish by-catch in association with this closure will provide greater resolution in regard to fishing pressure.

There is also a need to determine whether there are alternate spawning grounds for the Giant Australian Cuttlefish in northern Spencer Gulf to determine the relative conservational significance of Point Lowly and whether other areas within the region may require additional management consideration. It is clear that Giant Australian Cuttlefish aggregate on the reef fringing Point Lowly, however, the specific characteristics and preferred dimensions of their dens and spawning substrate is unknown. For example, the preferred orientation, surface texture, depth range and exposure of natural spawning dens are not understood. Also there is a requirement to understand whether coastal pollutants play a role in shaping the distribution and relative abundance of aggregating Giant Australian Cuttlefish. This level of information is required prior to the development and deployment of artificial spawning habitat that may be required to either mitigate habitat loss in the future or promote spawning in other areas where the habitat may be limited.

1.3 Objectives

1. To use the standard survey methodology described in Steer et al. (2013) to estimate Giant Australian Cuttlefish abundance and biomass of the Point Lowly spawning aggregation, characterise the spawning habitat and analyse the ambient water quality.
2. To explore and assess the potential of alternate Giant Australian Cuttlefish spawning areas in northern Spencer Gulf.
3. To characterise the natural spawning substrate during the 2013 spawning season. Use the 'natural spawning preference' information to design and develop artificial habitat with the intention of strategically deploying it in northern Spencer Gulf prior to the 2014 spawning season.
4. To assess whether there are abnormally high levels of metals accumulating in Giant Australian Cuttlefish in northern Spencer Gulf.
5. To determine the potential impact of fishing on Giant Australian Cuttlefish in northern Spencer Gulf.

2 GIANT AUSTRALIAN CUTTLEFISH ABUNDANCE AND BIOMASS SURVEY

MA Steer

2.1 Introduction

The 'cause' of the observed population decline is currently unknown and a range of potential factors have been considered and investigated in Steer et al. (2013). One hypothesis, however, relates to whether the current trend in the population reflects natural processes as cephalopod populations, in general, are renowned for their considerable fluctuations in abundance (Pierce and Guerra 1994). The extent of the temporal data that exists for the Point Lowly Giant Australian Cuttlefish population is relatively short and there has been no formal census of the spawning aggregation prior to 1998. Therefore, it is not certain whether the peak in abundance recorded in 1999 was a result of a population increase, or whether it was indicative of a natural population size that has persisted through time. This paucity of information highlights the requirement for an on-going, annual monitoring program to provide a greater understanding of the natural dynamics of the population.

Given the significance of the Point Lowly Giant Australian Cuttlefish population there is a need to provide a robust assessment of its annual status to inform management and the general public. In March 2013, management implemented a spatial closure for upper Spencer Gulf (north of Wallaroo) as a precautionary measure to ensure that the Giant Australian Cuttlefish population is not unnecessarily compromised by commercial and recreational fishing. Although, fishing has not been specifically identified to detrimentally affect the population, it was the most amenable factor to control. It is therefore important to assess the relative status of the Point Lowly Giant Australian Cuttlefish population to inform management and assist in the development of the most appropriate management strategies.

The relatively isolated stretch of rocky reef that fringes Point Lowly is considered to be an essential feature in attracting large numbers of spawning Giant Australian Cuttlefish to the area as it provides substrate upon which they can attach their eggs and seek shelter within northern Spencer Gulf. The proximity of this area to coastal urbanisation and industrial activity presents potential issues associated with water quality and eutrophication that may influence local productivity. As such, the historic Giant Australian Cuttlefish monitoring program was recently expanded to include an assessment of the relative condition of the spawning environment through routine characterisation of the habitat and analysis of the ambient water quality (Steer et al. 2013).

This Chapter aims to estimate Giant Australian Cuttlefish abundance and biomass of the Point Lowly spawning aggregation, characterise the spawning habitat, and analyse the ambient water quality for the 2013 and 2014 spawning seasons.

2.2 Methods

The methods have been extensively described in Steer et al. (2013). In summary, the survey used replicated 50 x 2 m belt-transects to determine the relative density and size of Giant Australian Cuttlefish across 10 sites that extend from False Bay to Fitzgerald Bay (Figure 2.1). An extra site, 'Backy Point', was included in the analysis to provide an indication of Giant Australian Cuttlefish activity outside of the main spawning area, however it was not considered in the overall estimate of abundance and biomass. Mean estimates of Giant Australian Cuttlefish density and weight (kg per m²) were calculated for each site and multiplied by the corresponding area of spawning substrate to provide an overall estimate of Giant Australian Cuttlefish abundance and biomass.

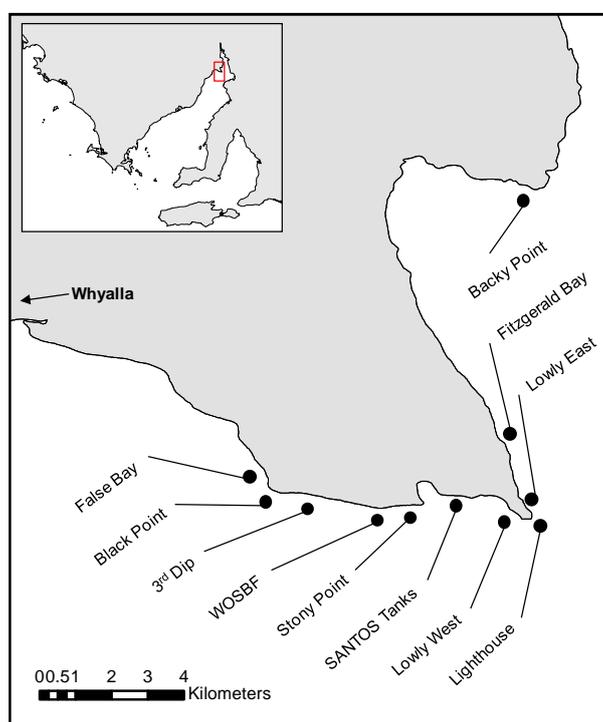


Figure 2.1. Location of survey sites fringing Point Lowly in Northern Spencer Gulf.

The habitat characteristics were determined from replicated underwater photo-quadrats taken along each of the belt-transects. From these digital images the percentage cover of the various algal functional groups and benthic invertebrates were quantified using image analysis software.

Replicated water samples were collected from each site and the concentration of inorganic (total ammonia) and total (nitrogen and phosphorus) nutrients, and chlorophyll *a* was analysed. A series of temperature loggers were deployed in northern Spencer Gulf (Plank Shoal, Western Shoal and Ward Spit) in October 2005 as part of an un-related project (see

Saunders 2009). Additional loggers were deployed at Black Point and Backy Point in July 2012 and were set to record hourly temperature. The time series of temperature data presented in this study has been extracted from two spatially separate (~20 km) data loggers from northern Spencer Gulf (i.e. Plank Shoal June 2009 – July 2012 and Black Point July 2012 onwards). Data were aggregated and presented as weekly averages. Previous comparisons of temperature data collected from over a 60 km area had shown similar seasonal trends (Saunders 2009). Trends in water temperature from June 2009 onwards were investigated in relation to the key developmental periods (i.e. embryonic development period and early life history) of Giant Australian Cuttlefish on the spawning ground.

Giant Australian Cuttlefish abundance and biomass surveys were undertaken in late May, mid June and early July in 2013 and 2014. The peak in spawning activity across the three monthly surveys provided the annual estimates of abundance and biomass for the 2013 and 2014 spawning seasons, respectively.

2.3 Results

2.3.1 Giant Australian Cuttlefish Abundance and Biomass

Over the past two years the density of Giant Australian Cuttlefish has displayed a clear, decreasing trend from west to east along the Point Lowly Peninsula. The 4 km stretch of reef extending from False Bay to Stony Point constituted the main spawning area with Giant Australian Cuttlefish densities peaking at 0.08 and 0.20 cuttlefish/m² in 2013 and 2014, respectively. Small pockets of spawning activity, consisting of <0.04 cuttlefish/m², were recorded within Fitzgerald Bay and Backy Point (Figure 2.2). Given the patchy distribution of Giant Australian Cuttlefish, as exemplified by the wide error variances surrounding each mean, no clear temporal trend was detected in 2013 ($F_{2, 188} = 0.55$, $p = 0.58$). This temporal stability was also evident in the overall estimate of Giant Australian Cuttlefish abundance, where the spawning population peaked at 13,492 individuals in June, which was moderately larger than the May and July estimates by 17.9% and 9.7%, respectively (Figure 2.3). Despite a marked increase in the overall density of Giant Australian Cuttlefish across the entire spawning area in 2014, particularly at False Bay, Black Point and West of the Santos Boundary Fence (WOSBF) in May and June where average density exceeded 0.1 cuttlefish/m², the general patchiness of the spawning activity continued to make it difficult to detect any temporal trends in aggregative behaviour ($F_{2, 188} = 3.1$, $p = 0.05$). The overall estimates of Giant Australian Cuttlefish abundance in 2014 indicated that, like 2013, the spawning population peaked in June at 57,317 individuals, which was marginally (6%) more than the May estimate and subsequently declined by 40% in July. Peak spawning was observed to occur in June in 2013 and 2014.

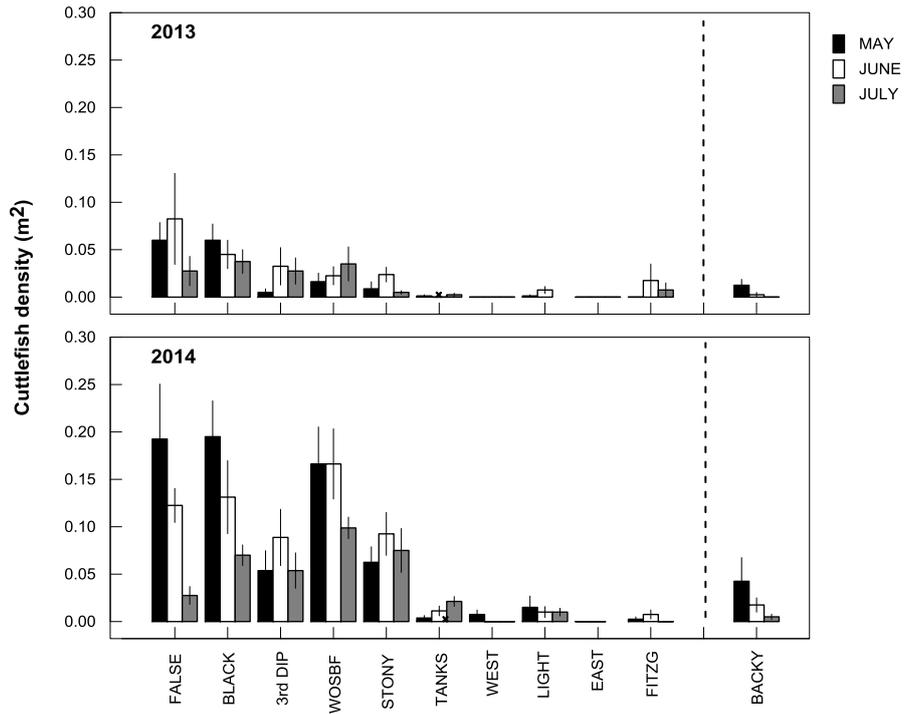


Figure 2.2. Monthly mean Giant Australian Cuttlefish density (\pm se) across each survey site for the 2013 (top) and 2014 (top) surveys. Note, data presented for Backy Point have not been included in the overall estimates of abundance.

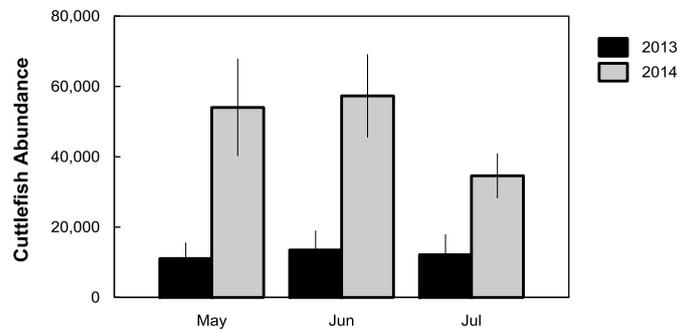


Figure 2.3. Extrapolated estimates of total Giant Australian Cuttlefish abundance (\pm se) for each month of the 2013 and 2014 surveys.

Estimates of peak Giant Australian Cuttlefish abundance over the last two years has indicated that the population declined to a historically recorded low of 13,492 individuals in 2013 before increasing to 57,317 in the following season. The 2013 estimate marked the culmination of a general population decline recorded over a 13-year period, from a peak of 182,585 cuttlefish in 1999. Within this period, from 2009 to 2013 the population reduced at an exponential rate (Figure 2.4). This trend, however, did not continue in 2014 as the population increased by 325% over the past year, providing evidence of the first increase in Giant Australian Cuttlefish abundance recorded since 2010. Although, this indicates that

2014 was a relatively strong recruitment year, the increase can only be considered moderate, as it represents 32% of the peak observed in 1999.

The decline in the annual estimate of biomass was most pronounced in 2013, with its estimate of 6.8 t representing a 96.8% reduction from the historic peak of 211.1 t in 1999, and a 37.7% decline in comparison to the previous year's estimate of 10.9 t (Figure 2.4). This decline in biomass, particularly from 2011 to 2013, has been driven by a truncation of the size composition of the spawning population. The average size of both males and females during these three years was considerably smaller in comparison to most years (Figure 2.5). This, however, was not maintained in 2014 as the average size of Giant Australian Cuttlefish measured on the spawning grounds significantly increased for both sexes (Males: $F_{11,12705} = 140.77$, $p < 0.01$; Females: $F_{11,3725} = 154.92$, $p < 0.01$), measuring 203.4 and 166.6 mm (ML), respectively. Such change in the overall size composition of the spawning population contributed to a 589% increase in biomass to 47.1 t in 2014 (Figure 2.5). The composition of the spawning population in 2013 was reminiscent of the late 1990s where females constituted approximately 22% (4.5:1) of the population. This has declined from a peak of 31% (3.2:1) in 2012 (Figure 2.5).

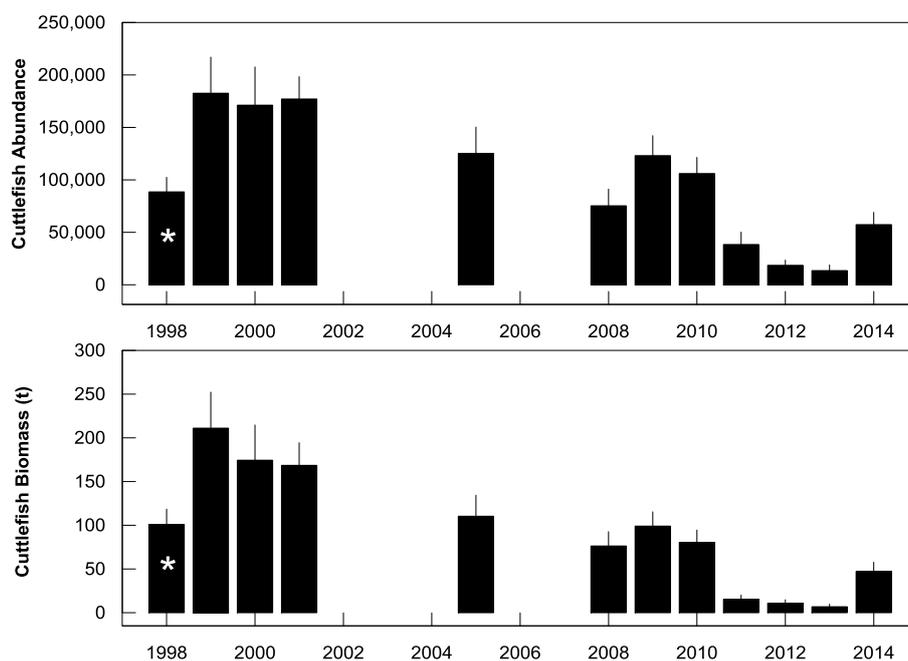


Figure 2.4. Annual estimates of total abundance and biomass (\pm SD) of Giant Australian Cuttlefish aggregating around Point Lowly during peak spawning from 1998 to 2014. * The fishing closure was not implemented until 1999, therefore the 1998 estimates were reflective of a population that was heavily fished. Historic data obtained from Hall and Fowler 2003.

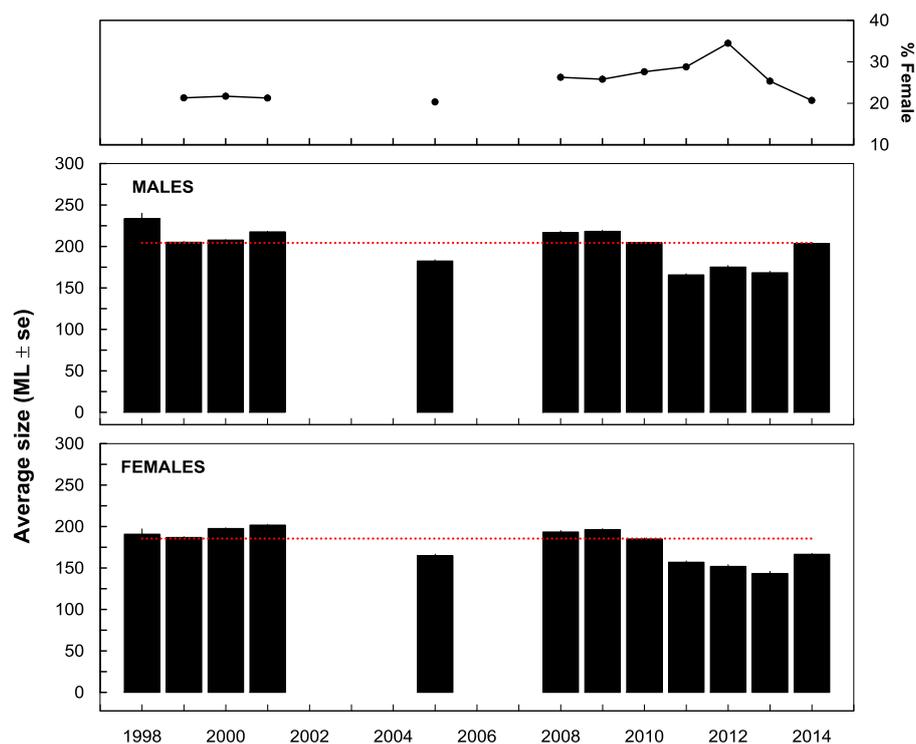


Figure 2.5. The population sex ratio presented as the percentage of females (top). The average size of Giant Australian Cuttlefish (\pm se) for males (middle) and females (bottom) from 1998 to 2014. The red line represents the overall average.

2.3.2 Habitat Characterisation

Four algal functional groups, highly branched robust algae (BRBRANCH); brown foliaceous (BRFOLI); green foliaceous (GFOLI); and *Hinckia* sp. (HINCK), were the most prominent throughout the survey generally accounting for >50% of the relative cover. The sea urchin (*Heliocidaris erythrogramma*) was the most common benthic invertebrate frequently observed at abundances >2 per m² across most of the sites. Although there was a diversity of other algal groups and benthic invertebrates observed throughout the survey (e.g. lobed green algae, membranous brown algae, gastropods, sponges and ascidians), their relatively low abundance precluded them from detailed analysis, particularly given that it is the large-scale temporal and spatial changes in the habitat characteristics that are of most interest in assessing the overall condition of the spawning environment.

A multivariate comparison of the relative cover/abundance of the key functional groups across the survey sites, months and years sampled indicated that there was significant variation in the habitat composition (Year*Site*Month: Wilks' $\lambda = 0.49$, $F = 1.61$, $p = 0.001$). The relative coverage of HINCK displayed the greatest variation throughout the surveys ($F_{15, 217} = 2.65$, $p = 0.001$). This was evident in 2014 where its relative coverage, ranged from <1% to >40% in four of the key spawning sites (Black Point, 3rd Dip, WOSBF and Stony

Point) (Figure 2.6). The pattern of this variation appeared to relate to a mid season (June) decrease, flanked by months of dense coverage (Figure 2.6). This pattern was also observed at the Tanks site, but was less pronounced. Unlike 2014, the relative coverage of HINCK in 2013 rarely exceeded 20% and the spatio-temporal pattern was unclear (Figure 2.6).

BRBRANCH was the most dominant algal function group covering >30% of the area surveyed at most sites. Its coverage was consistently greatest at Tanks and Fitzgerald Bay across both years, however the magnitude of the cover was highly variable ranging from 30% to 80% throughout the surveys ($F_{15, 217} = 1.82$, $p = 0.04$). Clear spatio-temporal differences in BRBRANCH coverage was also observed at Point Lowly West particularly during the June and July surveys in 2013 and the July survey in 2014 where the relative coverage accounted for <1% of the surveyed area (Figure 2.6).

With the exception of a relatively dense coverage at Point Lowly East in May 2013 (>65%) small patches of GFOLI were evident throughout most sites in both years (Figure 2.6). Although the average coverage of these patches often contributed to ~20% of the area surveyed they were typically highly variable and exhibited no clear spatio-temporal trend.

BRFOLI peaked at 36% at Stony Point during May 2013 and remained <20% for the other sites. The average area covered by this algae was typically highest during the beginning of the spawning season (May and June) for both of the surveyed years (month: $F_{2, 217} = 1.82$, $p = 0.04$).

The relative abundance of sea urchins varied across sites and between the two survey years ($F_{9, 217} = 6.58$, $p = 0.06$). They were common at all sites west of the Point Lowly Lighthouse, generally exceeding 2 per m^2 , whereas they were infrequently observed at the eastern most sites (Figure 2.7). With the exception of the False Bay, Stony Point and Tanks sites, urchin abundance was markedly higher in 2014, with average estimates exceeding the previous year by approximately 1.5 urchins per m^2 (Figure 2.7).

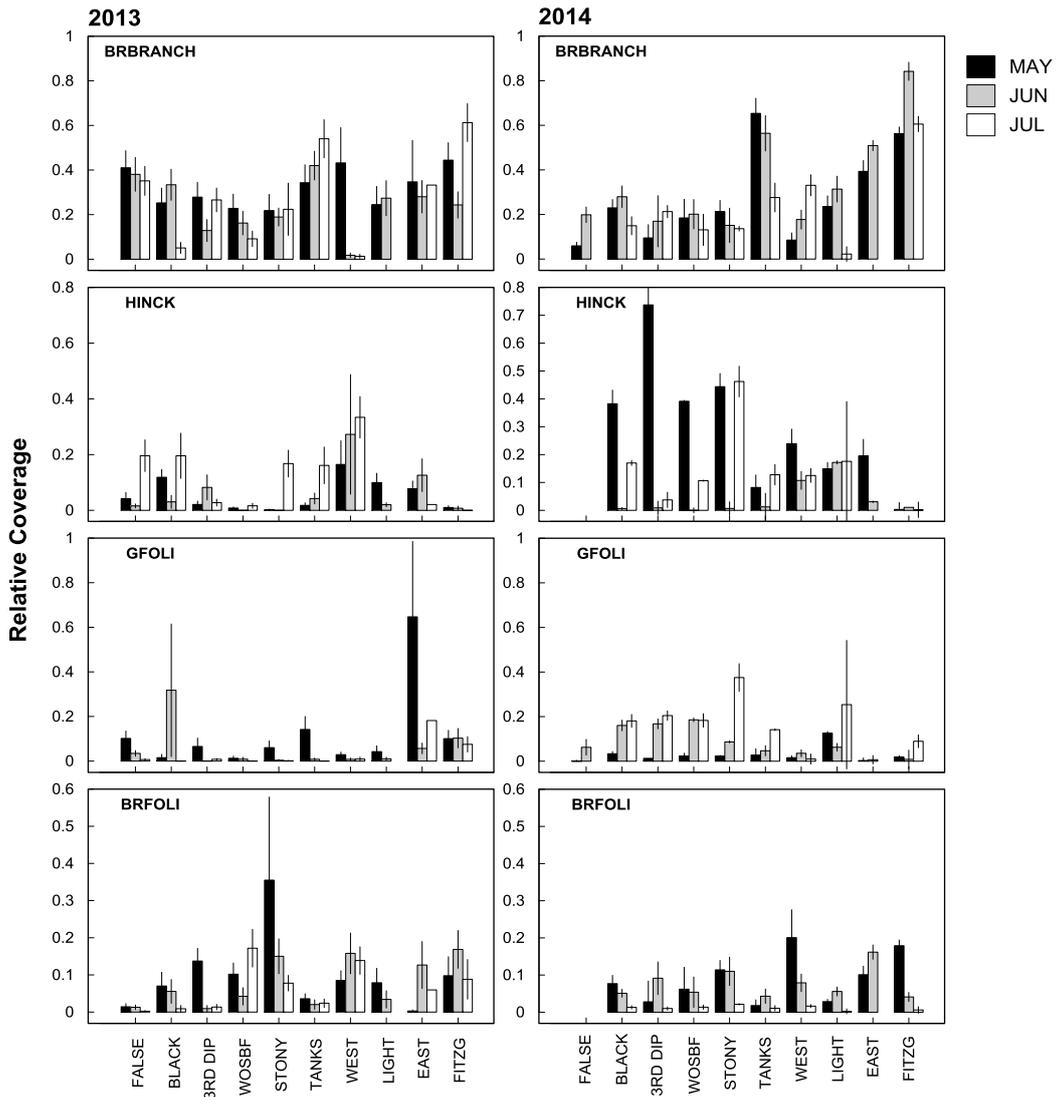


Figure 2.6. The average relative coverage (\pm se) of the four main algal groups across each survey site for the 2013 (left) and 2014 (right) surveys. Highly branched robust algae (BRBRANCH); brown foliaceous (BRFOLI); green foliaceous (GFOLI); and *Hincksia* sp. (HINCK).

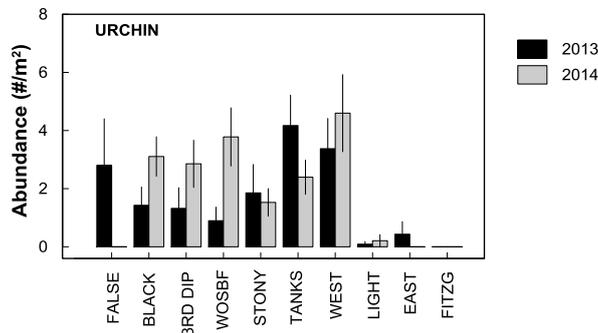


Figure 2.7. The average relative coverage (\pm se) of sea urchins across each survey site for the 2013 and 2014 surveys.

2.3.3 Ambient Water Quality

No clear spatial variation in total nitrogen and phosphorus was detected within each of the surveys, as all sites shared similar ambient concentrations, and were therefore indicative of an over-all 'broad-scale' Point Lowly profile. This profile, however, varied considerably through time, but did not conform to a clear seasonal pattern (significant Month*Year interactions). For example, the average concentration of total nitrogen was relatively high (>0.15 mg/L) in June 2013, but did not follow the same trend in June 2014 where the concentrations were at their lowest (<0.1 mg/L) (Figure 2.8). Similar aseasonal variation was seen for phosphorus (Figure 2.8).

The coastal water surrounding Point Lowly was generally devoid of ammonia throughout the two winter surveys, as in most cases ambient concentration levels did not exceed the detection limits (0.005 mg/L) of the analytical equipment. Concentration levels only exceeded the detection limits at six sites in June 2013, ranging from 0.0051 mg/L at Fitzgerald Bay to 0.0072 mg/L at Point Lowly West (Figure 2.8).

The raw chlorophyll concentration data were presented for each survey site as only a single filtered sample was collected. There was a general west to east increase in the concentration of chlorophyll in 2014, from approximately 0.5 ug/L at False Bay to 1.8 ug/L at Fitzgerald Bay (Figure 2.8). This was particularly evident in June and July and in contrast to 2013 where there was no clear spatial or temporal pattern.

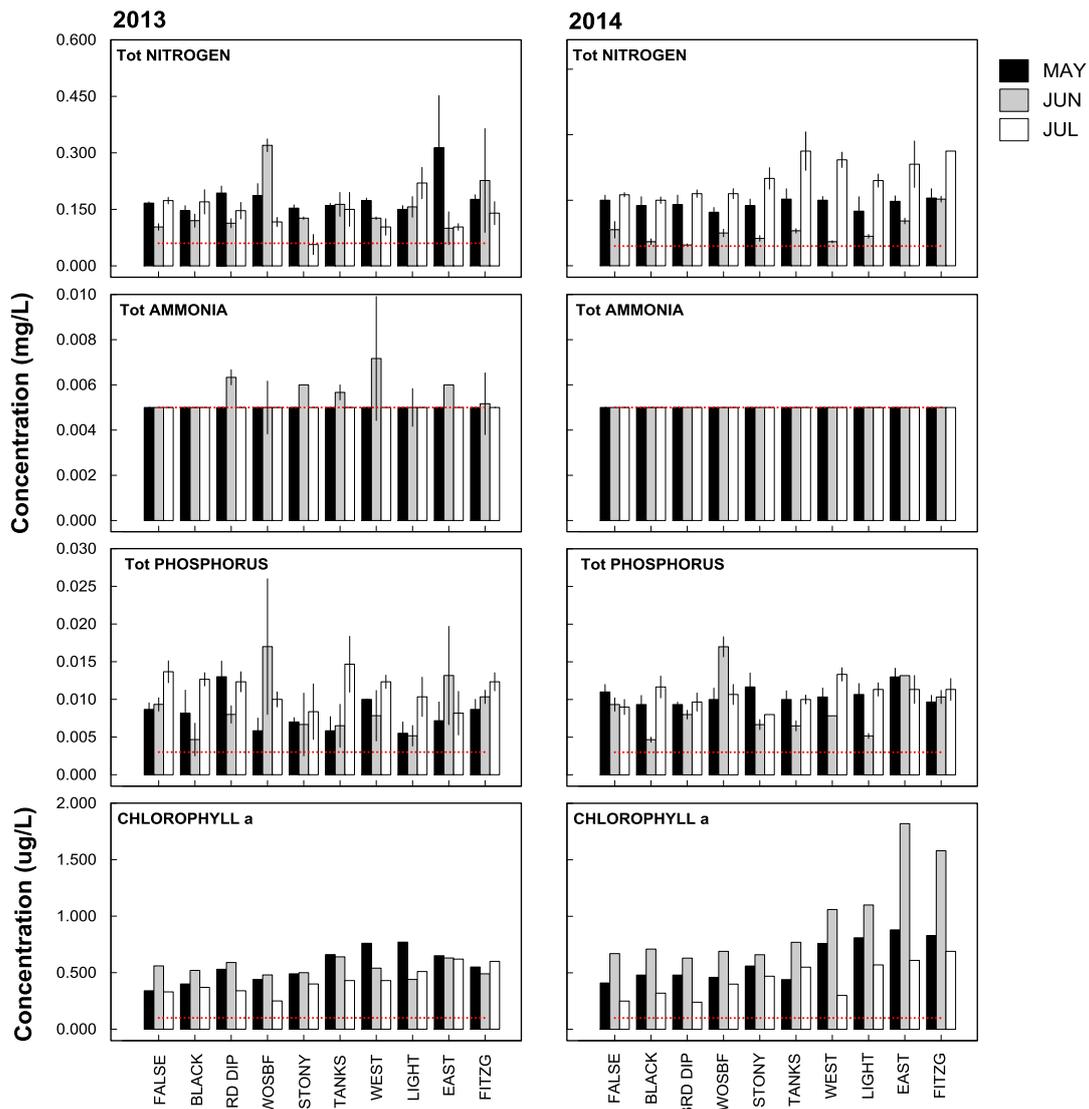


Figure 2.8. The average concentration (\pm se) of total nitrogen, ammonia, phosphorus and Chlorophyll a (note single value with no error variance) across each survey site for the 2013 (left) and 2014 (right) surveys. The red line indicates the limits of detection of the analytical equipment.

2.3.4 Temperature Profile

The weekly averaged summer maxima of 25.8°C in 2014 was the highest recorded over the past five years (Figure 2.9). Similarly the preceding winter minimums in 2012 and 2013 were moderately warm, remaining above 13°C for the first time since 2009. Peak spawning, over the past five years, occurred when winter temperatures dropped below 17°C and consequently the development of the resultant eggs and embryos occurred during the coldest time of the year (Figure 2.9). The duration of embryo development during this time is estimated to take approximately 120 days (Hall and Fowler 2003). The daily averaged temperature regime during this critical 120 day developmental period varied considerably over the past five years (Figure 2.10). The most recent cohort (2013) experienced the

warmest conditions, particularly during the later stages of development (i.e. >70 days) where average daily temperatures accelerated from 13.3°C to 18.5°C over a 37 day period (Figure 2.10). Relatively large temperature pulses were also observed during this time, with embryos regularly experiencing rapid 2°C changes over four day periods (Figure 2.10). Conversely, the 2010 cohort experienced a gradual and relatively symmetrical temperature profile declining from 15.6°C to 12.6°C over 74 days and then returning to 15.6°C by day 120 (Figure 2.10). The 2009 and 2011 cohorts experienced similar temperature profiles, whereas the 2012 cohort initially experienced relatively cool temperatures (<14°C) over the first 40 days before rapidly increasing to 18.1°C by day 114 (Figure 2.10).

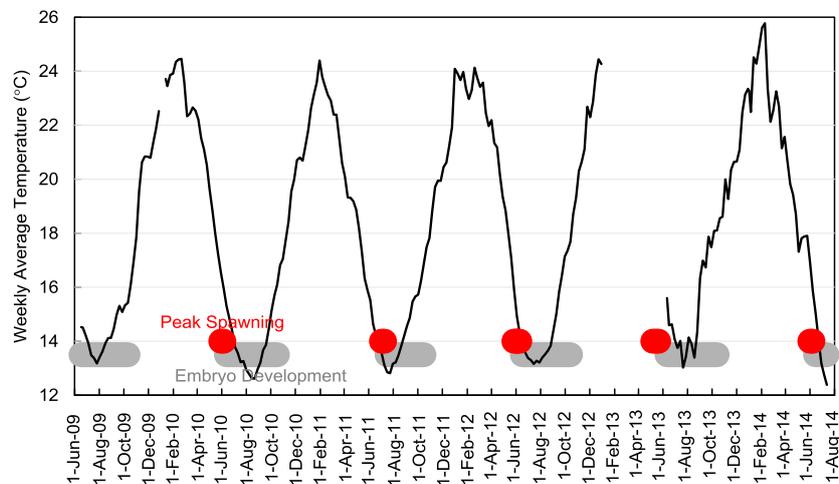


Figure 2.9. The weekly average temperature profile of Northern Spencer Gulf. Estimated periods of peak Giant Australian Cuttlefish spawning (red) and 120 day embryo development (grey) are overlaid.

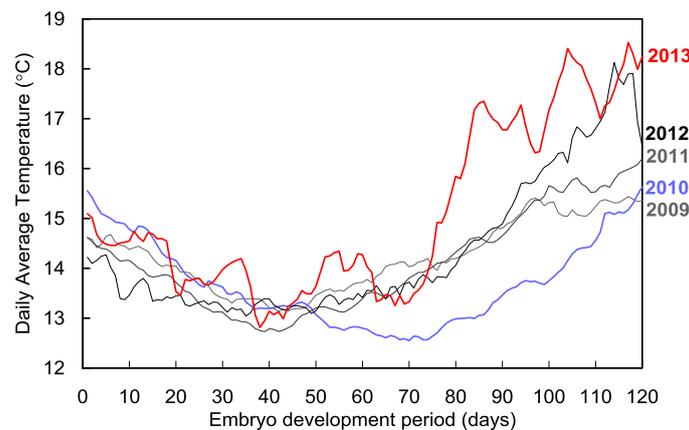


Figure 2.10. The daily average temperature profile of the first 120 days of embryo development over the past five seasons.

2.4 Discussion

The most recent increase in the spawning population after a steady period of decline, coupled with the return of larger animals, indicated that 2013/2014 was a relatively favourable year for Giant Australian Cuttlefish growth and survival. It is widely acknowledged within the cephalopod literature that changing environmental conditions (especially temperature) throughout the early life history stages, results in different conditions for growth and consequently individual size in the population (Forsythe 1993; Boyle and Rodhouse 2005). Both laboratory and field studies have demonstrated that warming temperatures during the early life history phase accelerates growth and confers survival, provided that temperatures do not exceed beyond the species' thermal tolerance and food is not limited (Forsythe 1993). Indeed, the increased relative abundance and larger individual size of the 2014 aggregation experienced relatively warm temperatures during its early critical developmental period (2013). Conversely, the 2010 cohort experienced the coolest conditions and resulted in the greatest annual reduction of spawning abundance (63%) and biomass (79%) in 2011. Although, temperature is often considered the governing environmental factor that shapes cephalopod populations, there have been other potential influencing factors identified in the past. These include, but are not limited to: increased nutrient loading; continued pressure from fishing (see Chapter Six for a detailed investigation); or repercussions from an altered mating system.

There were no large-scale temporal and/or spatial changes in the relative condition of the Point Lowly spawning habitat over the past two years. Similarly, the ambient water quality appeared to be relatively consistent throughout the surveys and typical of the area (EPA pers comm.). Rapid 'opportunistic' blooms of *Hinckesia sordida* have been suggested by local divers to prevent Giant Australian Cuttlefish from spawning in the area in the past or interfere with embryo development as a result of increased fouling of the egg capsules (Steer et al. 2013). *Hinckesia sordida* is a fast growing macroalgae that rapidly blooms in response to coastal eutrophication and favourable temperatures. As such, it can be considered a relatively useful barometer of 'real-time' environmental conditions (Campbell 2001). The coverage of *Hinckesia* in 2013 was relatively sparse (i.e., <20%), particularly on the western end of the Point Lowly peninsula where most of the spawning occurred. Similarly, its relative coverage was lowest during the peak spawning period (June). When present, this opportunistic alga rapidly absorbs soluble nutrients (ammonia and nitrogen) from the water column, therefore the moderately increased concentration of nitrogen and ammonia during the 2013 water sampling program further corroborated the lack of *Hinckesia* in the area. It is not known whether *Hinckesia* is a limiting factor, or at what density it is required to reach to inhibit spawning activity and hatching success, but it appeared to have

had a negligible effect on the 2013 spawning population and subsequent, successful recruitment. The relative coverage, however, increased significantly throughout the main spawning sites in 2014, peaking above 30% in May. Next year's (2015) population estimate may provide some insight as to whether the 2014 *Hincksia* bloom was significant enough to warrant further investigation.

It has been previously suggested that the erosion of the spawning biomass through an initial period of high exploitation (i.e., late 1990s) may have compromised the effectiveness of the mating system through the removal of larger, and potentially 'fitter' individuals from the population (Hall pers. comm.). Despite the virtual elimination of commercial fishing from the main spawning area in 1999, legal fishing in adjacent areas may have continued to contribute to an already weakened mating system. The relative impact of the commercial fishing on the Giant Australian Cuttlefish population, however, has been negligible since the implementation of the initial 1998 closure, with a recent study unable to associate the decline in abundance with fishing (Steer et al. 2013). A more recent investigation also indicated that the commercial by-catch of Giant Australian Cuttlefish, which has previously been unquantified, has also had a negligible effect on the spawning population (see Chapter Six for more detail). Given the short lifespan (approximately annual) of Giant Australian Cuttlefish, the spawning population has progressed through 16 generations since the period of high exploitation. Although the prolonged population effect is unknown, the recent increase in the size of the spawning aggregation, in association with a marked increase in the average individual body size and the return of the 5:1 female bias in the population, indicates that the contemporary mating system is reminiscent of the 1999 population structure.

3 IDENTIFICATION OF ALTERNATE SPAWNING AGGREGATIONS

MA Steer

3.1 Introduction

The continuous rocky reef that fringes Point Lowly is considered to be the only area capable of supporting high densities of spawning Giant Australian Cuttlefish in northern Spencer Gulf as the remaining coastline is largely dominated by mangroves, tidal flats and salt marshes. However, the possibility of Giant Australian Cuttlefish aggregating to spawn elsewhere, or widely distributing their spawning activity within Spencer Gulf, needs to be considered as an alternate hypothesis in explaining the decline of the Point Lowly population.

In recent years, several coastal residents and local fishers have reported large quantities of Giant Australian Cuttlefish turning up in areas where they were not expected. However, it is not known whether these animals were actively spawning. Point Douglas and Two Hummock Point, which are located approximately 30 km north of Point Lowly, are two areas where locals have indicated high numbers of Giant Australian Cuttlefish in 2011 and 2012. Similarly, commercial and recreational fishers have reported increased catches of Giant Australian Cuttlefish around Port Augusta during the 2012 spawning season. Point Riley (approximately 6 km north of Wallaroo) has also been reported to support commercial quantities of Giant Australian Cuttlefish in the past. Given these anecdotal reports it can be speculated that there may be other areas within northern Spencer Gulf that can accommodate smaller pockets of spawning Giant Australian Cuttlefish, similar to those observed in Backy Point and Fitzgerald Bay, and more typical of *S. apama* that occurs outside of northern Spencer Gulf (Rowling 1994) and other cuttlefish species worldwide (Hanlon and Messenger 1996).

So far the research on the aggregation around Point Lowly has overshadowed any attempt to explore and document other 'less productive' spawning areas within northern Spencer Gulf to determine their relative contribution to the overall population. If Giant Australian Cuttlefish have a strong propensity to return to their natal area to spawn then it is possible that other spawning areas may have become more productive over the past few years and have accounted for a greater proportion of the spawning population, resulting in a more diffuse spawning pattern and reducing the dominance of the Point Lowly aggregation. If movement and migration patterns are more passive, then unknown changes in the local hydrodynamics or proximate cues may have directed Giant Australian Cuttlefish away from Point Lowly to spawn elsewhere.

There is a need to determine whether there are alternate spawning grounds for the Giant Australian Cuttlefish in northern Spencer Gulf to determine the relative significance of Point

Lowly and whether other areas within the region may require additional management consideration. The aim of this study was to target specific areas within northern Spencer Gulf to search for spawning Giant Australian Cuttlefish during the 2013 spawning season.

3.2 Methods

3.2.1 Video Surveys

A towed waterproof video camera has been used to film Giant Australian Cuttlefish and successfully characterise their spawning habitat in the past (Steer et al. 2013). Although diver-based surveys are the preferred method to accurately estimate Giant Australian Cuttlefish abundance, the use of remote video is a more effective method in undertaking large scale exploratory surveys.

Using digitised habitat maps (Bryars 2003) and aerial photography (e.g. Google Earth), four zones within northern Spencer Gulf were identified to share similar characteristics to known Giant Australian Cuttlefish spawning grounds (Figure 3.1). Each zone was visited during early June (2013) to coincide with the most likely peak in spawning activity in the area. Numerous potential spawning sites were identified on-site within each of the four zones. The criteria used to identify these sites included their coastal geography (i.e. boulderous/rocky coast), level of exposure to the prevailing conditions (i.e. medium to low energy environments), depth range (1 – 8 m), and/or advice by local fishers. A towed waterproof video camera secured within a protective cage was used to search for Giant Australian Cuttlefish at each site. The video camera was connected to a portable digital recorder integrated with a GPS system, depth transducer and a GeoStamp[®] audio encoder capable of recording continuous time and positional data. The video camera was mounted at a 45° angle and lowered over the side of the vessel to approximately 0.5 m above the sea floor. The camera's field of view was estimated to cover an average width of approximately 1.6 m (Steer et al. 2013). The vessel drifted over the habitat and the depth of the camera was manually adjusted in relation to the benthic topography. The duration of each survey depended on the extent of the available habitat within each site.

All digital footage was replayed through a computer monitor and the GPS position, depth and time was recorded for each encountered Giant Australian Cuttlefish. The habitat was also qualitatively assessed as being either 'optimal' or 'sub-optimal' spawning habitat. Optimal habitat was characterised on the basis of adequate rocky reef habitat that would accommodate spawning Giant Australian Cuttlefish, whereas sub-optimal habitat typically consisted of extensive sandy areas and/or seagrass meadows (Figure 3.2).

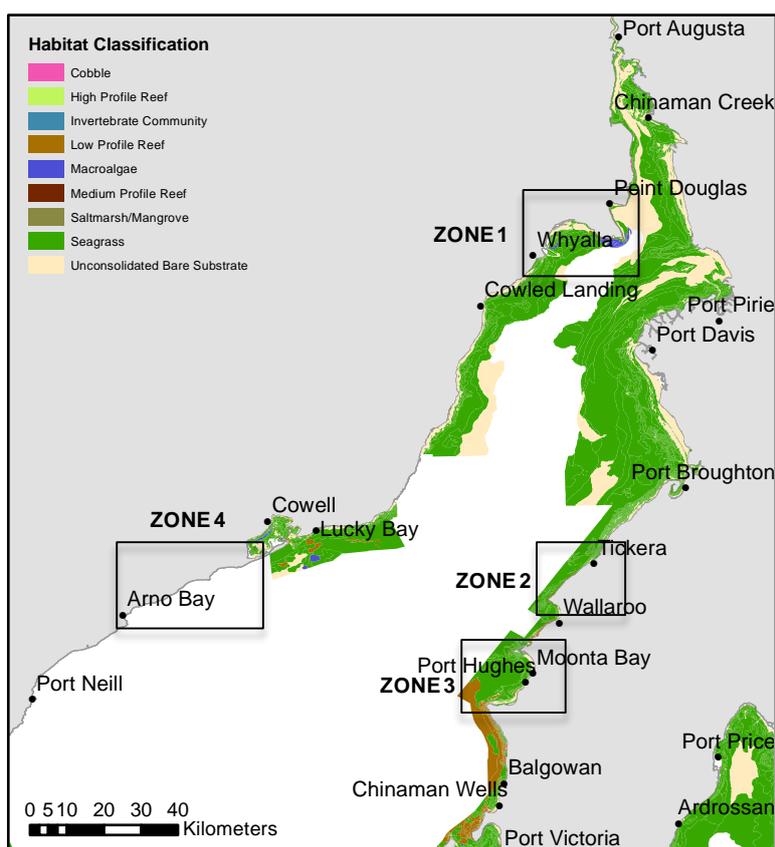


Figure 3.1. The extent of the exploratory video surveys partitioned into four zones within Northern Spencer Gulf.

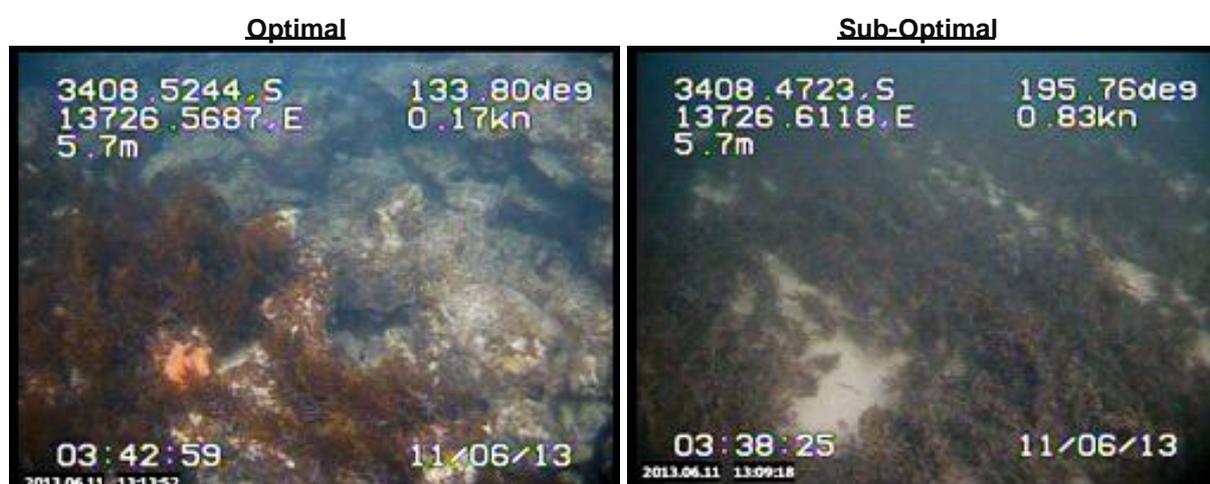


Figure 3.2. Video captured examples of 'optimal' (left) and 'sub-optimal' (right) spawning habitats.

3.3 Results

Overall, approximately 10.5 hrs of footage was recorded, covering an estimated 33.4 km². Of this, 19.3 km² (68%) was considered to be optimal spawning habitat. Giant Australian

Cuttlefish were only observed in Zone 1 and were confined to the known spawning grounds surrounding Point Lowly and Backy Point (Figure 3.3).

A total of 4:43 hrs of digital footage was recorded within Zone 1, covering 9.6 km². A total of 4.0 km² of this survey extended north of Backy Point and explored new areas outside of the known spawning ground. Most of this habitat (75.8%) consisted of dense seagrass and interspersed sandy patches and deemed sub-optimal for spawning Giant Australian Cuttlefish. Small areas of optimal reef (24.2%) were surveyed, however, no cuttlefish were detected (Figure 3.3). A total of 10 Giant Australian Cuttlefish were clearly identified in the remaining footage taken throughout the known spawning area extending from Black Point to Backy Point.

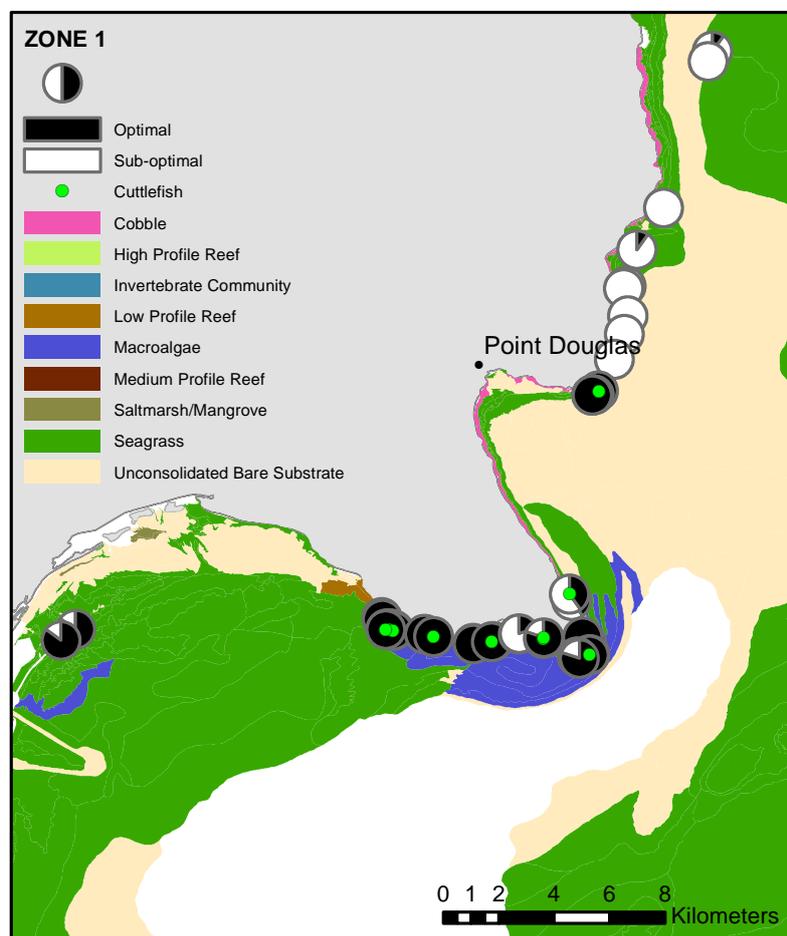


Figure 3.3. Location of tow video transects within Zone 1. Pie diagrams indicate the relative proportion of 'optimal' spawning habitat for each transect. Green dots indicate the presence of Giant Australian Cuttlefish.

A total of 1:45 hrs of footage was recorded within Zone 2 covering a total area of 4.9 km². The most optimal habitat was identified around Point Riley, consisting of reef that most closely resembled that of the key spawning habitat fringing Point Lowly (Figure 3.4). Despite the conducive spawning habitat no Giant Australian Cuttlefish were recorded in the area.

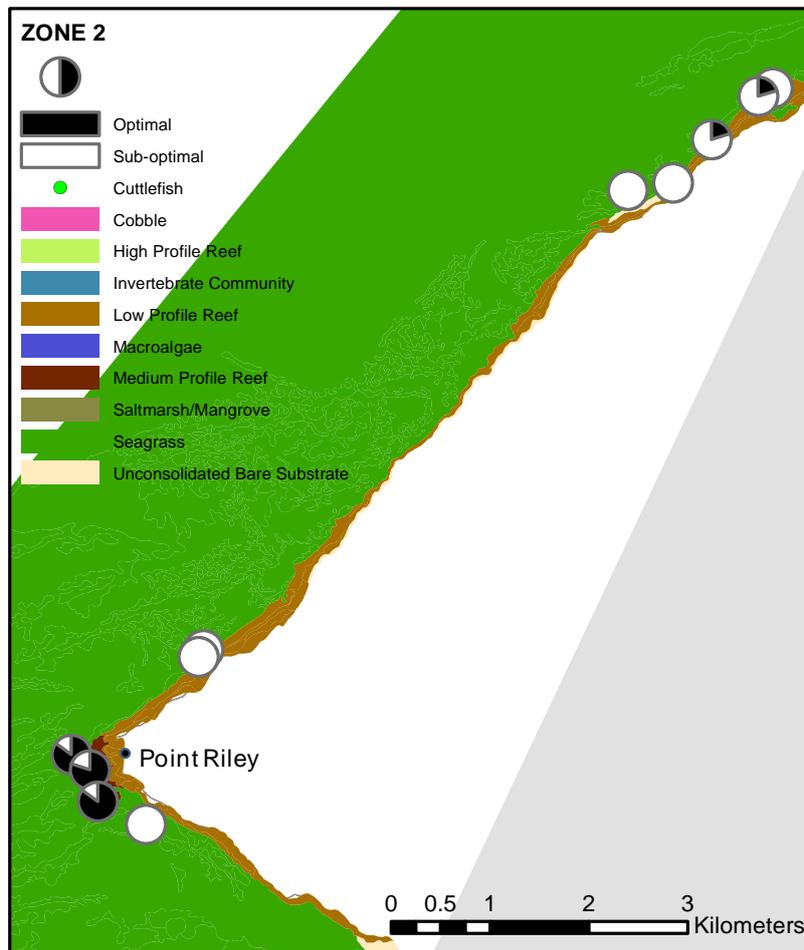


Figure 3.4. Location of tow video transects within Zone 2. Pie diagrams indicate the relative proportion of 'optimal' spawning habitat for each transect. Green dots indicate the presence of Giant Australian Cuttlefish.

Approximately 39% of optimal habitat was identified within 1:05 hr of footage that covered a total of 2.8 km² within Zone 3. Most of it was located around Cape Elizabeth (Figure 3.5). No Giant Australian Cuttlefish were detected.

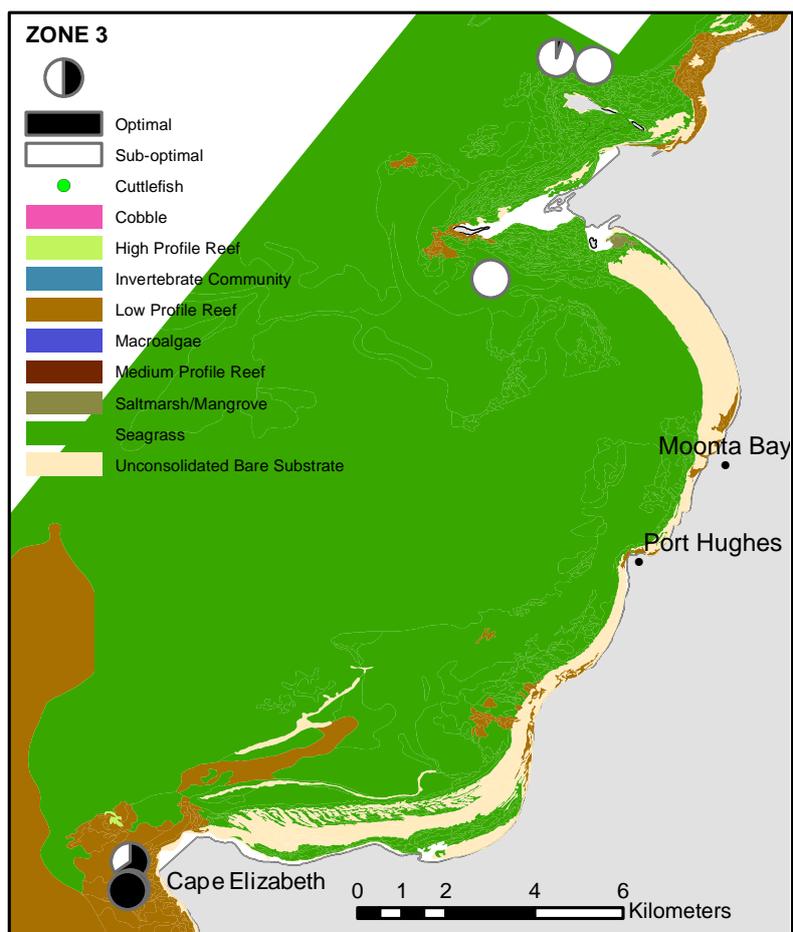


Figure 3.5. Location of tow video transects within Zone 3. Pie diagrams indicate the relative proportion of 'optimal' spawning habitat for each transect. Green dots indicate the presence of Giant Australian Cuttlefish.

Approximately 62% of the 16.1 km² surveyed in Zone 4 was considered optimal spawning habitat (Figure 3.6). No Giant Australian Cuttlefish were sighted.

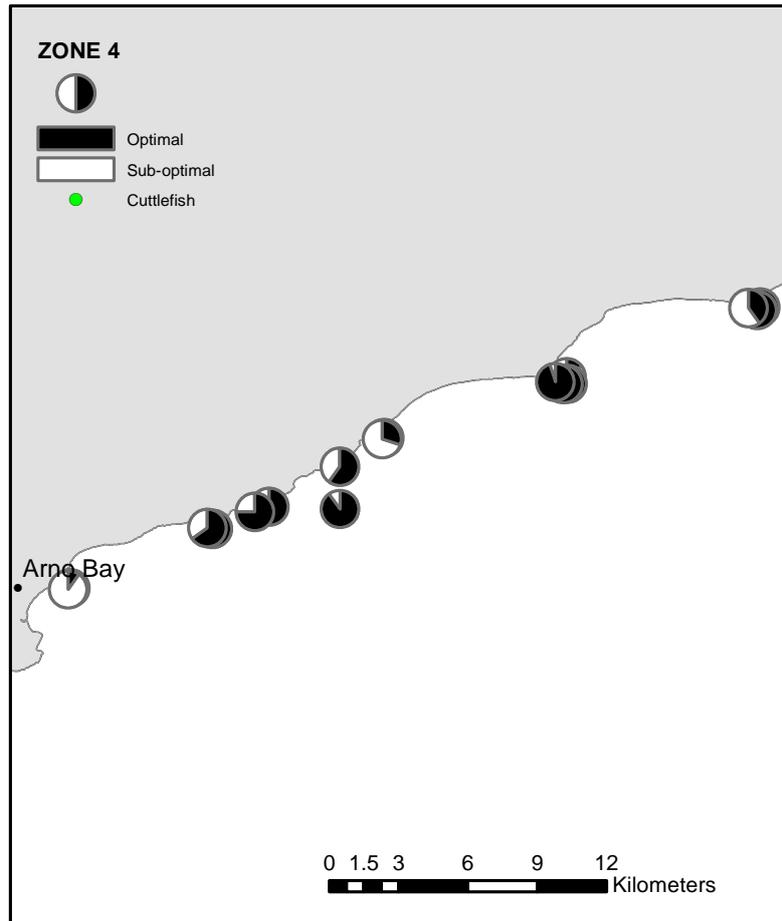


Figure 3.6. Location of tow video transects within Zone 4. Pie diagrams indicate the relative proportion of 'optimal' spawning habitat for each transect. Green dots indicate the presence of Giant Australian Cuttlefish.

3.4 Discussion

This exploratory survey found no evidence of Giant Australian Cuttlefish spawning activity outside of the known spawning grounds. Considerable effort was made to target the most likely areas within northern Spencer Gulf that could support spawning Giant Australian Cuttlefish and at a time when they were expected to be in peak reproductive condition (June). The survey, however, coincided with the lowest estimate of Giant Australian Cuttlefish abundance on record (see Chapter 2), but it was anticipated that if Giant Australian Cuttlefish were forming smaller pockets of spawning activity elsewhere within the region then they were likely to be detected by the towed camera. The camera successfully detected cuttlefish on the known spawning grounds confirming the effectiveness of the survey methodology.

Although no Giant Australian Cuttlefish were observed outside of the Point Lowly spawning grounds, this survey clarified the extent of available spawning habitat throughout northern Spencer Gulf. In particular, it reaffirmed that the continuous, shallow, boulderous reef that fringes Point Lowly is not widespread. This was exemplified by the difficulties encountered in our study to find similar habitats within the region. Despite anecdotal reports from local residents that aggregations of Giant Australian Cuttlefish have been observed north of the known spawning grounds, very little suitable spawning habitat was detected, as the area was dominated by extensive sandy environments and seagrass patches. Although no cuttlefish were observed in these four zones during this survey, this northernmost area may be temporarily inhabited by Giant Australian Cuttlefish but appears unlikely to support any substantial spawning activity. A similar expanse of sub-optimal spawning habitat that covered most of the shallow water environment on the north western side of the gulf between Port Augusta and Wallaroo was identified from archived habitat maps (Bryars et al. 2003) and aerial photography. Point Riley was the only area along this stretch of coastline that contained a continuous medium profile rocky reef reminiscent of the Point Lowly fringing reef. Most of this reef was surveyed and no Giant Australian Cuttlefish were observed. Commercial quantities (1 – 4 t) of cuttlefish have historically been taken from the area surrounding Point Riley (Marine Fishing Area 23, Zone 2), particularly during the late 1990s and early 2000s when cuttlefish were at their peak abundance (Hall and Fowler 2003). A similar exploratory video survey was undertaken in June 2012 which also extensively covered the Point Riley reef, and like the most recent survey, no Giant Australian Cuttlefish were detected (Steer unpublished data). Furthermore, divers unsuccessfully searched the area for Giant Australian Cuttlefish eggs in August 2013 and more recently in July 2014 as part of two companion studies (Gillanders et al. FRDC 2013/010; Chapter 4). Although no Giant Australian Cuttlefish have been observed on the reef in recent surveys, historic information suggests that it may have supported spawning aggregations in the past.

Optimal spawning habitat continued to be sparse south of Point Riley until Cape Elizabeth (Zone 3). Although this zone falls outside of the suspected range of the northern Giant Australian Cuttlefish 'sub-population' (Gillanders and Donnellan, unpublished data), it was still considered important to survey, particularly as it represented an area where the habitat appeared to become more conducive to spawning Giant Australian Cuttlefish. Cape Elizabeth marked the northern-most point of an extensive stretch of low profile reef that continued along the coast to Port Victoria. While the limited time and resources prohibited extending the exploratory survey further south, the time invested in surveying the optimal habitat adjacent to Cape Elizabeth failed to detect any Giant Australian Cuttlefish. Minor quantities of cuttlefish (<1.2 t) have been caught by commercial fishers in this region in the

past (Hall and Fowler 2003), so it remains possible that spawning may occur along this extensive low profile reef system.

The shallow water environment within Zone 4 had not been previously surveyed by either the National or State benthic mapping initiatives (Miller et al. 2014), consequently this component of the study was purely exploratory. Similar patches of low profile reef to that observed around Cape Elizabeth were observed amongst expanses of broken rocky bottom and dense macroalgae along the western side of the gulf extending north of Arno Bay. This habitat was most prominent within the shallow (<6 m) depth zone. Despite the habitat appearing optimal for spawning Giant Australian Cuttlefish, none were sighted. On site observation of high quantities of organic wrack that had accumulated along the intertidal zone indicated that the area was relatively exposed to high energy wave action. It is possible that although the habitat appeared optimal, the local oceanographic conditions may deter Giant Australian Cuttlefish from aggregating within the area. This may also explain why there have been virtually no reported catches of cuttlefish from commercial marine scalefish fishers operating within the area since 1996 (Hall and Fowler 2003).

4 USING ARTIFICIAL SUBSTRATE TO PROMOTE SPAWNING ACTIVITY

MA Steer

4.1 Introduction

The sub-tidal rocky reef fringing from Black Point to Point Lowly is unique in northern Spencer Gulf and its heterogeneous structure along with its west to east aspect, are likely to be the underlying factors that attract high densities of spawning Giant Australian Cuttlefish to the area. The plate-like fragmented slabs of bedrock that comprise the reef create numerous dens and crevices in which female Giant Australian Cuttlefish attach their eggs. These dens are vital for successful reproduction and recruitment as they provide both a stable structure for egg attachment over a long embryonic developmental period (up to four months) and a refuge for resultant hatchlings. Artificial structures have also provided suitable substrates for Giant Australian Cuttlefish to spawn, the most significant of which has been the OneSteel (formerly BHP) sea wall in Whyalla which has supported relatively high densities of Giant Australian Cuttlefish during the spawning season (Hall and Fowler 2003, Steer and Hall 2005). A pilot study undertaken by BHP Billiton as part of their Olympic Dam expansion Environmental Impact Statement (EIS) investigated the potential for establishing artificial habitat to mitigate habitat loss associated with the construction of a desalination plant at Point Lowly (BHP Billiton 2009). These artificial habitats, constructed from concrete pavers, were successful in attracting spawning Giant Australian Cuttlefish and provided an appropriate substrate for egg attachment at a time when cuttlefish were relatively abundant.

There is currently no evidence to suggest that habitat loss has contributed to the decline in Giant Australian Cuttlefish abundance as extensive habitat surveys carried out by SARDI and BHP Billiton during the 2012, 2013 and 2014 spawning seasons provided no clear indication that the spawning habitat has been structurally compromised (Steer et al. 2013; Chapter 2). Although spawning habitat does not currently appear to be a limiting factor for Giant Australian Cuttlefish aggregating around Point Lowly, there may be a requirement to provide an artificial alternative in the future. This requirement may be due to the mitigation of habitat loss through coastal development, or to be used to promote spawning in other areas where habitat may be limited.

It is clear that Giant Australian Cuttlefish aggregate on the reef fringing Point Lowly, however, the specific characteristics and preferred dimensions of their dens and spawning substrate is unknown. For example, the preferred orientation, surface texture, depth range and exposure of natural spawning dens are not understood. This level of information is required prior to the development and deployment of artificial spawning habitat that may be required to either mitigate habitat loss in the future or promote spawning in other areas

where the habitat may be limited. This component of research therefore aims to firstly characterise the natural spawning substrate during the 2013 spawning season, then use that information to design and develop artificial habitat and strategically deploy the structures throughout northern Spencer Gulf to investigate whether Giant Australian Cuttlefish utilised them as spawning substrate during the 2014 season.

4.2 Methods

4.2.1 Characterisation of Natural Spawning Substrate.

Two divers were deployed along the main spawning ground, extending from Black Point to False Bay, in June and July 2013 to search for dens containing Giant Australian Cuttlefish eggs. Once discovered, each den was digitally photographed and its dimensions measured *in situ*. Den measurements included: maximum width, height and depth of the main entrance; number of potential entrances; orientation of the entrance; water depth; and number of eggs present (Figure 4.1).

4.2.2 Deployment and Monitoring of the Artificial Habitat

The dimensions of the natural dens, subsequently informed the design and construction of the artificial 'reefs' (see Section 4.3.1). Three replicate artificial reefs were deployed at five sites within northern Spencer Gulf: Black Point; North Backy Point, Point Douglas, OneSteel Wall and Point Riley, in late March 2014 (Figure 4.2). These sites were selected as they were known to have either supported spawning Giant Australian Cuttlefish in the past or shared similar coastal geography and exposure to prevailing oceanographic conditions to known spawning areas. Each artificial reef was positioned on sand and orientated towards the incoming swell. All three reefs were placed within 50 m of each other to ensure they can be easily monitored during a single dive.

Artificial reefs deployed at the OneSteel Wall and Black Point were inspected by SARDI divers in late May 2014, coinciding with the routine Giant Australian Cuttlefish abundance and biomass survey (Chapter 2). Five Reef Watch volunteer divers inspected all artificial reefs on 21-22 September 2014. The timing of this inspection ensured that the artificial reefs were exposed to the entire 2014 spawning season. Each construction was examined for any evidence of spawning activity (i.e. presence of either developing eggs or hatched egg casings); presence of any other species; and integrity and condition of the structure.

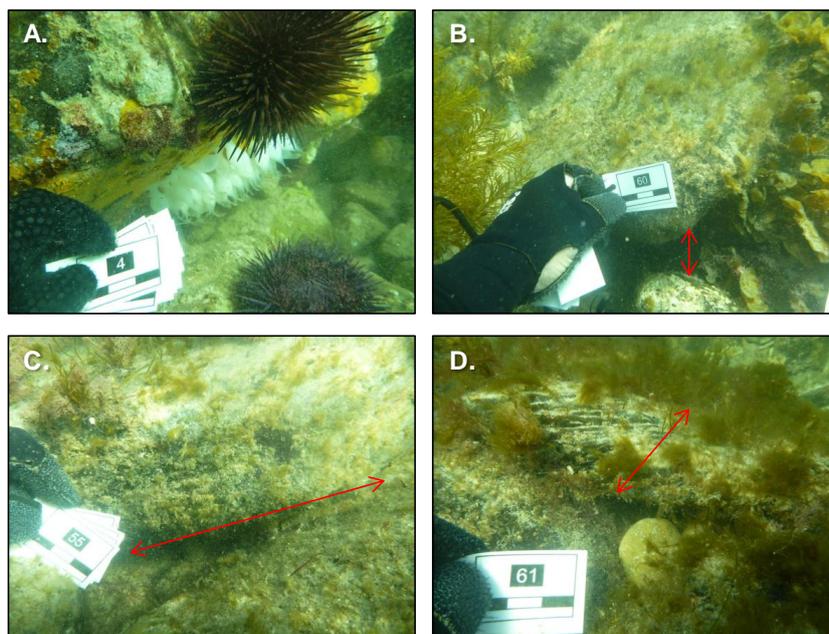


Figure 4.1. Examples of den measurements taken *in situ*. A. Identifying a successful den through the presence of Giant Australian Cuttlefish eggs. B. Arrow indicates the maximum height of the den entrance. C. Maximum width of the entrance. D. Maximum depth of the den.

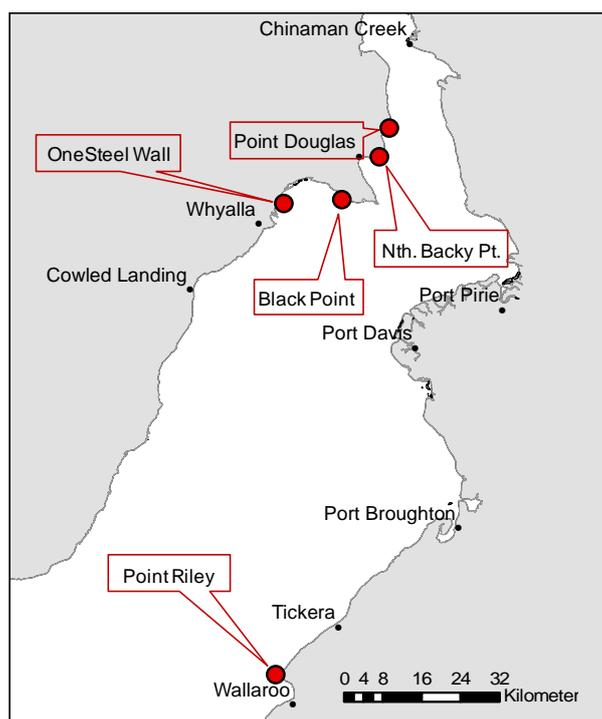


Figure 4.2. The sites within northern Spencer Gulf where the constructed artificial dens were deployed in 2014.

4.3 Results

4.3.1 Dimensions of Natural and Artificial Dens

The dimensions of 41 dens were measured, the majority of which (95%) were located within the shallow rocky reef (<5 m depth) extending along False Bay, whereas the remaining 5% were located within a similar depth at Black Point. Although eggs were observed in all of the inspected dens, it was not always possible to estimate the entire clutch size as it was often obscured from sight. When the entire clutch was visible they ranged in size from four to ~300 eggs, averaging 55.7 eggs per den. Most (90.2%) of the dens had an obvious single entrance, on average measuring a maximum of 13.4 cm high and 25.5 cm wide (Figure 4.3). The maximum depth of the dens was often difficult to measure, particularly when the structure of the rocky reef was complex; however, it was estimated to average 42.5 cm. The entrances of the dens were predominantly orientated towards the south eastern quadrant, with an average compass bearing of 158.9° (Figure 4.3).

Three concrete pavers (60 x 60 x 4 cm) and four bricks (23 x 11 x 8 cm) were assembled to form a discreet artificial reef (Figure 4.3). The concrete pavers were concertinaed together to provide two opposing dens with a maximum entrance height of 12.5 cm and 40 cm wide. The maximum depth of the den was 55 cm. These specifications conformed to the measured dimensions of the natural dens (Figure 4.3). The entire structure measured 60 x 60 x 30 cm and weighed approximately 150 kg.

4.3.2 Monitoring of artificial dens

During the May 2014 survey, paired Giant Australian Cuttlefish were observed at two of the three artificial dens deployed at Black Point. No eggs, however, were observed within the structure (Figure 4.3). All dens were successfully inspected on 21-22 September 2014. Seven of the 15 dens were damaged. Three had completely collapsed and four had slightly displaced sections (i.e. displaced brick). Most of the damaged dens were located at the OneSteel Wall and Point Riley sites. One of the dens at the OneSteel site appeared to be broken as a result of a fishing snag as fishing line was wrapped around the structure. The inspecting divers repaired all of the damaged dens.

A single Giant Australian Cuttlefish was observed occupying an artificial den at Point Riley. All other dens were devoid of cuttlefish, but many housed a variety of other fauna, such as abalone, sea urchins, starfish, crabs, ascidians and small fish. Despite observing Giant Australian Cuttlefish eggs amongst the natural habitat at Black Point none were attached to the artificial structure in the area. Similarly, no eggs were deposited on any of the other artificial reefs deployed within northern Spencer Gulf.

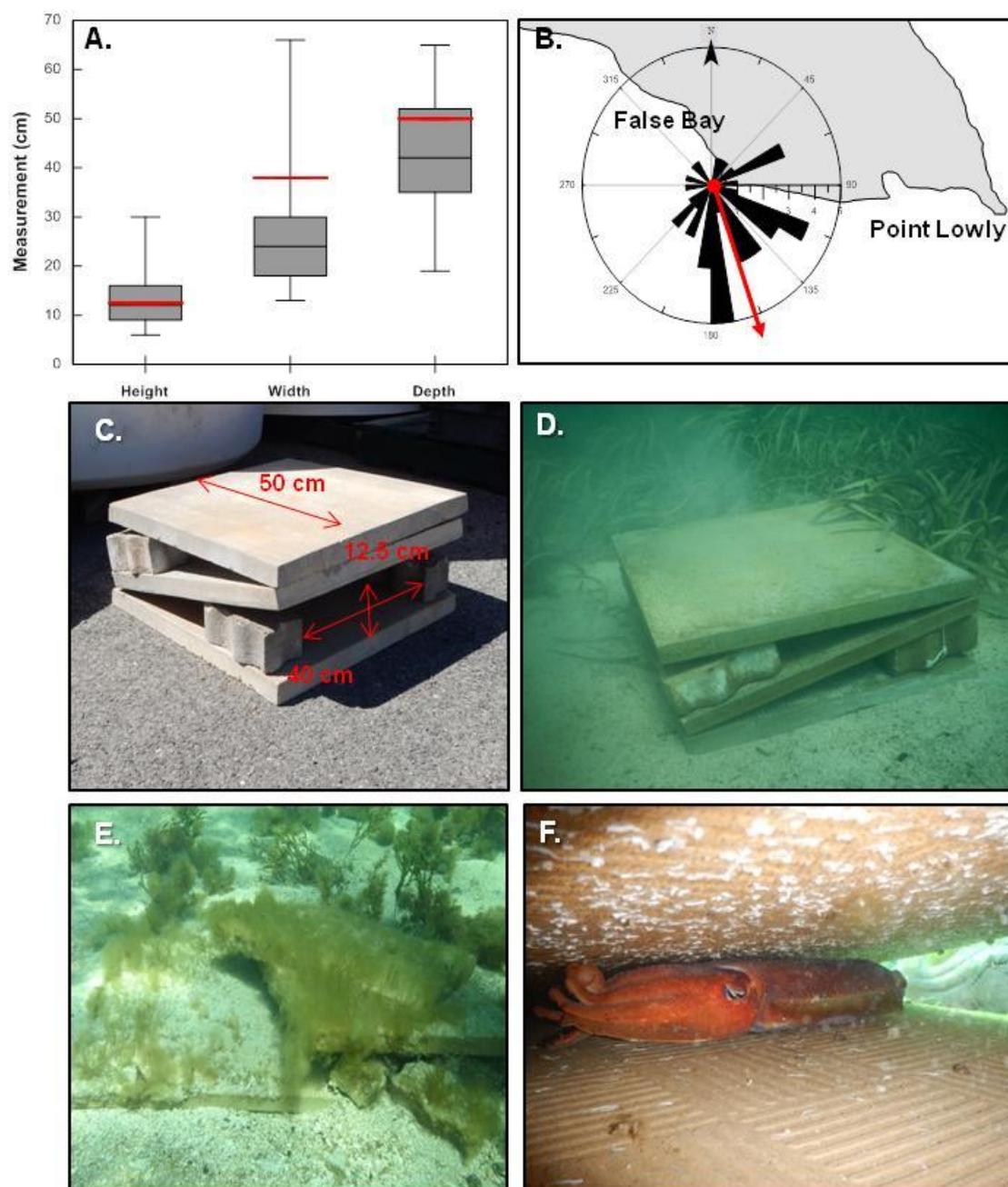


Figure 4.3. (A.) Box and Whisker plots (indicating the mean, quartiles and range) of the entrance height, width and depth of natural dens surveyed in 2013. The red lines represent the relative dimensions of the constructed artificial dens. (B.) A rose diagram of the relative orientation of the natural dens at False Bay. (C.) An example of a constructed artificial den. (D.) *In situ* artificial den deployed at Point Douglas. (E.) Damaged den at the OneSteel Wall site. (F.) Paired Giant Australian Cuttlefish occupying an artificial den at Black Point in May 2014.

4.4 Discussion

The artificial dens were unsuccessful in supporting spawning Giant Australian Cuttlefish during the 2014 season. The dens deployed at Black Point had the greatest chance of being used as they were situated in an area that supported the highest density of spawning

Giant Australian Cuttlefish throughout the season (Figure 2.2). Furthermore, pairs of Giant Australian Cuttlefish were observed occupying the structures on multiple occasions. Black Point accounts for approximately 20% of the total available spawning habitat along the Point Lowly peninsula, covering an area of approximately 97,000 m² and has supported record levels of Giant Australian Cuttlefish in the past (0.8/m² in 1999) (Hall and Fowler 2003). At its most recent peak, the density of cuttlefish inhabiting Black Point was estimated at 0.2 cuttlefish/m² (Figure 2.2). This indicated that spawning habitat was not a limiting factor at this site during 2014. Given the expanse of optimal spawning habitat at Black Point the addition of three small structures within the area would have provided an insignificant increase in the available substrate. Although they did not support any spawning activity it was encouraging to see the structures temporarily occupied by multiple Giant Australian Cuttlefish throughout the breeding season.

Backy Point has generally supported relatively high numbers of spawning Giant Australian Cuttlefish each year, however, their relative densities are considerably lower than the sites fringing Point Lowly. The structure of the reef at this site is slightly different from the main spawning area, as it is composed of a narrow coastal fringe of angular boulders arranged in a honeycomb-like matrix with deep interstitial crevices. This reef accounts for approximately 4.2% of the known spawning area within the Point Lowly area and supported a maximum of 0.04 cuttlefish/m² in 2014 (Figure 2.2). The reef at Point Riley on the eastern side of the gulf, shares similar characteristics to Backy Point and although is not routinely surveyed for Giant Australian Cuttlefish, it has supported commercial fishers targeting the species in the past (Hall and Fowler 2003). The artificial OneSteel wall has also historically supported spawning Giant Australian Cuttlefish, and accounts for approximately 0.6% of the total known spawning area (Hall and Fowler 2003). The deployment of artificial dens at these three sites added marginally more spawning habitat in comparison to those at Black Point, however, with the exception of a single Giant Australian Cuttlefish occupying an artificial den at Point Riley, there was no evidence of the structures supporting spawning cuttlefish during the 2014 season.

Exploratory video surveys confirmed that the coastal area at Point Douglas was devoid of low profile reef and was unlikely to support spawning cuttlefish (Chapter 3). The addition of artificial reefs in the area, however, would have provided some spawning substrate, but were unsuccessful in attracting Giant Australian Cuttlefish in 2014.

Given that pairs of Giant Australian Cuttlefish that appeared to be in spawning condition were observed sheltering in some of the artificial dens throughout the peak season, there was some expectation that these animals would have subsequently used the structures as a substrate for spawning. It is possible that the concrete pavers used in the construction of the

dens may not have provided an appropriate surface for egg attachment, with eggs either shedding away from the surface post spawning or the Giant Australian Cuttlefish preferring other more natural substrates. Similar sandstone pavers have successfully attracted spawning Giant Australian Cuttlefish in a previous study (BHP Billiton 2009), and a variety of artificial structures, such as sheets of corrugated iron (i.e. at Fitzgerald Bay, Steer personal observation), ropes, buoys and fishing traps have also supported cuttlefish eggs (Sykes et al. 2014, Nabhitabhata 2014). Given this diversity of spawning substrates, the lack of spawning activity on the deployed structures in 2014 may be due to chance and it is possible that they may be utilised over successive seasons. All of the dens were serviced in late September 2014 and left *in situ* to investigate whether Giant Australian Cuttlefish use them as spawning substrate throughout the upcoming 2015 season.

5 METAL LOADS IN CEPHALOPODS (*SEPIA APAMA* AND *SEPIOTEUTHIS AUSTRALIS*) IN SPENCER GULF

S. Gaylard (EPA)

5.1 Introduction

Many metals occur naturally in the Earth's crust, but are considered as pollutants when discharged from industry. They can be naturally released to soil and water during physical and chemical weathering of rocks, or released through extraction, mining and smelting of ore bodies (Walker et al. 2003). Metals in the Spencer Gulf marine environment have resulted from natural sources and from both recent and historical anthropogenic discharges. The Port Pirie smelter is one of the largest lead and zinc smelters in the world and has been operating on the shores of Port Pirie Creek since 1889. Similarly the Whyalla Steelworks has smelted and manufactured steel at Whyalla since 1958. These facilities have discharged substantial amounts of metals into the marine environment during a period where there were little to no environmental regulations, although significantly improved from historical loads, both facilities still discharge metals into the marine environment (Gaylard 2014).

Studies of metals in sediments showed that within approximately 30 km (~600 km²) of the Port Pirie lead smelter there were elevated levels of cadmium, lead, manganese and zinc and that levels decreased with distance from First Creek (Ward and Young 1981). Approximately 20% of this area (120 km²) was considered to be significantly contaminated, with lead, zinc and cadmium levels greater than 10 times background levels. Metal levels were typically 200–300 times higher than background for lead and zinc, and up to 1000 times higher than background for cadmium were recorded in areas adjacent to the First Creek discharge site (Ward and Young 1981; Ward and Hutchings 1996). Seagrasses growing near the smelter had elevated levels of cadmium, lead and zinc in the leaves, were less productive, and had less epiphytes (Ward 1987). Metals had reduced the abundance or totally eliminated 20 of the most common fish species that lived among the seagrasses in the contaminated area (Ward 1984; Ward et al 1986; Ward and Hutchings 1996), suggesting a widespread ecological effect of the smelter. In 1996, in response to concerns over human consumption of metal contaminated shellfish, the SA Government prohibited the taking of shellfish from the majority of Germein Bay.

In 2006-08, work undertaken by the EPA showed that metal levels in marine waters near First Creek had decreased by an order of magnitude compared to results reported by Ferguson (1983). However, this work suggested that the metals were still adversely affecting the ecology of the harbour and possibly the adjacent mangrove areas, and being

transported through the deep-water shipping channel into Germein Bay (EPA unpublished). Separate studies on both *in situ* and translocated bivalves showed that within the Shellfish Fishing Exclusion Zone, shellfish were found to still exceed food safety guidelines and that animals taken from northern Spencer Gulf were typically higher in some metals than other locations throughout South Australia (Corbin and Wade 2004; Gaylard et al. 2011).

Anthropogenic discharges of lead, manganese and zinc, as well as ammonia and total nitrogen reported by the individual facility or through the National Pollutant Inventory (www.npi.gov.au) for the study period (1994-2012), were investigated by Steer et al. (2013) and showed no clear association with the decline in Giant Australian Cuttlefish at Point Lowly. Notwithstanding this finding, the Giant Cuttlefish Working Group considered that further work to investigate metal accumulation was warranted and whether this was a risk to the aggregating population at Point Lowly.

The aims of this survey were to: (1) Investigate whether there are any spatial patterns in metal concentrations in Giant Australian Cuttlefish and Southern Calamary, and (2) Investigate whether there are differences in metal concentrations between the two species. Calamary were investigated for comparative purposes as this cephalopod co-occurs with the Giant Australian Cuttlefish and has appeared to be relatively abundant over the period in which the cuttlefish population had declined. This survey was not intended as a comprehensive assessment of the safety of Giant Australian Cuttlefish or Southern Calamary for human consumption. Comparisons of metal concentrations to the Australia and New Zealand Food Standards Code and previous researchers' data have been made to provide perspective on the relative proportion of contamination in these two cephalopods.

5.2 Methods

5.2.1 Sample Collection

In May 2013, five adult Giant Australian Cuttlefish were sampled from commercial prawn trawling surveys from the waters off Wallaroo approximately 100 kilometres south of Point Lowly (Figure 5.1). In July 2013, 18 adult Giant Australian Cuttlefish from five sites and 21 adult Southern Calamary from three sites along the Point Lowly peninsula were captured using lures on a line from the SARDI research vessel "Toro" (Figure 5.1). Animals were placed immediately in an ice bath and subsequently frozen at -20°C until dissection.

All animals collected from Point Lowly were dissected, separating the digestive gland, mantle and viscera for metal analysis to determine whether there was any variation in metal concentration between different organs. Only the digestive glands were removed

from Giant Australian Cuttlefish collected from Wallaroo. Greater emphasis was placed on collecting and interpreting the results from the digestive gland as this organ is known to accumulate >80% of the total metal burden in cephalopods (Bustamante et al. 2002a, b; Bustamante et al. 2006; Miramand et al. 2006; Lacoue-Labarthe et al. 2009; Rjeibi et al. in press).

Each dissected component was freeze dried and then thoroughly homogenised and digested with concentrated nitric acid by heating on a digestion block with a temperature controller. The metal levels were analysed in triplicate by inductively coupled plasma mass spectrometry (ICP-MS) (Perkin Elmer Elan DRC II, USA). Samples were analysed in blocks of tissue type with at least one blank, one duplicate, one blank spike, one sample spike and one laboratory control sample for every batch of 20 samples using a 20 ppb internal indium standard. All quality control (QC) recoveries and certified standards were recorded as 93–106 % of the reference values, which was deemed acceptable (National Measurement Institute 2011). Any unexpected results were cross verified using inductively coupled plasma-atomic emission spectrometry (ICP-AES) to ensure accuracy. All analytical methods were undertaken at the National Measurement Institute, North Ryde, Sydney, which is a National Association of Testing Authorities (NATA) accredited laboratory.

The limits of reporting (LOR) of the method were $0.05 \mu\text{g.g}^{-1}$ for arsenic (As), and selenium (Se), and $0.01 \mu\text{g.g}^{-1}$ for cadmium (Cd), copper Cu, lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni) and zinc (Zn). Both gold (Au) and silver (Ag) LOR were $0.02 \mu\text{g.g}^{-1}$.

5.2.2 Statistical Analysis

A permutational multivariate ANOVA (PERMANOVA) was used to test for differences between species and sites in the multi element matrix of digestive gland concentrations (Anderson et al. 2008). The PERMANOVA was undertaken on a resemblance plot of Euclidian distances from the $\log(X+1)$ transformed metal data and was normalised. The PERMANOVA was run using unrestricted permutations of the data using 4999 permutations to draw inferences at a significance level of 0.01 (Anderson et al. 2008). If the overall test was significant then pairwise analysis were performed using Gosset's t-statistic to determine statistical significance between species and sites (Anderson et al 2008). For the test between species, only sites where both Giant Australian Cuttlefish and Southern Calamary were captured were used for the comparison.

Principal Components Analysis (PCA) was used to discriminate the degree of association in metal concentrations and different sampling sites in the Giant Australian Cuttlefish and

Southern Calamary digestive glands. Digestive gland metal concentrations were log (x+1) transformed and normalised to account for different scales in the metal data. In addition to the PERMANOVA, the PCA provides a qualitative representation of the data to allow relationships between metals and sites to be seen.

Non-parametric Mann Whitney U tests were used to compare individual elements between the two species. The Kruskal Wallace multiple comparisons test, which incorporated a Bonferroni correction, was used to quantify differences between sites in individual metal concentrations (Orlich 2000).

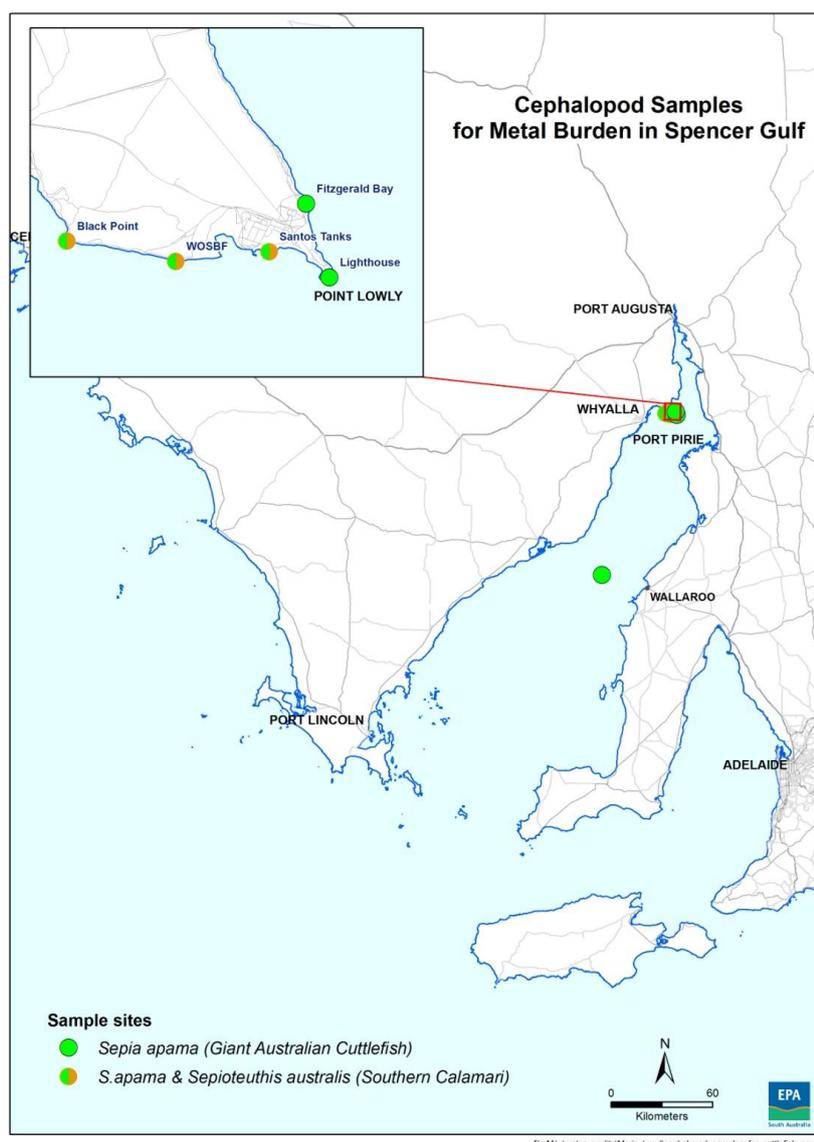


Figure 5.1. Map of Giant Australian Cuttlefish and Southern Calamary sampling sites in Spencer Gulf.

5.3 Results

The concentration of eleven different metals (Arsenic (As), Cadmium (Cd), Copper (Cu), Lead (Pb), Manganese (Mn), Mercury (Hg), Nickel (Ni), Selenium (Se), Silver (Ag), Gold (Au) and Zinc (Zn)) in three different body components (mantle, digestive gland and viscera) were investigated in cuttlefish and calamary. Giant Australian Cuttlefish were sampled at five sites in northern Spencer Gulf (Black Point (n=5), West of Santos Boundary Fence (WOSBF) (n=5), Santos Tanks (n=2) and Fitzgerald Bay (n=5)) and one site at Wallaroo (n=5) located approximately 100 km from Point Lowly as a reference site (Figure 5.1). Southern Calamary were sampled from three sites; Black Point (n=7), Santos Tanks (n=7) and WOSBF (n=7).

5.3.1 Sites of Accumulation

The digestive gland was the site of highest metal concentration in both species, which is consistent with previous studies (Bustamante et al. 2002a; Bustamante et al. 2002b; Miramand et al. 2006; Lacoue-Labarthe et al. 2009; Rjeibi et al. in press). With the exception of mercury, more than 70% of each metal was found in the digestive gland of the Giant Australian Cuttlefish and Southern Calamary. Mercury levels were lower in the digestive gland representing 58.0% and 52.1% of total body burden in both cuttlefish and calamary, respectively, with the mantle and viscera being approximately evenly split between the remaining metal concentrations (Table 5.1).

Table 5.1. Proportion of total metal concentration in body components of Giant Australian Cuttlefish (n = 18) and Southern Calamary (n = 21) collected around Point Lowly in July 2013.

Metal	Giant Australian Cuttlefish			Southern Calamary		
	Digestive gland	Mantle	Viscera	Digestive gland	Mantle	Viscera
Cadmium (Cd)	99.7	0.1	0.3	97.6	0.9	1.5
Copper (Cu)	97.5	1.2	1.3	98.5	0.5	1.0
Lead (Pb)	93.6	3.0	3.3	92.0	3.3	4.7
Manganese (Mn)	76.1	10.4	13.5	80.6	6.1	13.3
Mercury (Hg)	58.0	23.0	19.0	52.1	25.1	22.8
Nickel (Ni)	83.4	8.2	8.3	95.5	1.8	2.8
Selenium (Se)	82.6	8.9	8.6	87.6	5.9	6.6
Silver (Ag)	98.7	0.4	0.9	99.4	0.2	0.4
Zinc (Zn)	97.6	1.0	1.3	74.2	12.3	13.4

5.3.2 Differences between species

Given the highest metal levels were consistently found in the digestive gland, comparisons between species were only made for this tissue type and only with the samples from sites where both species were captured (Black Point, WOSBF and Santos Tanks).

The PERMANOVA analysis detected significant differences in metal concentrations in the digestive gland between species ($P_{\text{perm}} < 0.001$) but did not detect significant differences between sites ($P_{\text{perm}} = 0.219$) or between site and species ($P_{\text{perm}} = 0.233$). Individual elements were significantly different in almost each case: Giant Australian Cuttlefish had higher Cd, Pb and Zn, while Southern Calamary had higher As, Mn, Hg, Se and Ag (Figure 5.2, Table 5.2). Zn concentrations showed a large difference between species ($\bar{x} = 1158.5 \pm 144.3 \mu\text{g.g}^{-1}$) in cuttlefish compared to $93.4 \pm 6.5 \mu\text{g.g}^{-1}$ in calamary (Figure 5.2). Cd in cuttlefish ($\bar{x} = 31.8 \pm 2.7 \mu\text{g.g}^{-1}$) was over 13 times higher than in calamary ($\bar{x} = 2.3 \pm 0.2 \mu\text{g.g}^{-1}$). Cu and Ni were not different between the two species (Figure 5.2).

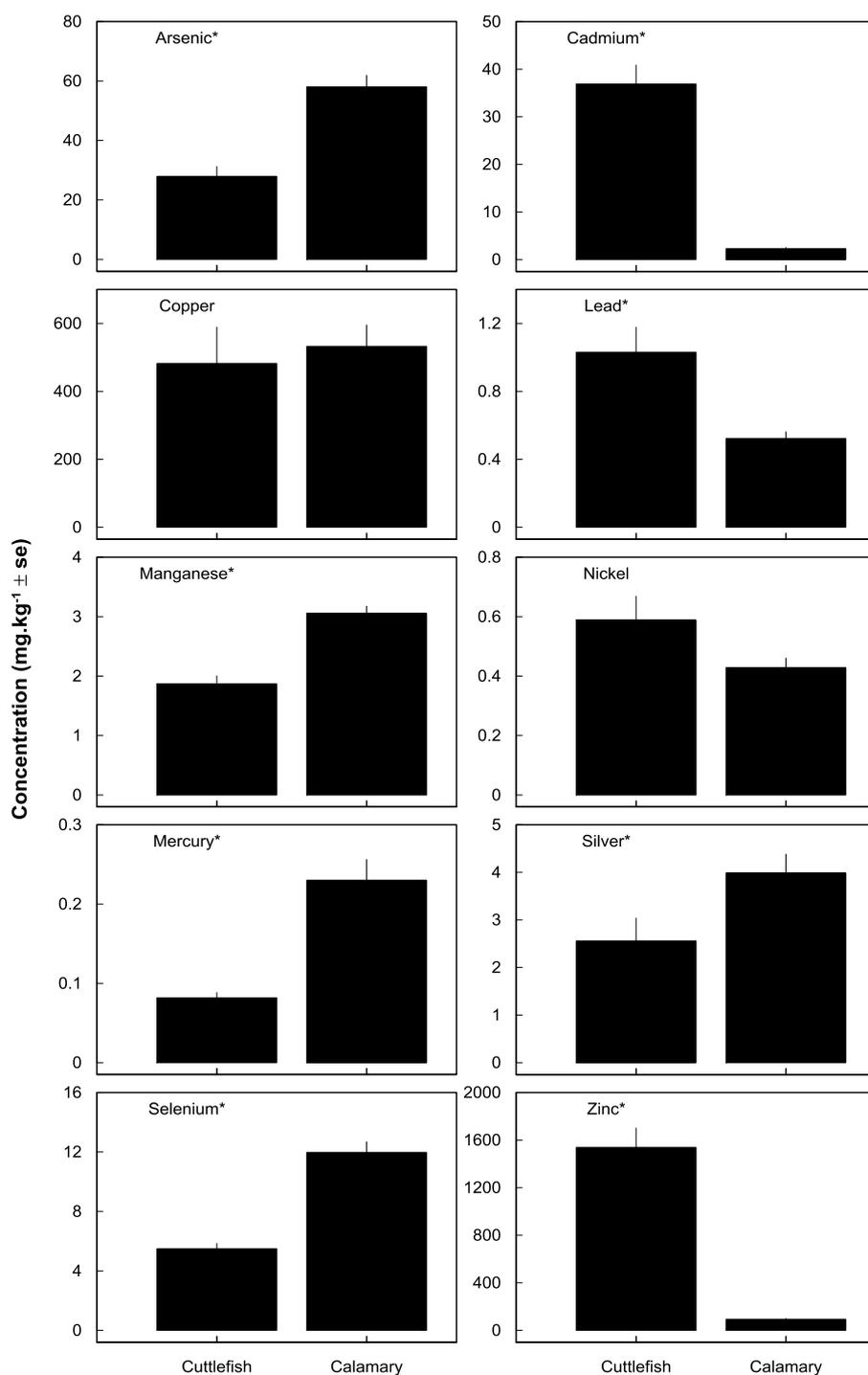


Figure 5.2. Average (\pm se) metal concentration in the digestive gland of Giant Australian Cuttlefish and Southern Calamary. Significance level. ** $p < 0.001$, * $p < 0.05$.

5.3.3 Differences Between Sites

PCA was used to show spatial differences in metal concentration among sites for both species. The first axis (PC1) accounted for 48.9% of the variability, while the second axis (PC2) accounted for a further 18.2% of the variability. There was a separation between

the samples from Wallaroo and the remainder of the sites, with the Wallaroo points skewed towards the right of the plot influenced by Ni and negative associations with the Pb, Cd, and Zn vectors (Figure 5.3). The dissimilarity in the Wallaroo sites is also influenced by the negative association with length, which is further supported by the higher proportion of female Giant Australian Cuttlefish at Wallaroo (Figure 5.3). This finding was further tested using the Kruskal Wallace one way analysis of variance. This indicated that the Wallaroo cuttlefish were significantly shorter and weighed less compared to animals from all other sites with the exception of Santos Tanks ($p < 0.05$).

Differences in metal concentration in digestive glands among sites was tested using PERMANOVA. The overall test was significant (pseudo $F = 2.911$ $P_{perm} = 0.001$); pairwise tests showed that Giant Australian Cuttlefish from Wallaroo were significantly different to all other sites tested (Table 5.2), while Black Point, Santos Tanks, WOSBF and Fitzgerald Bay were similar.

Table 5.2. Giant Australian Cuttlefish site comparisons indicating the pseudo t statistic and the significance level. ** $p < 0.001$, * $p < 0.05$.

Groups	Pseudo T	P(perm)
Black Point, Fitzgerald Bay	0.9077	0.594
Black Point, Santos Tanks	0.79998	0.617
Black Point, Wallaroo	3.2178	0.009**
Black Point, WOSBF	1.2671	0.135
Fitzgerald Bay, Santos Tanks	0.58865	0.86
Fitzgerald Bay, Wallaroo	2.3695	0.009**
Fitzgerald Bay, WOSBF	0.80169	0.596
Santos Tanks, Wallaroo	1.8703	0.047*
Santos Tanks, WOSBF	0.53591	0.954
Wallaroo, WOSBF	2.8172	0.008**

The Kruskal-Wallace multiple comparisons test was used to quantify differences between sites in individual metal concentrations. Giant Australian Cuttlefish from Black Point had significantly higher Cd, Pb, Ag, and Zn compared to those from the putative reference site at Wallaroo, while animals from Fitzgerald Bay had significantly higher Pb and Ag compared to those from Wallaroo. WOSBF had higher Pb, Ag and Zn and Santos Tanks had significantly higher Pb compared to Wallaroo (Figure 5.4). Giant Australian Cuttlefish from Wallaroo had significantly higher Ni compared to Black Point and Fitzgerald Bay, while this site was also higher than WSOBF in Hg. Apart from the differences noted

between the sites around Point Lowly and Wallaroo, there were no statistical differences between Black Point, Fitzgerald Bay, Santos Tanks and WSOBF sites for any metal in the digestive gland.

The PCA of metal concentration in Southern Calamary digestive glands suggests that there were subtle differences in metal concentration among sites (Figure 5.5). The PCA is a reasonable representation of the data with the first axis (PC1) accounting for 37.4% of the variability, and the second axis accounted for a further 26.2% (i.e., 63.7% of the total variability in the data can be explained within these two axis). Calamary from WOSBF are orientated in the centre and to the right of the plot, while animals from Santos Tanks are spread throughout the left of the plot and at the outer margins (Figure 5.5) suggesting some dissimilarity between these sites. Pairwise testing indicated that animals from WOSBF were statistically different to those from Santos Tanks ($P_{\text{perm}} = 0.007$).

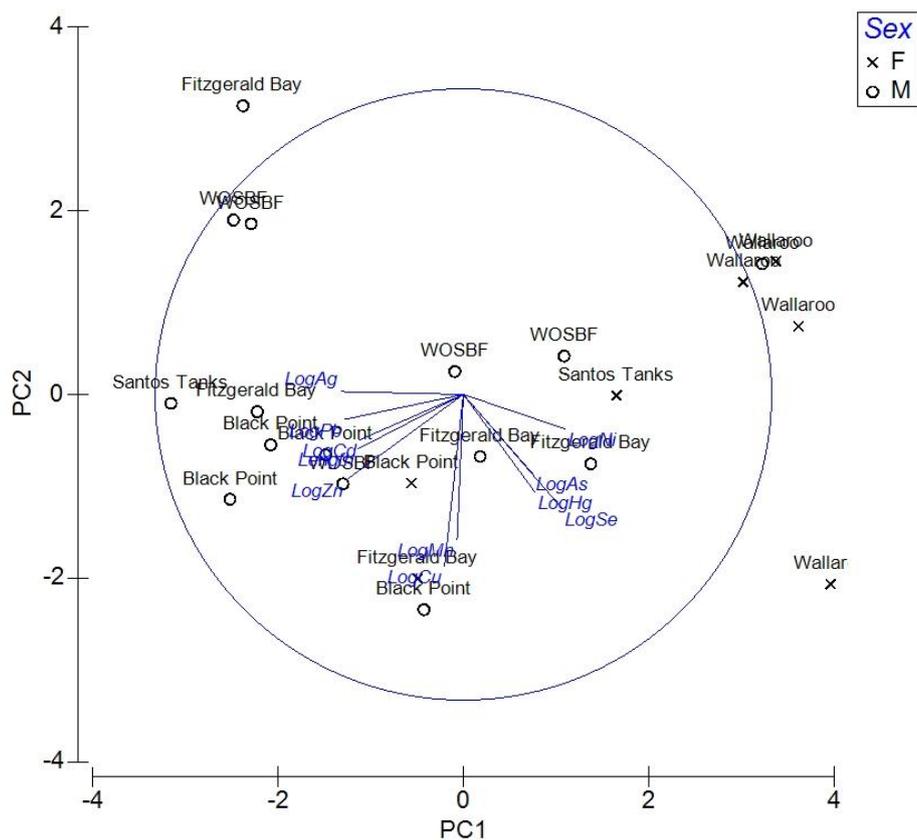


Figure 5.3. Principal components analysis (PCA) plot of metal concentration in the digestive glands of Giant Australian Cuttlefish throughout Point Lowly and Wallaroo. The vectors indicate the elements driving dissimilarity between replicates. Each symbol represents an individual.

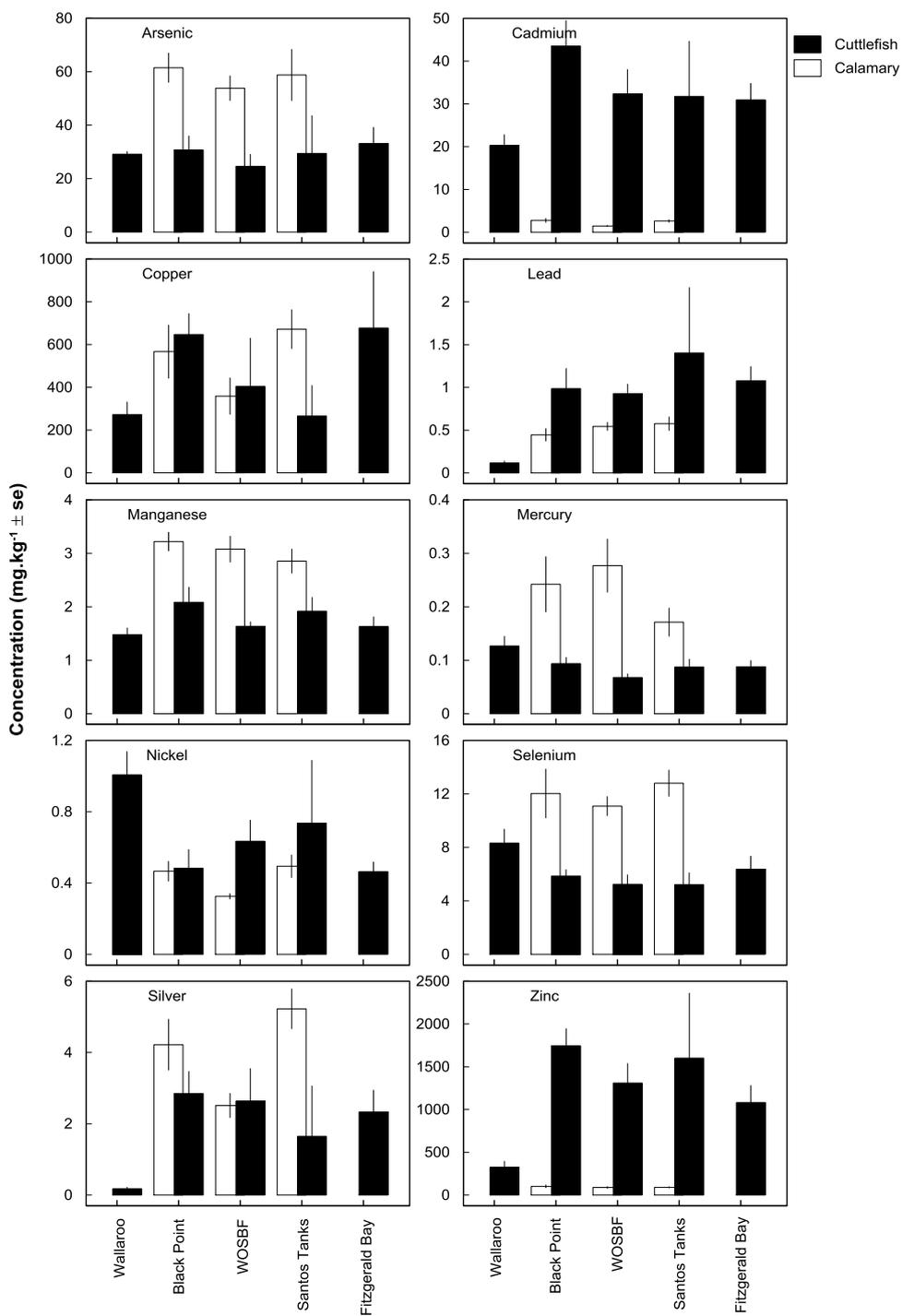


Figure 5.4. Average (\pm se) metal concentration in the digestive gland of Giant Australian Cuttlefish and Southern Calamary around Point Lowly and Wallaroo.

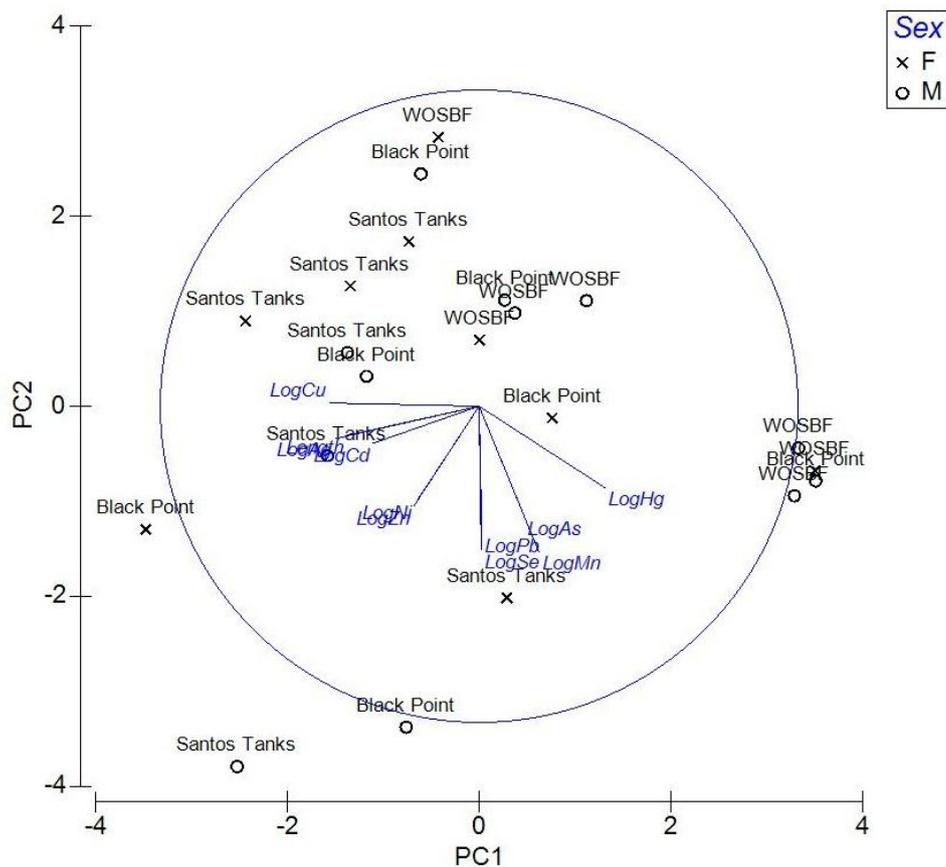


Figure 5.5. Principal components analysis (PCA) plot of metal levels in the digestive glands of Southern Calamary throughout Point Lowly and Wallaroo. The vectors indicate the elements driving dissimilarity between replicates. Each symbol represents an individual.

5.3.4 Food Safety

Analysis of the mantle of these species indicated that at all sites the animals sampled were below the Food Standards Australia Contaminants and Natural Toxicants standard 1.4.1 Maximum Levels (ML) (Commonwealth of Australia 2013) and therefore safe for human consumption.

In 1975, the South Australian Fisheries Department surveyed numerous fish and seafood species throughout South Australia including the mantle of both Giant Australian Cuttlefish and Southern Calamary from Douglas Bank, located approximately 10 km north of Point Lowly in the northern Spencer Gulf (Olsen 1983). While the sample sizes of the Giant Australian Cuttlefish and Southern Calamary were very low (n=1-2 depending on species), their results can be compared to the current study. With the exception of mercury, there has generally been a slight reduction in some of the metal concentrations between 1975 and 2013 (Figure 5.6).

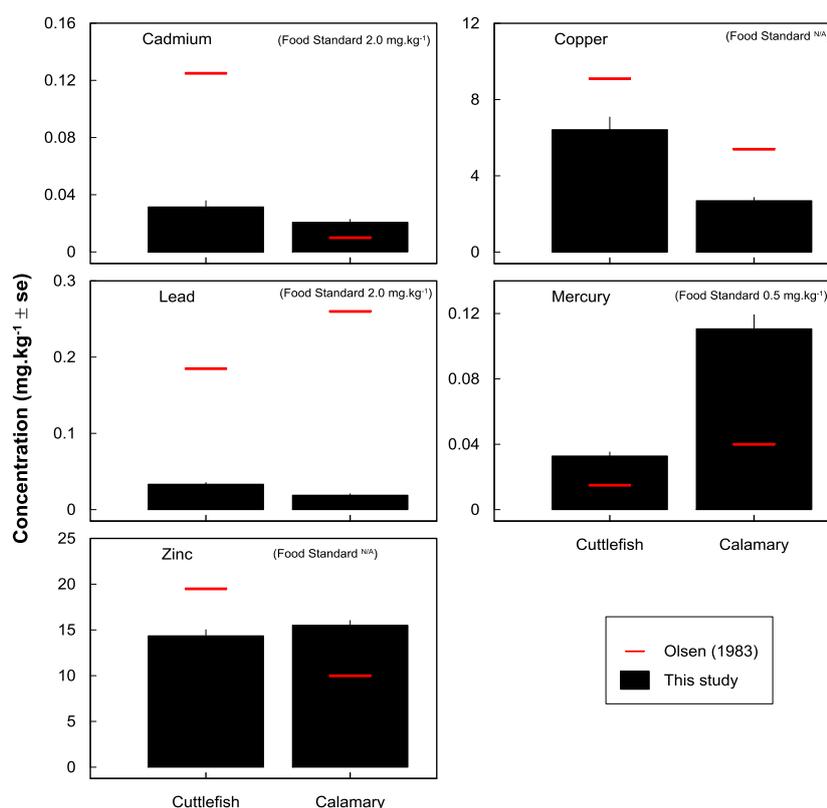


Figure 5.6. Comparison of mantle metal concentrations between this study and Olsen (1983) for Giant Australian Cuttlefish and Southern Calamary. Food standard value also shown where available.

5.4 Discussion

A number of metals are essential to life as they are involved in vital biochemical pathways, and as such, are often termed 'micronutrients'. Essential metals are therefore needed at relatively low concentrations for normal metabolism, growth and reproduction. However, at higher concentrations they can become toxic, while non-essential metals have no recognised biological function (e.g. mercury and cadmium) and can be toxic at very low concentrations (Depledge and Rainbow 1990). All aquatic invertebrates accumulate trace metals in their tissues, and metals accumulate in different concentrations, and different tissues depending on the organisms. Thus, aquatic invertebrates living in the same habitat may well have very different body metal concentration, even within closely related taxa (Rainbow 1990; Rainbow 2002). As such, the disparity observed in metal burden between Giant Australian Cuttlefish and Southern Calamary may not be surprising given the slight differences in the exposure, trophic position, ability of the organism to regulate a metal, organism age, size and sex between the two (Bryan et al 1979; Rainbow 1993). These factors also differ between individuals of

the same species at the same site, which contributes to substantial variability in metal concentrations (Depledge and Rainbow 1990). The metal concentrations varied between both species with Southern Calamary having significantly higher As, Mn, Hg, Se and Ag, while Giant Australian Cuttlefish were significantly higher in Cd, Ni and Zn (Figure 5.2). Both species have short life cycles (years) and live in similar locations suggesting that their exposure to any land based pollution source would be similar.

Loliginid squid such as Southern Calamary have different digestive gland cells to those of other cephalopods as they do not have “boules” structures which are characteristic of cephalopods (Boucher-Rodoni and Boucaud-Camou 1987). In cuttlefish, some of these boules are considered as heterolysosomes and heterophagosomes involved in intracellular digestion (Boucaud-Camou and Yim 1980) and are likely to be involved in the long term storage of Cd and Zn, which have been shown to have very long retention times (Bustamante et al. 2002b). Beyond physiological differences, cuttlefish generally spend most of their time close to the seafloor, which has been shown to be contaminated with metals (Ward and Young 1981; Ward et al. 1986b), although Bustamante et al. (2002b) has demonstrated that uptake of metals from contaminated sediment is a small proportion of total uptake, with the majority of metals coming through food. It is possible that cuttlefish feed on lower trophic level animals such as invertebrates, compared to squid which are likely to feed on higher trophic level species including fish (Chouvelon et al 2011). These differences in the diet between the two species may be contributing to the differences observed in metal loads between the two species, particularly when the presence of boules in cuttlefish which accumulate Cd and Zn is taken into account.

The Giant Australian Cuttlefish digestive gland metal concentrations of Cd, Pb, Ag and Zn were all significantly higher in the Point Lowly animals compared to Wallaroo. This finding is consistent with the long and extensive contamination of the northern Spencer Gulf due to the historic industrialisation of the region spanning over 120 years (Ward et al 1986b; Gaylard 2014). However, there were a number of potentially confounding factors that need to be considered. As stated above, the Wallaroo animals were significantly smaller and weighed significantly less than all other animals tested and they were also represented by more female animals than in any other site. The Wallaroo animals were caught two months earlier than the remainder of the sites and from deeper water (>10 m). The uptake of many metals is a function of exposure time (Viarengo and Nott 1993; Bustamante et al 2002b), therefore the smaller size and more offshore location of capture could indicate lower terrigenous pollutant exposure and would also explain the large difference in Ag between the Point Lowly animals and Wallaroo. Ag is typically taken up via water, and Giant Australian Cuttlefish will accumulate Ag while in nearshore coastal

waters, when they move into deeper waters, Ag is rapidly excreted from their system (Bustamante et al 2004; Miramand et al. 2006). Further work may be warranted to improve understanding of the differences between the Point Lowly and Wallaroo sites and the uptake of these metals, and whether this is an artefact of the smaller size of the Wallaroo samples or related to the contamination in northern Spencer Gulf.

With the exception of Giant Australian Cuttlefish and Southern Calamary from Wallaroo, the metal concentrations in the digestive gland of the Giant Australian Cuttlefish were very similar with no differences between sites (Figures 5.3 and 5.4). This finding is not surprising given the movement of Giant Australian Cuttlefish throughout the Point Lowly rocky habitat and their movement away from this region outside of the aggregation period (Kassahn et al. 2003). Giant Australian Cuttlefish are not considered to be site attached with studies showing that males will remain at the breeding aggregation for approximately 40 days (Payne et al. 2011) but can have home ranges between 909 m² to 17,526 m² (Gillanders and Payne 2014) and therefore they are likely to inhabit a range of locations along the Point Lowly rocky reef where they may be exposed to ambient metal concentrations.

The metal concentration in Southern Calamary digestive glands showed significant differences around Point Lowly with individuals from both Black Point and Santos Tanks significantly higher in Cd and Ag than those from WOSBF, while animals from Santos Tanks were also higher in Ni than those from WOSBF. There are no clear reasons behind these differences with respect to proximity to land based pollution sources suggesting that this could reflect the significantly larger length of the Santos Tanks animals compared to both the WOSBF (Kruskal Wallace $p=0.002$) and Black Point animals ($p=0.047$).

There have been few surveys of metal burden in Giant Australian Cuttlefish and Southern Calamary, therefore specific comparisons to other sites or periods of time are difficult. The metal burdens found in this study are within the bounds of other published literature from Australia (Port Philip Bay, Vic. *Nototodarus gouldi*; Finger and Smith 1987) and overseas (Bay of Seine, France, *Sepia officinalis*; Miramand et al. 2006, and Portugal; Raimundo et al. 2005; Pereira et al. 2009). This suggests that, at least compared to similar species, the animals sampled here are likely to be within their range of metal tolerance.

While there have been differences in tissue metal concentration in both cephalopod species observed between Point Lowly and Wallaroo, this does not infer a negative biological effect. There is not necessarily a relationship between the total amount of accumulated metal in an invertebrate and toxic effect. On entry of a trace metal to an invertebrate, the metal is typically biologically available until the physiology of the

invertebrate excretes it or binds it to a particular molecule of high affinity where the metal is unlikely to escape (i.e., detoxified) (Rainbow 2002). As a broad generalisation, the accumulated metal burden in an aquatic invertebrate can be separated into two categories – firstly, metal in a metabolically available form, and secondly, a metal that has been detoxified and is no longer available for metabolism (Rainbow 2002). Toxicity can occur when the rate of uptake into the body exceeds the combined rate of excretion and detoxification of the metabolically active metal, and so long as the rate of uptake does not exceed the rate of excretion and detoxification, the concentration of detoxified metal in the body can increase almost indefinitely without effect (Rainbow 2002).

Common detoxification pathways often involve metals binding to proteins such as metallothioneins or insoluble metaliferous granules (Bustamante et al. 2002a; Rainbow 2002; Bustamante et al 2006). These pathways play a role in the homeostasis of the essential metals including copper and zinc. In cephalopods, Bustamante et al. (2006) found that for the metals Ag and Cu, there was a direct relationship between cytosolic metal and metallothioneins suggesting binding to metallothioneins as the likely method of detoxification, whereas Cd and Zn appeared to mainly bind to high (>70 kDa) and low (<4 kDa) molecular weight proteins, suggesting a slightly different detoxification pathway (Tanaka et al. 1983; Finger and Smith 1987).

Recent work has shown that the loads of metals discharged into the northern Spencer Gulf have significantly reduced over the last decade (Gaylard 2014) and there have been some indications of reduction in metal loads in sediments and seagrass, particularly in close proximity to Port Pirie (EPA, unpublished data).

6 QUANTIFICATION OF GIANT AUSTRALIAN CUTTLEFISH BY-CATCH IN SPENCER GULF BY COMMERCIAL FISHERIES

MA Steer, C Noell & S Barrett

6.1 Introduction

There are three cuttlefish species that occur in Spencer Gulf; *Sepia apama* (Giant Australian Cuttlefish), *S. novaehollandiae* (Nova Cuttlefish), and *S. braggi* (Slender Cuttlefish) (Jereb and Roper 2005), all of which can be legally targeted, or retained, under the generic classification of cuttlefish (*Sepia* spp.) within South Australia's Marine Scalefish Fishery (PIRSA 2013). Within this fishery they are prioritised as 'tertiary' species as they are of low-medium value and make a minor contribution to the total production value of the commercial sector. The most recent estimate (2013-14) of the State-wide commercial catch of cuttlefish was 3.9 t (Fowler et al. 2014), which represented <0.2% of the total multi-species harvest in the Marine Scalefish Fishery. A spatial closure, implemented in March 2013, prohibited the capture of Giant Australian Cuttlefish north of Wallaroo, and effectively removed the Marine Scalefish Fishery's impact on the northern Spencer Gulf cuttlefish population.

There are three other fisheries that operate within Spencer Gulf that incidentally encounter cuttlefish species as by-catch: Spencer Gulf Prawn Fishery, Blue Crab Fishery, and Northern Zone Rock Lobster Fishery. Of these, the 46 year-old trawl-based prawn fishery has historically been the most heavily scrutinised having undergone an intensive gulf-wide by-catch survey in 2007 (Currie et al. 2009) and a series of other smaller-scale spatial surveys since the late 1990s (Carrick 1997; Dixon et al. 2005; Svane et al. 2007; Dixon et al. 2013). Small scale by-catch surveys have also been undertaken for the pot-based Blue Crab (Currie et al. 2007) and Rock Lobster (Brock et al. 2007) fisheries. Although there has been a considerable amount of effort invested into describing and assessing the overall multi-species composition of by-catch within these fisheries it is difficult to isolate the relative impact on Giant Australian Cuttlefish, as in most cases this species has been reported either in combination with other cephalopod taxa (i.e. Southern Calamary, *Sepioteuthis australis*; Svane et al. 2007) or grouped with its congeneric species (Dixon et al. 2005). This is most likely due to the difficulties in discriminating between *Sepia apama* and *S. novaehollandiae* which share similar morphologies and have overlapping geographic ranges. The gulf-wide by-catch survey of the Spencer Gulf Prawn Fishery, however, discriminated between the two species and reported their average abundance as 1.38 and 0.51 individuals per hectare for *S. novaehollandiae* and *S. apama*, respectively (Currie et al. 2009). These estimates of abundance were derived from a standardised sampling program that encompassed most of

the gulf, including areas outside of traditional trawling grounds, and the results were interrogated to identify species that may be vulnerable to trawling activity

In a recent study, University of Adelaide researchers used fishery independent trawl data collected between 2000 and 2010 and Bayesian hierarchical models to investigate spatial and temporal variation in Giant Australian Cuttlefish abundance and the ability of fishery-dependent catch and effort data to predict the status of the population (T. Prowse *et al.*, in press). The main findings of the study were: (1) that there was evidence for a broad-scale decline in the northern Spencer Gulf (not just around Point Lowly); and (2) that the decline could not be attributed to commercial harvesting. Similarly, Steer *et al.* (2013) found no clear association between the decline in the Giant Australian Cuttlefish population and fishing intensity by the Marine Scalefish and Spencer Gulf Prawn fisheries. While these studies have not provided any evidence for any particular factor(s) that caused the decline, fishing effort remains the factor most amenable to control. Consequently, the SA Government initiated a spatial closure for upper Spencer Gulf (north of Wallaroo) as a precautionary measure to ensure a maximum level of protection for Giant Australian Cuttlefish during a period of low abundance while continuing and new research investigates the cause of the decline at the Point Lowly aggregation site (this study; Gillanders FRDC 2013/010). Furthermore, the Spencer Gulf Prawn Fishery imposed a code of conduct to isolate Giant Australian Cuttlefish in good condition from the catch, maintain them alive within onboard holding tanks and release them at a time when scavenging predators (i.e. dolphins) are absent.

Our study aims to quantify Giant Australian Cuttlefish by-catch in association with the spatial closure to provide greater resolution in regard to the current levels of fishing pressure. Although all three fisheries were investigated, particular emphasis was directed towards investigating the spatio-temporal trends in Giant Australian Cuttlefish by-catch of the Spencer Gulf prawn fleet. Furthermore, an identification guide will be produced to distinguish between the three cuttlefish species that occur in Spencer Gulf.

6.2 Methods

6.2.1 Species Identification

Taxonomic descriptions of the cuttlefish species that are known to inhabit the South Australian gulfs were referred to prior to the biological sampling program (Zeidler and Norris 1989, Lu 1992, Jereb and Roper 2005). All sampled cuttlefish were identified to species, measured (ML, mm), weighed (g), sexed and staged according to their status of reproductive maturity (see Lipinski 1979). Cuttlebones (sepions) were dissected from a sub-sample of specimens spanning a broad size range and their maximum length, width and rostrum length

was measured in mm. A general description of the whole archetypal specimen was also recorded for each species. Given that fishing gear can damage the external appearance of soft-bodied animals greater emphasis was placed upon describing the diagnostic features of the internal sepions.

6.2.2 Northern Zone Rock Lobster Fishery

The Northern Zone Rock Lobster Fishery (NZRLF) is extensive, covering all South Australian waters between the mouth of the Murray River and the Western Australian border, an area of approximately 207,000 km² (Linnane and McGarvey 2014). The extent of fishing within this area is intrinsically linked to confined patches of aeolianite limestone reefs where Rock Lobster occurs; consequently the fishery does not extend north of the Middle Zone of Spencer Gulf (Figure 6.2) where the substrate is expansively sandy.

In addition to logbook data, some commercial Rock Lobster fishers participate in a voluntary catch sampling program. This program was implemented in 1991 to estimate the relative abundance and reproductive condition of undersize lobsters. By-catch has also been recorded. Fishers are encouraged to sample up to three research pots per trip where the escape gaps are closed and are often supported by on-board scientific observers. Participation in this program is neither random nor systematic and can vary among areas within the fishery (Linnane et al. 2014).

The voluntary catch sampling database, extending from 2000 to 2013, was investigated to provide a monthly indication of the quantity of cuttlefish that has been incidentally caught and recorded in this pot-based fishery. The fishing season extends from 1 November to 31 May of the following year.

6.2.3 Spencer Gulf Blue Crab Fishery

Fishery-independent surveys have been conducted in the Blue Crab Fishery since 2002 for stock assessment purposes. Surveys are conducted using industry vessels, skippers and crews, with independent observers collecting data on Blue Swimmer Crab sizes, catch rates and by-catch. These surveys are generally undertaken during winter (June/July) and have conformed to a standardised sampling regime consisting of a maximum of 108 stations distributed north of Wallaroo (Figure 6.1). At each survey site, both commercial crab pots and small-mesh (research) pots were set and hauled daily (see Noell et al. 2014). Surveys were not undertaken in 2011 or 2013.

Annual cuttlefish catch rates (number per pot) were calculated for both survey pot types for each fishing region within Spencer Gulf. These spatially and temporally resolved catch rates

were then applied to the entire fleet on the basis of their reported fishing effort (number of potlifts) to estimate the overall by-catch of cuttlefish for the Spencer Gulf Blue Crab Fishery.

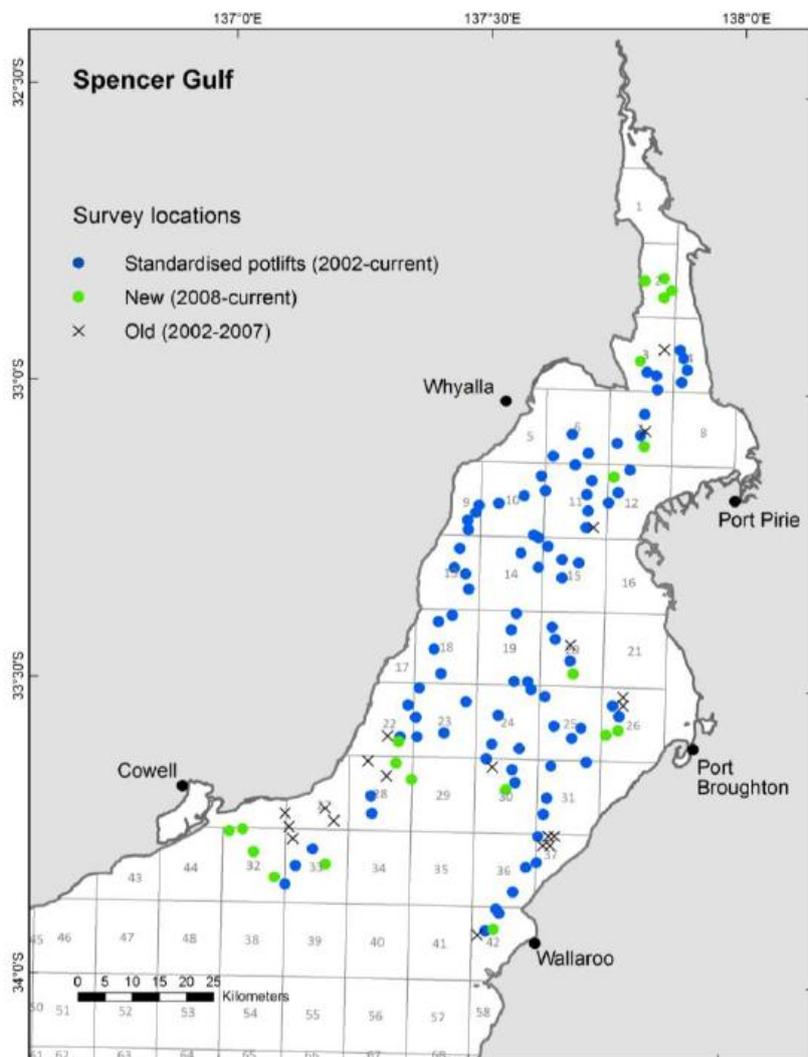


Figure 6.1. Commercial fishing blocks (grid) and fishery-independent survey locations in Spencer Gulf of the Blue Crab Fishery.

6.2.4 Spencer Gulf Prawn Fishery

Fishery-independent and -dependent programs were run to quantify cuttlefish by-catch in the Spencer Gulf Prawn Fishery. The fishery independent program relied on scientific observers to count, weigh and sub-sample cuttlefish that were incidentally caught during routine stock assessment surveys. Historically these surveys have been carried out three times per year as part of this fishery's real-time management obligations. They have coincided with the dark lunar phase in November, February (or March) and April and consist of approximately 200 fixed 30 minute trawl shots that are distributed throughout the gulf (Figure 6.2). Onboard scientific observers counted and weighed all cuttlefish by-catch from each survey

shot. From every second shot, all cuttlefish caught on one side of the trawl net were retained for biological analysis (see Section 4.2.1).

The fishery dependent program relied on the commercial fishers to assess cuttlefish catch during their regular fishing activity, extending from May 2013 until June 2014, inclusive (Table 6.1). This involved 5-12 representative vessels out of the entire fishing fleet ($n = 39$). All cuttlefish caught on one side of the trawl net were counted and recorded after each shot (including zeros). Initially, all cuttlefish from one shot (the 4th of the night) were retained for biological analysis (see Section 4.2.1), however this was increased to two shots per night (3rd and 7th) from November 2013 to improve the data resolution. The GPS location and duration of each shot was also recorded.

Quantification of total cuttlefish by-catch in the Spencer Gulf Prawn Fishery was calculated at the finest spatial and temporal resolution possible (i.e. at the level of fishing block, region, or zone; Figure 6.2). Cuttlefish catch rates (number per hour) were calculated for each survey shot. A species-specific catch rate was also derived through calculating the relative proportion of each of the three species (*S. apama*, *S. novaehollandiae* and *S. braggi*) from each biological sample. These spatially and temporally resolved catch rates were then applied to the entire fleet on the basis of their reported fishing effort (hours fished) to estimate the overall by-catch of cuttlefish for each fishing period throughout the season. The estimate did not account for potential localised depletion rates as a result of routine fishing practices.

The fishery independent surveys typically precede commercial fishing as they are relied on to develop the in-season fishing strategies for the commercial fleet. Furthermore, the spatial distribution of the fixed survey sites often extends outside of the commercial fishing area. Consequently, there is little spatial and temporal overlap between the two surveys, preventing any broad-scale comparison of their respective catches of cuttlefish. Throughout the course of this study, however, there were two occasions where the independent and dependent surveys occurred within the same month and vessels trawled a number of common fishing blocks (17 blocks in March 2014 and 28 in April 2014) (Table 6.1). Although the timing of these paired surveys did not coincide, the month long time-frame was the finest temporal resolution available, consequently providing the only opportunity to investigate whether the two survey programs were comparable in terms of their spatial and temporal estimates of cuttlefish catch rates and species composition. Given their spatial and temporal differences, the two sampling programs were analysed and interpreted separately.

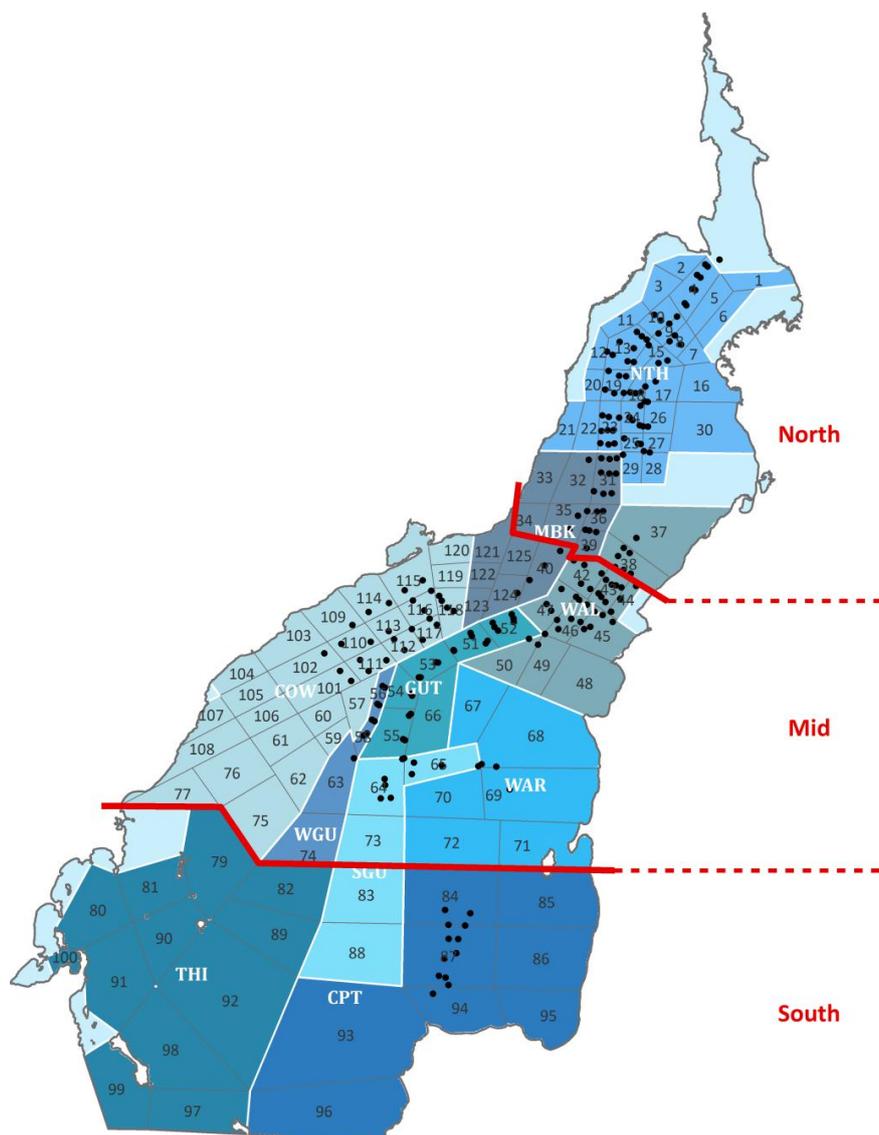


Figure 6.2. Distribution of the cuttlefish by-catch sampling programs in the Spencer Gulf Prawn Fishery. Black dots indicate the fishery independent shot location. The fishery dependent program was partitioned into fishing blocks (numbers), regions (coloured areas) and zone (red boundaries). Abbreviations: COW, Cowell; CPT, Corny Point; GUT, Gutter; MBK, Middlebank; NTH, North; SGU, South Gutter; THI, Thistle; WAL, Wallaroo; WAR, Wardang; WGU, West Gutter.

Table 6.1. Summary of cuttlefish by-catch samples collected from the Spencer Gulf Prawn Fishery.

Month	Survey	Vessels	Shots	Bio. Samples
Feb-13	Independ.	5	96	39
Apr-13	Independ.	9	150	59
May-13	Depend.	9	665	55
Jun-13	Depend.	10	412	34
Nov-13	Independ.	9	181	58
Dec-13	Depend.	6	432	24
Mar-14	Independ.	7	189	45
	Depend.	8	161	21
Apr-14	Independ.	12	161	54
	Depend.	9	590	56
May-14	Depend.	8	662	77
Jun-14	Depend.	5	366	22

6.2.5 Estimating Harvest Fraction

Comparing the estimates of by-catch of Giant Australian Cuttlefish from the commercial fishery with the monthly estimates of Giant Australian Cuttlefish abundance on the Point Lowly spawning grounds during the peak breeding season (derived in Chapter 2) provided an indication of the fishery's harvest fraction. This, however, relied on the assumptions that (1) all Giant Australian Cuttlefish within northern Spencer Gulf aggregate at Point Lowly to spawn during the peak breeding season; and (2) natural mortality rates, which are currently unknown, were not considered in the estimate. The lack of spatial resolution of the cuttlefish by-catch estimates from the Northern Zone Rock Lobster Fishery and the absence of corresponding data from the Blue Crab Fishery (Table 6.2) precluded the calculation of the harvest fraction for these two fisheries. The spatial and temporal scale of data collected from the Spencer Gulf Prawn Fishery, however, was sufficient to calculate a relative harvest fraction for the peak spawning periods (May and June) of 2013 and 2014.

6.3 Results

6.3.1 Species Identification

A total of 7,217 specimens were processed during our by-catch study, consisting of 1,170 *S. apama*, 6,043 *S. novaehollandiae* and 4 *S. braggi*. The size structure of *S. apama* ranged from 44 to 248 mm and the overall sex ratio was close to equal (0.9:1, F:M). *Sepia novaehollandiae* were typically smaller ranging from 49 to 136 mm and also exhibited a relatively unbiased sex ratio (1.2:1). *Sepia braggi* were uncommon, and considerably smaller, ranging in size from 27 to 57 mm.

6.3.1.1 *Sepia apama*

Whole animals can be typically identified by their broadly ovoid mantle; wide fins that extend anteriorly along the mantle margin; a short, broad head that is narrower than the mantle; and attain a maximum body size of 248 (ML) and weight of 1.9 kg in Spencer Gulf (Figure 6.3). The feeding tentacles are often retracted within tentacular pockets located deep within the arm crown. Specimens are reddish brown in colour. All arms have faint white transverse bars and spots bordered by darker pigment. The dorsal mantle has a fine, faint white, irregular, reticulated pattern throughout (Figure 6.4).

Sepions are broadly ovoid and wider along the anterior half. The dorsal surface is flat anteriorly, typically vivid white in colour and the rib structure is relatively faint. The posterior end of the dorsal surface is slightly granulose with irregular longitudinal ridges. The rostrum is present in juveniles and sub-adults and typically curves ventrally, however, it is lost in adults, or is a rounded, knob-like structure. The striated zone on the ventral surface is flat, or slightly concave with a faint longitudinal groove along the midline. The anterior striae form an inverted 'U' shape in smaller animals and become straight in larger animals. The inner cone is narrow along the anterior margin broadening posteriorly and forms a thickened rough 'V' shaped callus on the posterior inner edge (Figures 6.4 and 6.7).

6.3.1.2 *Sepia novaehollandiae*

Nova cuttlefish are smaller than *S. apama* rarely exceeding 125 mm (ML) in length and 521 g in weight. They have an oblong shaped mantle and narrow fins that extend laterally along the length of the mantle. Specimens are brown in colour, their arms do not have any distinct patterning, whereas the mantle is speckled with small white blotches (Figure 6.5).

Sepions are elongate-oval, acutely narrowing at both ends. The posterior dorsal surface typically has a pinkish tinge and is covered with fine denticulate projections that diminish anteriorly. The ribs are sharply concentric and become more pronounced anteriorly. The rostrum appears as a prominent spike that projects on a slight dorsal angle. The striated zone of the ventral surface is long extending greater than two-thirds of the length of the sepion. The striae are broadly 'V'-shaped and are wavy across the midgroove. The median sulcus is wide and deep along the striated zone. The outer cone slightly scallops inwards before expanding posteriorly (Figures 6.5 and 6.7).

6.3.1.3 *Sepia braggi*

Slender cuttlefish are small (<65 mm ML) and rarely encountered. Their mantle is cigar-shaped and triangular along the anterior margin. Narrow fins extend along the lateral margin of the mantle and are widest along the posterior third. The head is short, slender and narrower than the mantle. The terminal ends of the arms are fine and contain widely

dispersed, minute, suckers. Specimens are pale buff to pinkish brown in colour. Arms have short transverse darker purplish bars and blotches (Figure 6.6).

Sepions are lanceolate in shape, broadest along the anterior third and accurately rounded at the anterior end and strongly curved ventrally. The dorsal surface is rose colour posteriorly fading to white and weakly granulose with irregular longitudinal ridges. The ribs are narrow and distinct. A chitinous band borders the lateral margin of the sepion. The spine is distinct, resembling a ball-horn that projects upwards. The ventral surface has a distinct medial groove that extends the entire length of the sepion. The anterior striae are an inverted 'U'-shape, incurved medially extending to a more pronounced 'V'-shape towards the posterior sulcus. The inner cone appears strap-like expanding posteriorly into two short 'wings' to form a cup-like structure (Figure 6.6).

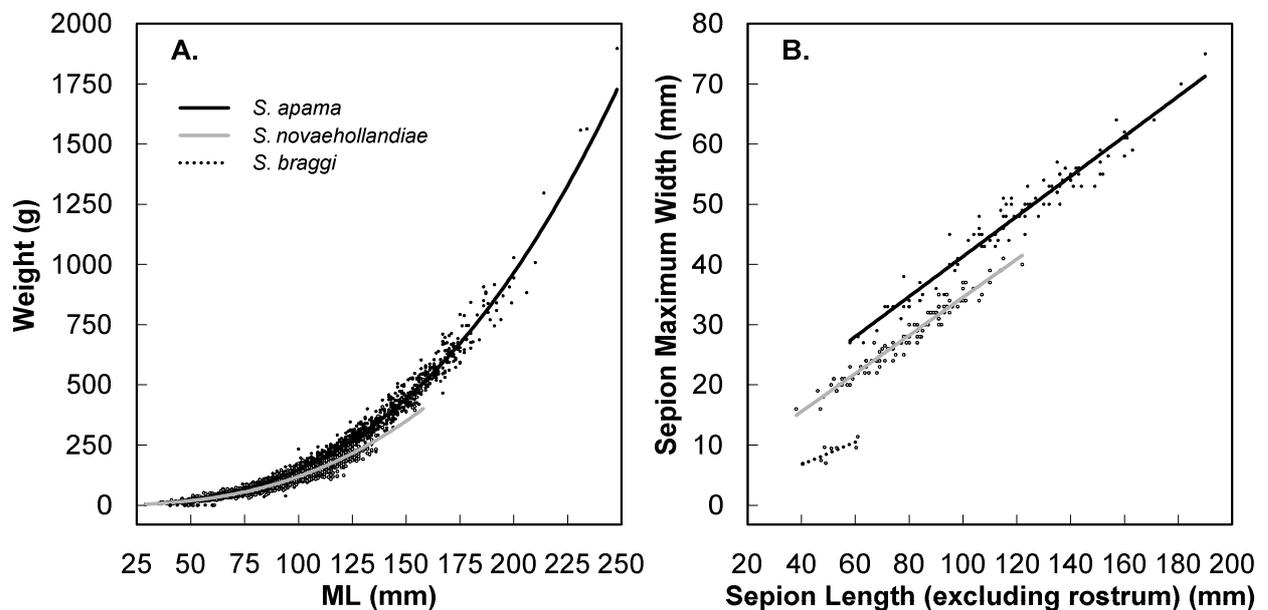


Figure 6.3. Length-weight relationships (A) and sepion length-width relationships (B) for the three species of cuttlefish incidentally caught by the Spencer Gulf Prawn Fishery.

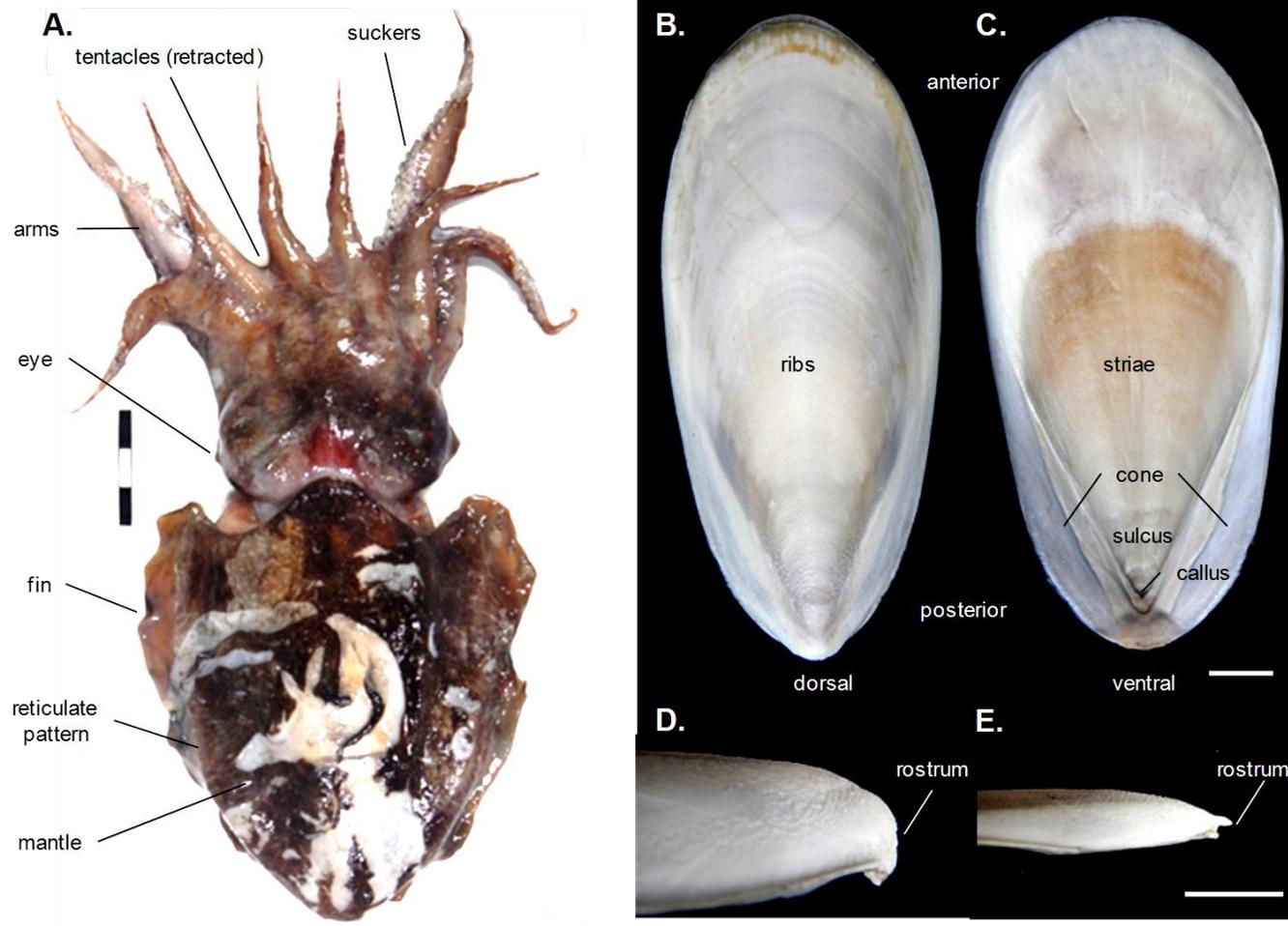


Figure 6.4. (A.) Whole specimen of the Giant Australian Cuttlefish (*Sepia apama*) (Scale bar = 50 mm). Internal cuttlebone (sepion) (B.) Dorsal view, (C.) Ventral view, (D.) Lateral view of the reduced rostrum from a mature adult specimen and (E.) Prominent rostrum from an immature specimen (Scale bars = 20 mm).

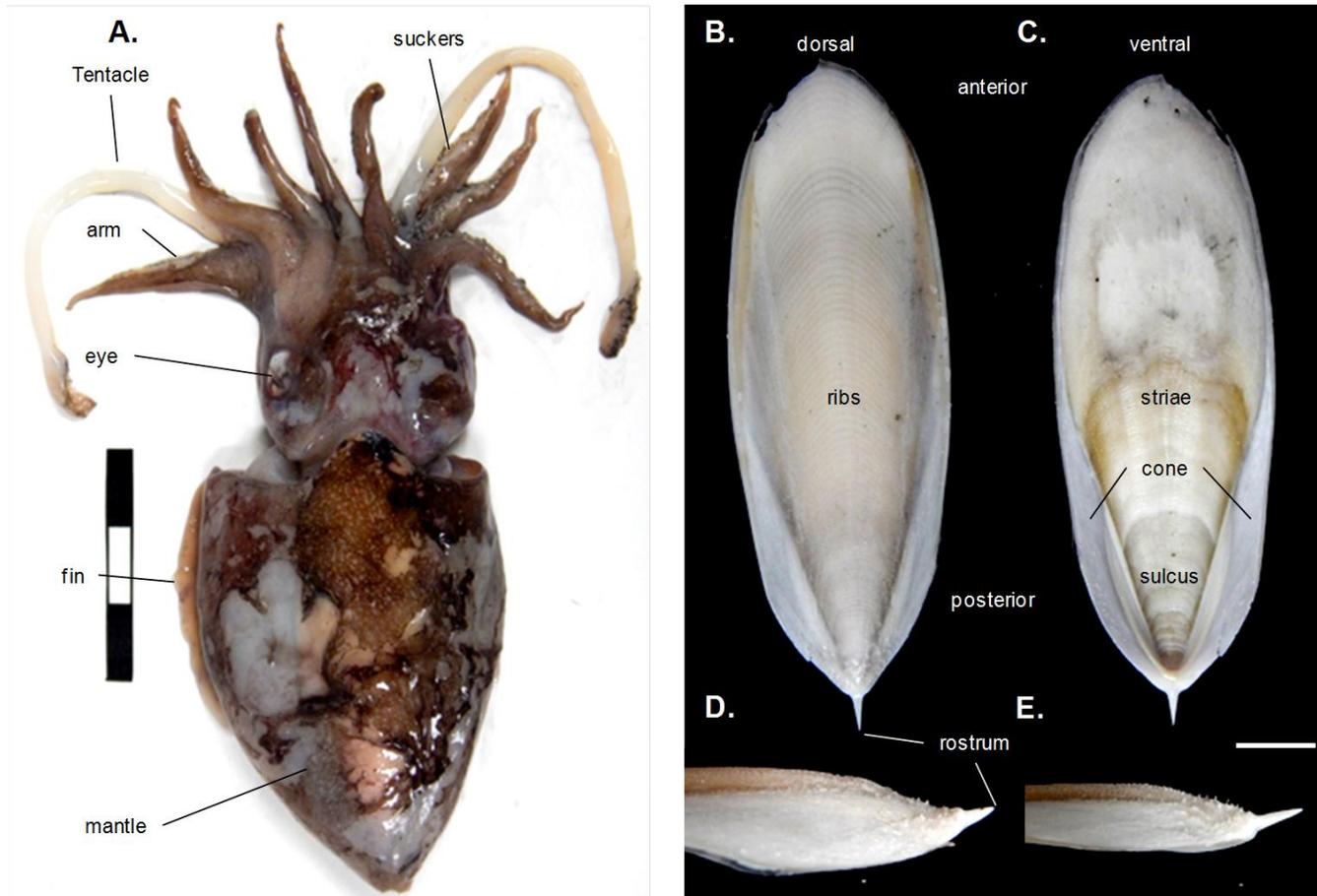


Figure 6.5. (A.) Whole specimen of the Nova's Cuttlefish (*Sepia novaehollandiae*) (Scale bar = 50 mm). Internal cuttlebone (sepion) (B.) Dorsal view,(C.) Ventral view, (D & E.) Lateral view indicating the variations of the rostrum (Scale bars = 12 mm).

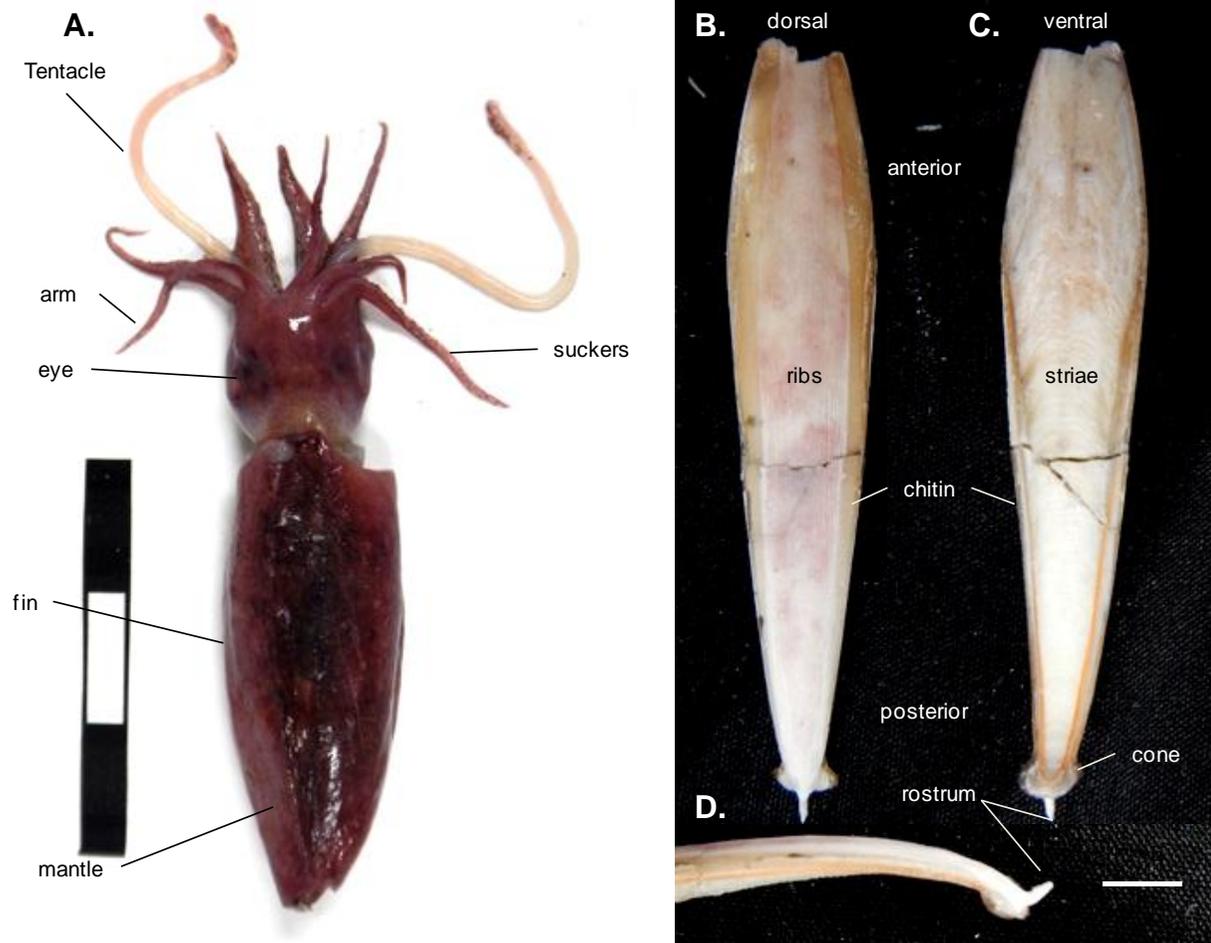


Figure 6.6. (A.) Whole specimen of the Slender Cuttlefish (*Sepia braggi*) (Scale bar = 50 mm). Internal cuttlebone (sepion) (B.) Dorsal view, (C.) Ventral view, (D.) Lateral view of the rostrum (Scale bars = 10 mm).



Figure 6.7. Species comparison of the sepions collected from specimens of similar size (approximately 90 mm ML). (A.) Dorsal view, (B.) Ventral view (Scale bars = 10 mm).

6.3.2 Northern Zone Rock Lobster Fishery (NZRLF)

The species composition of cuttlefish by-catch is unknown for the NZRLF, but given fishing predominantly occurs over reef habitat it is likely to be dominated by *S. apama*. Recorded catches were highest during 2000 and 2001, peaking at 93 individuals in May 2000 and have remained below six since December 2002 (Figure 6.8). Despite the negligible quantities of cuttlefish being captured and recorded, there was evidence of a consistent increase during March, April and May, when cuttlefish are expected to aggregate over reefal habitat to spawn.

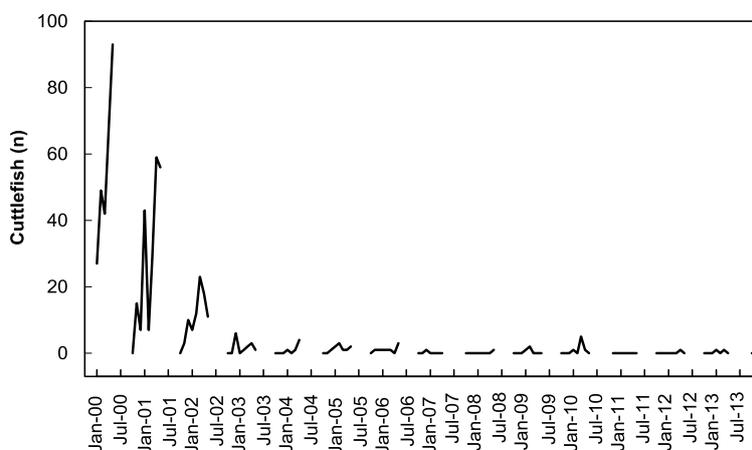


Figure 6.8. Numbers of cuttlefish incidentally caught in commercial Rock Lobster pots recorded monthly from January 2000 as part of the voluntary catch sampling program.

6.3.3 Spencer Gulf Blue Crab Fishery

The species composition of cuttlefish by-catch is unknown for the Blue Crab Fishery and given fishing effort is distributed throughout the gulf it is likely to comprise *S. apama* and *S. novaehollandiae*. Average annual cuttlefish catch rates were negligible remaining <0.03 cuttlefish/potlift. Highest catch rates were recorded in 2006 and 2004, at 0.025 and 0.021 cuttlefish/potlift, respectively (Figure 6.9). The most recent 2014 estimate of 0.015 cuttlefish/potlift was 166% greater than the 2012 estimate of 0.006 cuttlefish/potlift.

Extrapolated estimates of total cuttlefish catch ranged from 109 in 2002 to 2,483 in 2004 (Table 6.2). Catches remained above 1,000 cuttlefish from 2006 to 2010. The lack of surveys in 2011 and 2013 precluded an estimate of catch in these years and the 2014 fishing season (calendar year) was still in progress during the development of this report (Table 6.2).

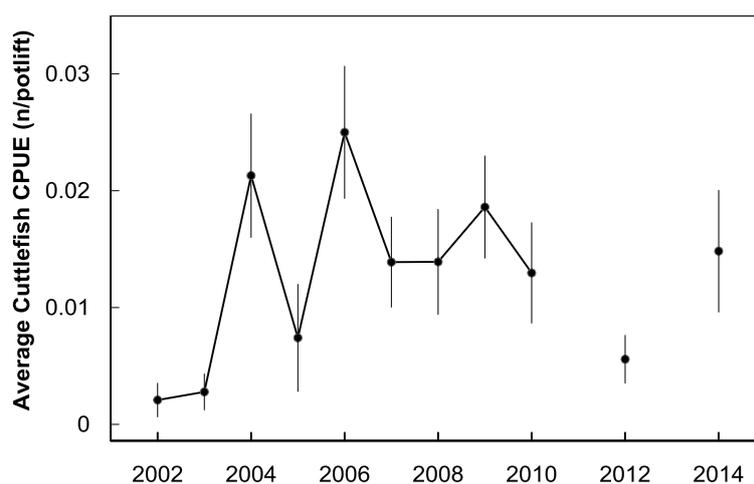


Figure 6.9. Average (\pm se) catch rate of cuttlefish incidentally caught in commercial and research pots in the Spencer Gulf Blue Crab Fishery during the annual fishery independent pot-sampling program. Note, surveys were not undertaken in 2011 and 2013.

Table 6.2. Annual estimate of total cuttlefish caught by the Spencer Gulf Blue Crab Fishery, calculated from spatio-temporally resolved catch rates from fishery independent surveys.

Year	Potlifts	Est. Cuttlefish
2002	73,436	108.9
2003	101,145	305.5
2004	104,888	2482.6
2005	109,298	760.3
2006	122,471	1323.5
2007	112,340	1392.5
2008	130,048	2033.9
2009	97,746	1418.8
2010	92,957	1029.3
2011	47,285	No Survey
2012	57,131	560.8
2013	63,651	No Survey
2014	Incomplete	Incomplete

6.3.4 Spencer Gulf Prawn Fishery

6.3.4.1 Dependent vs. Independent Surveys

There was no clear trend that indicated that the estimates of cuttlefish by-catch within the Spencer Gulf Prawn Fishery were biased by the type of survey during March and April 2014. There were instances where the fishery dependent survey yielded higher catch rates of cuttlefish within a fishing zone (i.e. *S. apama* in March 2014) and vice versa (i.e. *S. apama* in

April 2014) (Figure 6.10). Similarly, there were differences but no clear biases in the size composition of the two cuttlefish species collected throughout the study. With the exception of the *S. novaehollandiae* sample collected in March 2014, the size composition of the cuttlefish by-catch was relatively similar for both survey types (Figure 6.11)

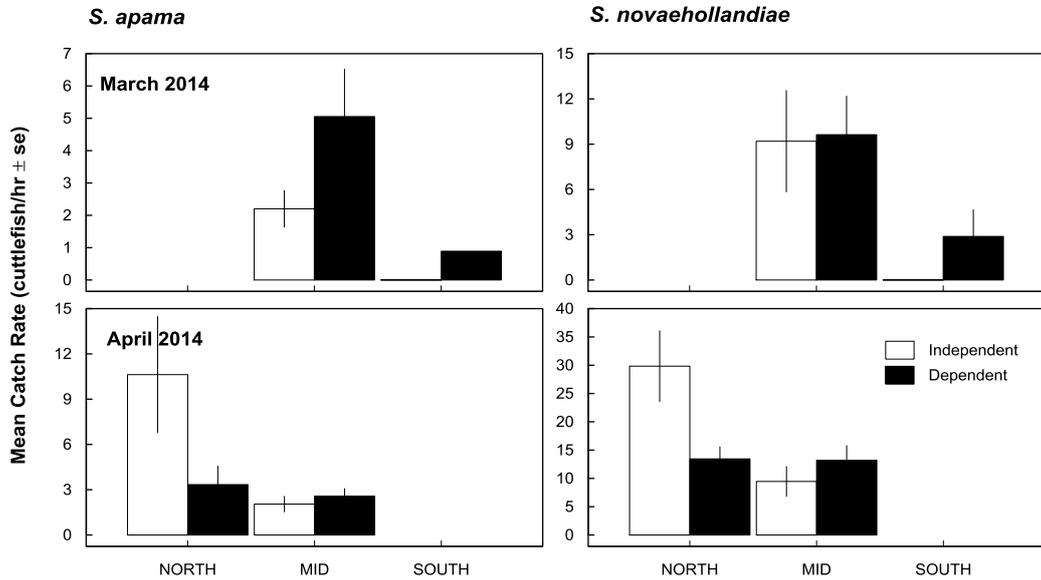


Figure 6.10. Comparison of the estimates of mean (\pm se) cuttlefish catch rates determined from fishery-dependent and -independent surveys in March and April 2014.

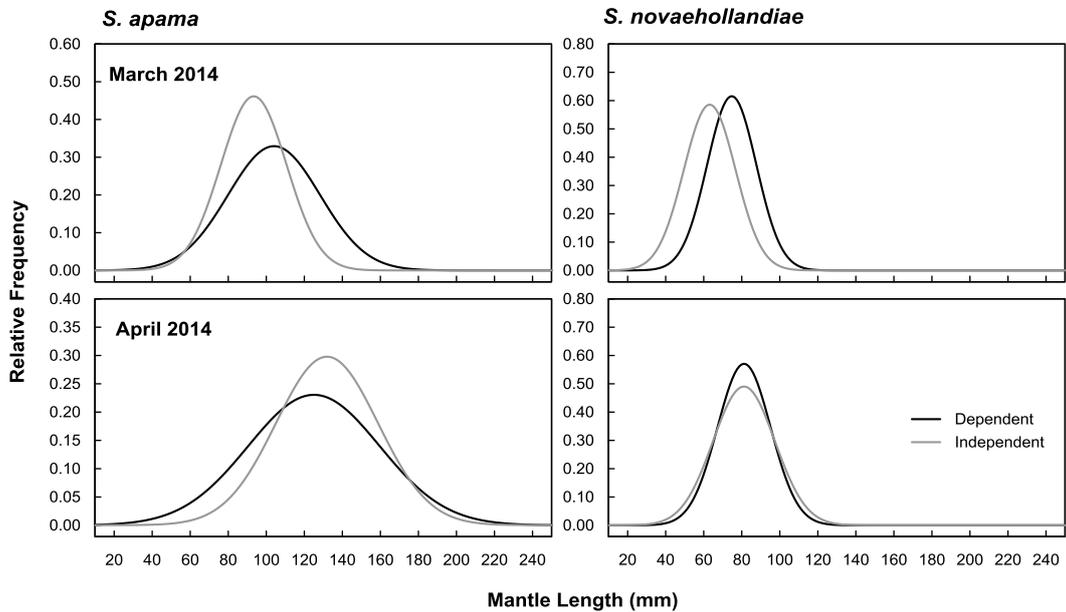


Figure 6.11. Comparison of the size distribution of cuttlefish caught from fishery-dependent and -independent surveys in March and April 2014.

6.3.4.2 Fishery-Independent Survey Estimates

A total of 5,654 cuttlefish were incidentally caught over the course of the five fishery independent stock assessment surveys conducted between February 2013 to April 2014 (Table 6.3). Catches ranged from 136 cuttlefish in February 2013 to 1,767 in April 2014. *Sepia novaehollandiae* accounted for the majority (72.4%) of the catch, with *S. apama* and *S. braggi* contributing 27.3% and 0.2%, respectively. The largest quantities of cuttlefish (>1,460 individuals) were caught during the March and April surveys.

Most of the cuttlefish (64%) were incidentally captured from the Northern Zone, whereas the Middle and Southern Zone accounted for 35.3% and 0.1%, respectively (Table 6.3). Catches of *S. apama* peaked during the cooler months in the Northern Zone, exceeding 400 individuals in March and April 2014 coinciding with the peak abundance of the Point Lowly spawning aggregation (Figure 2.3). *Sepia novaehollandiae* catch was consistently greater but followed the same trend. *Sepia braggi* were rarely encountered appearing in low numbers in April 2013 and March 2014 in the Middle and Southern Zones of Spencer Gulf, respectively (Table 6.3). Given its infrequent occurrence, *S. braggi* was excluded from all subsequent analysis.

The patterns of distribution and relative abundance (number caught per hour) of *S. apama* and *S. novaehollandiae* differed slightly over the course of these independent surveys. *Sepia apama* were more sparsely distributed throughout the Northern and Middle Zones of Spencer Gulf in February and November 2013 in comparison to *S. novaehollandiae*, with catch rates rarely exceeding 6 hr^{-1} (averaging $\sim 1.5 \text{ hr}^{-1}$) (Figure 6.12). Catch rates of *S. novaehollandiae* during this time, however, averaged 2.1 and 6.6 hr^{-1} , respectively, and were highest throughout the deeper sections of the gulf that extended from the northern boundary of the survey to the central gutter of the Middle Zone. Although *S. novaehollandiae* catch rates were consistently higher than *S. apama* their respective patterns of distribution merged during the March and April surveys. Both species were consistently more abundant within the deeper channel of the Northern Zone and throughout the north-eastern corner of the Middle Zone (Figure 6.12).

Table 6.3. Estimates of total cuttlefish caught during the fishery-independent surveys of the Spencer Gulf Prawn Fishery. Estimates are presented in total for Spencer Gulf and partitioned into the Northern, Middle and Southern Zones. Numbers in parentheses indicate the relative proportion (as a percent) of each species of the catch.

SPENCER GULF	Month	<i>S. apama</i>		<i>S. novaehollandiae</i>		<i>S. braggi</i>		Total
	Feb-13	54.0	(39.7%)	81.9	(60.3%)	0.0	(0%)	135.9
Apr-13	406.7	(27.6%)	1,065.3	(72.2%)	4.0	(0.3%)	1,476.0	
Nov-13	94.9	(11.8%)	707.1	(88.2%)	0.0	(0%)	802.0	
Mar-14	484.6	(32.9%)	978.4	(66.4%)	10.0	(0.7%)	1,473.0	
Apr-14	505.6	(28.6%)	1,261.4	(71.4%)	0.0	(0%)	1,767.0	
<i>Totals</i>	1,545.9	(27.3%)	4,094.0	(72.4%)	14.0	(0.2%)	5,653.9	
NORTH	Month	<i>S. apama</i>		<i>S. novaehollandiae</i>		<i>S. braggi</i>		Total
	Feb-13	34.9	(42.5%)	47.1	(57.5%)	0.0	(0%)	81.9
	Apr-13	246.0	(28.2%)	626.0	(71.8%)	0.0	(0%)	872.0
	Nov-13	13.8	(4.3%)	303.2	(95.7%)	0.0	(0%)	317.0
	Mar-14	402.2	(39.5%)	616.8	(60.5%)	0.0	(0%)	1,019.0
	Apr-14	425.1	(31.2%)	938.9	(68.8%)	0.0	(0%)	1,364.0
	<i>Totals</i>	1,122.0	(30.7%)	2,532.0	(69.3%)	0.0	(0%)	3,653.9
MID	Month	<i>S. apama</i>		<i>S. novaehollandiae</i>		<i>S. braggi</i>		Total
	Feb-13	19.2	(35.5%)	34.8	(64.5%)	0.0	(0.1%)	54.0
	Apr-13	158.7	(26.5%)	439.3	(73.5%)	0.0	(0%)	598.0
	Nov-13	81.1	(16.7%)	403.9	(83.3%)	0.0	(0%)	485.0
	Mar-14	82.4	(18.1%)	361.6	(79.7%)	10.0	(2.2%)	454.0
	Apr-14	80.5	(20%)	322.5	(80%)	0.0	(0%)	403.0
	<i>Totals</i>	421.9	(21.2%)	1,562.1	(78.3%)	10.0	(5%)	1,994.0
SOUTH	Month	<i>S. apama</i>		<i>S. novaehollandiae</i>		<i>S. braggi</i>		Total
	Feb-13	0.0	(0%)	0.0	(0%)	0.0	(0%)	0.0
	Apr-13	2.0	(33.3%)	0.0	(0%)	4.0	(66.7%)	6.0
	Nov-13	0.0	(0%)	0.0	(0%)	0.0	(0%)	0.0
	Mar-14	0.0	(0%)	0.0	(0%)	0.0	(0%)	0.0
	Apr-14	0.0	(0%)	0.0	(0%)	0.0	(0%)	0.0
	<i>Totals</i>	2.0	(33.3%)	0.0	(0%)	4.0	(66.7%)	6.0

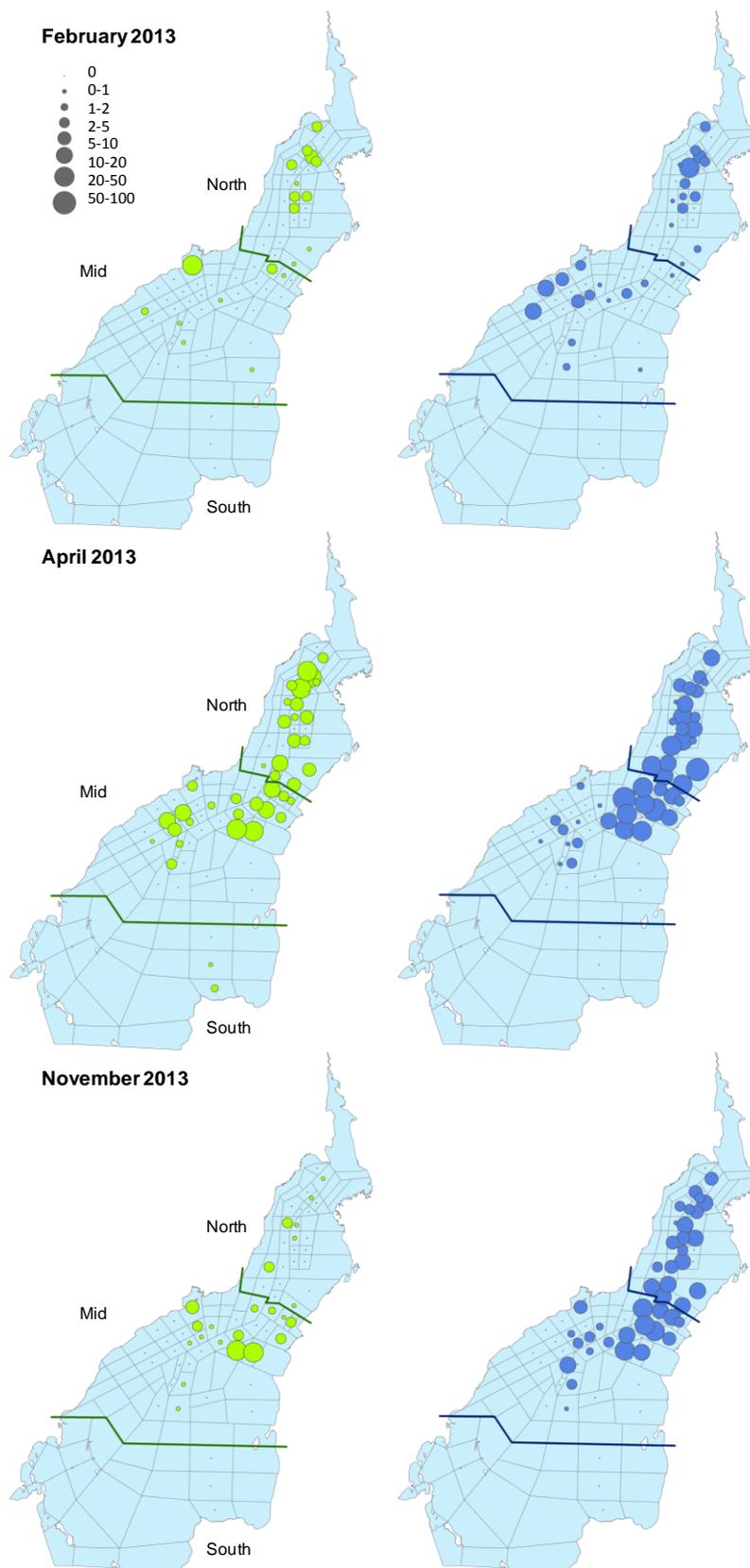


Figure 6.12. Catch rates of cuttlefish (number per hour) (*Sepia apama* on the left (green), *S. novaehollandiae* on the right (blue)) determined from fishery-independent surveys.

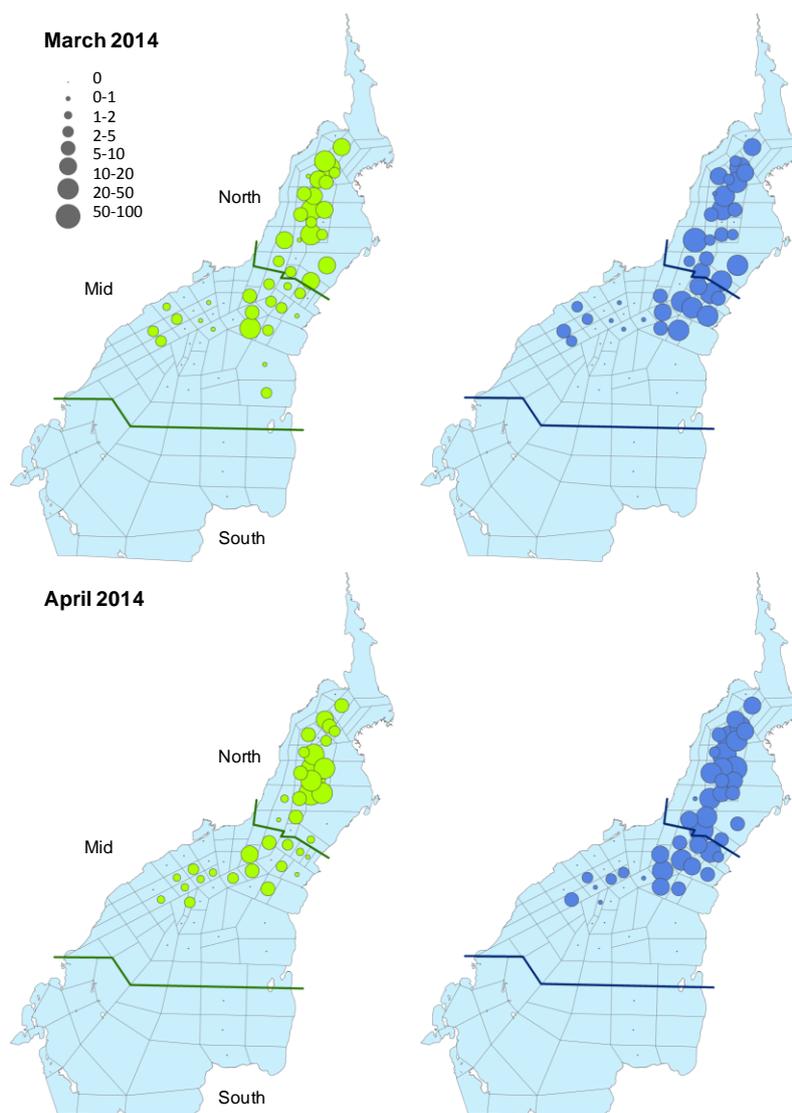


Figure 6.12 (cont.). Catch rates of cuttlefish (number per hour) (*Sepia apama* on the left (green), *S. novaehollandiae* on the right (blue)) determined from fishery-independent surveys.

6.3.4.3 Fishery-Dependent Survey Estimates

An estimated total of 212,000 cuttlefish were incidentally caught over the course of eight commercial fishing periods extending from March 2013 to June 2014 (Table 6.4). The collective trawl effort expended by the entire fishing fleet during this period equated to 26,164.41 hours. Catches ranged from 5,274 cuttlefish in December 2013 to 73,176 in May 2014. *Sepia novaehollandiae* accounted for the majority (88.7%) of the catch, with *S. apama* contributing the remaining 11.3%. No *S. braggi* were detected in any of the fishery-dependent samples. The largest quantities of cuttlefish (>20,000 individuals) were caught during the late autumn and winter fishing periods.

Most of the cuttlefish (59.8%) were incidentally captured from the Northern Zone, whereas the Middle and Southern Zone accounted for 38.3% and 1.9%, respectively (Table 6.4). The greatest proportion of *S. apama* (61.7%), however, was captured from the Middle Zone and they were least encountered (2.8%) in the Southern Zone. Catches of *S. apama* peaked during the cooler months, particularly in 2014, where catch estimates exceeded 3,000 individuals in March, April and May (Table 6.4). *Sepia novaehollandiae* continued to dominate the catch composition and followed the same seasonal trend as *S. apama*.

The patterns of distribution and relative abundance (number caught per hour) of *S. apama* and *S. novaehollandiae* differed slightly during these surveys. *Sepia apama* were more sparsely distributed throughout the Northern and Middle Zones from June to December 2013 and June 2014 in comparison to *S. novaehollandiae*, with catch rates rarely exceeding 2 hr⁻¹ (averaging ~0.4 hr⁻¹) (Figure 6.13). Catch rates of *S. novaehollandiae* during this time averaged ~5.0 hr⁻¹, and were highest throughout the Northern Zone and central gutter of the Middle Zone (Figure 6.13). No cuttlefish were caught in the Northern Zone during March 2014 as the commercial fleet concentrated all fishing effort further south, as usually is the case. Catches increased for both species during April and May 2014. *Sepia apama* were relatively evenly distributed throughout the deeper, central sections of the Middle and Northern Zones. No cuttlefish were caught north of Whyalla and around the Point Lowly Peninsula during the 2013 and 2014 commercial fishing seasons.

Table 6.4. Estimates of total cuttlefish caught during the fishery-dependent surveys of the Spencer Gulf Prawn Fishery. Estimates are presented in total for Spencer Gulf and partitioned into the Northern, Middle and Southern Zones. Numbers in parentheses indicate the relative proportion (as a percent) of each species of the catch.

SPENCER GULF	Month	<i>S. apama</i>		<i>S. novaehollandiae</i>		<i>S. braggi</i>		Total
	May-13	3,629.1	(14.9%)	20,743.6	(85.1%)	0.0	(0%)	24,372.6
Jun-13	1,003.0	(4.3%)	22,546.8	(95.7%)	0.0	(0%)	23,549.8	
Nov-13	883.2	(6.1%)	13,513.1	(93.9%)	0.0	(0%)	14,396.3	
Dec-13	905.4	(17.2%)	4,369.5	(82.8%)	0.0	(0%)	5,274.9	
Mar-14	3,251.7	(22.3%)	11,308.8	(77.7%)	0.0	(0%)	14,560.5	
Apr-14	5,840.5	(17.6%)	27,359.5	(82.4%)	0.0	(0%)	33,200.0	
May-14	6,908.9	(9.4%)	66,267.2	(90.6%)	0.0	(0%)	73,176.1	
Jun-14	1,436.9	(6.1%)	22,033.1	(93.9%)	0.0	(0%)	23,470.0	
<i>Totals</i>	<i>23,858.6</i>	<i>(11.3%)</i>	<i>188,141.7</i>	<i>(88.7%)</i>	<i>0.0</i>	<i>(0%)</i>	<i>212,000.3</i>	
NORTH	Month	<i>S. apama</i>		<i>S. novaehollandiae</i>		<i>S. braggi</i>		Total
	May-13	1,169	(7.1%)	15,316	(92.9%)	0.0	(0%)	16,485.0
Jun-13	894	(4.2%)	20,305	(95.8%)	0.0	(0%)	21,198.6	
Nov-13	73	(0.8%)	8,796	(99.2%)	0.0	(0%)	8,869.2	
Dec-13	103	(4.1%)	2,440	(95.9%)	0.0	(0%)	2,543.0	
Mar-14	0	(0%)	0	(0%)	0.0	(0%)	0.0	
Apr-14	1,687	(15.7%)	9,068	(84.3%)	0.0	(0%)	10,755.4	
May-14	3,808	(6.8%)	52,492	(93.2%)	0.0	(0%)	56,299.6	
Jun-14	741	(6.9%)	9,967	(93.1%)	0.0	(0%)	10,708.7	
<i>Totals</i>	<i>8,474.9</i>	<i>(7.7%)</i>	<i>118,384.5</i>	<i>(93.3%)</i>	<i>0.0</i>	<i>(0%)</i>	<i>126,859.4</i>	
MID	Month	<i>S. apama</i>		<i>S. novaehollandiae</i>		<i>S. braggi</i>		Total
	May-13	2,347.0	(34.4%)	4,468.0	(65.6%)	0.0	(0%)	6,815.1
Jun-13	78.1	(4.7%)	1,578.7	(95.3%)	0.0	(0%)	1,656.8	
Nov-13	810.3	(14.7%)	4,716.8	(85.3%)	0.0	(0%)	5,527.1	
Dec-13	773.9	(30.1%)	1,794.4	(69.9%)	0.0	(0%)	2,568.3	
Mar-14	3,225.7	(22.7%)	10,980.5	(77.3%)	0.0	(0%)	14,206.1	
Apr-14	3,677.5	(17.7%)	17,080.1	(82.3%)	0.0	(0%)	20,757.6	
May-14	3,101.1	(18.4%)	13,723.8	(81.6%)	0.0	(0%)	16,824.8	
Jun-14	695.5	(5.4%)	12,065.8	(94.6%)	0.0	(0%)	12,761.3	
<i>Totals</i>	<i>14,709.1</i>	<i>(18.1%)</i>	<i>66,408.0</i>	<i>(81.9%)</i>	<i>0.0</i>	<i>(0%)</i>	<i>81,117.0</i>	
SOUTH	Month	<i>S. apama</i>		<i>S. novaehollandiae</i>		<i>S. braggi</i>		Total
	May-13	112.9	(10.5%)	959.6	(89.6%)	0.0	(0%)	1,072.5
Jun-13	31.3	(4.5%)	663.2	(95.5%)	0.0	(0%)	694.5	
Nov-13	0.0	(0%)	0.0	(0%)	0.0	(0%)	0.0	
Dec-13	28.2	(17.2%)	135.5	(82.8%)	0.0	(0%)	163.7	
Mar-14	26.0	(7.3%)	328.4	(92.7%)	0.0	(0%)	354.4	
Apr-14	476.2	(28.2%)	1,210.9	(71.8%)	0.0	(0%)	1,687.1	
May-14	0.0	(0%)	51.7	(100%)	0.0	(0%)	51.7	
Jun-14	0.0	(0%)	0.0	(0%)	0.0	(0%)	0.0	
<i>Totals</i>	<i>674.6</i>	<i>(16.8%)</i>	<i>3,349.2</i>	<i>(83.2%)</i>	<i>0.0</i>	<i>(0%)</i>	<i>4,023.8</i>	

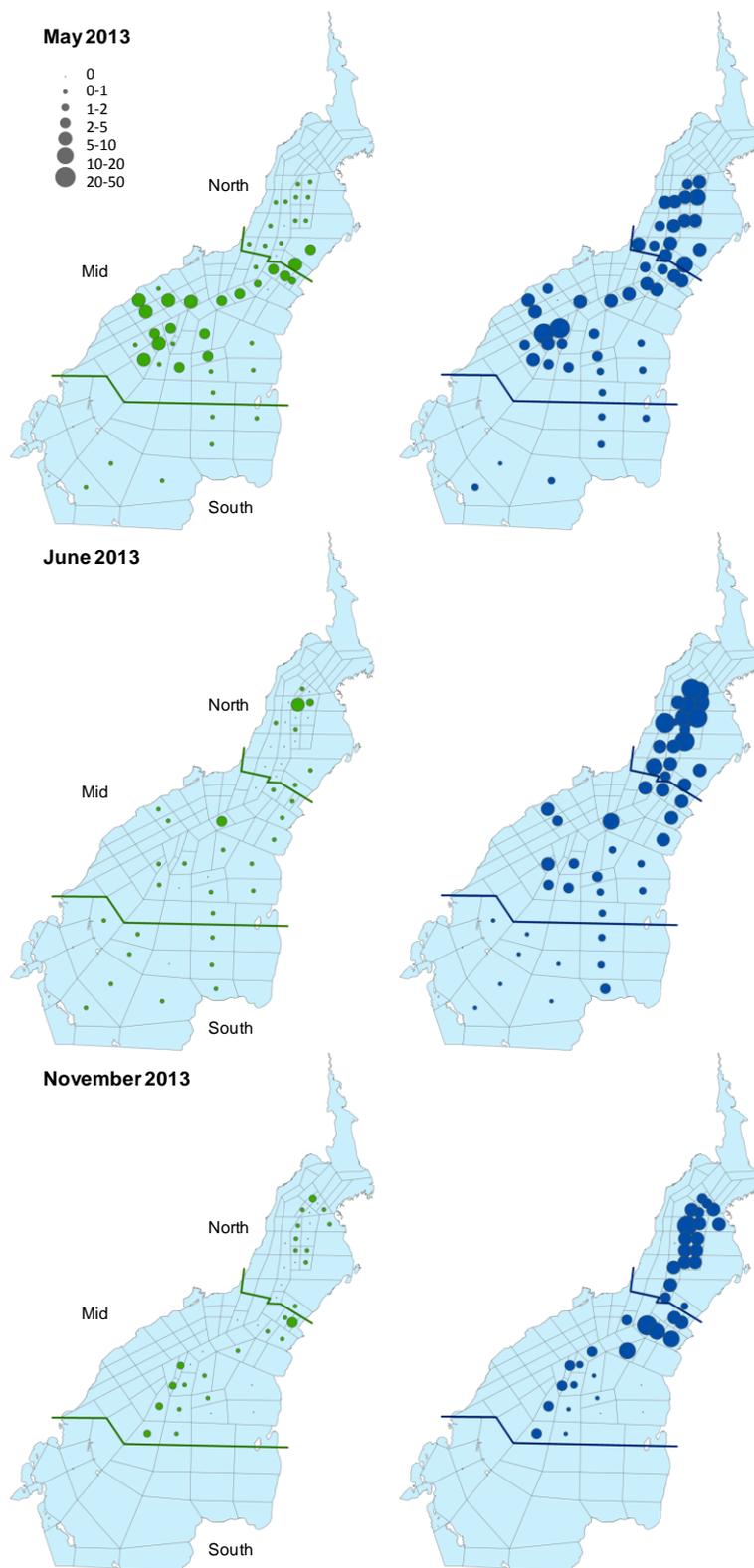


Figure 6.13. Catch rates of cuttlefish (number per hour) (*Sepia apama* on the left (green), *S. novaehollandiae* on the right (blue)) determined from fishery-dependent surveys.

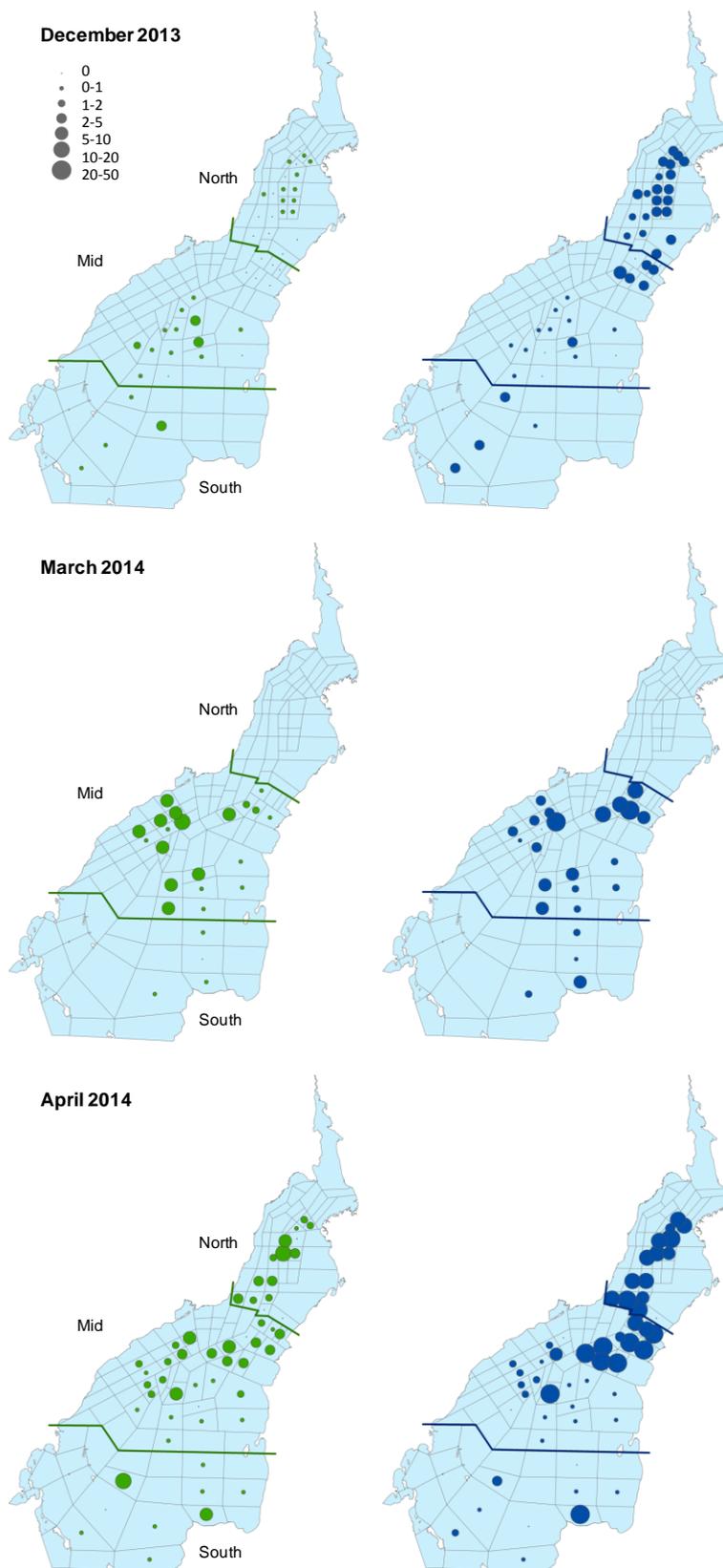


Figure 6.13 (cont.). Catch rates of cuttlefish (number per hour) (*Sepia apama* on the left (green), *S. novaehollandiae* on the right (blue)) determined from fishery-dependent surveys.

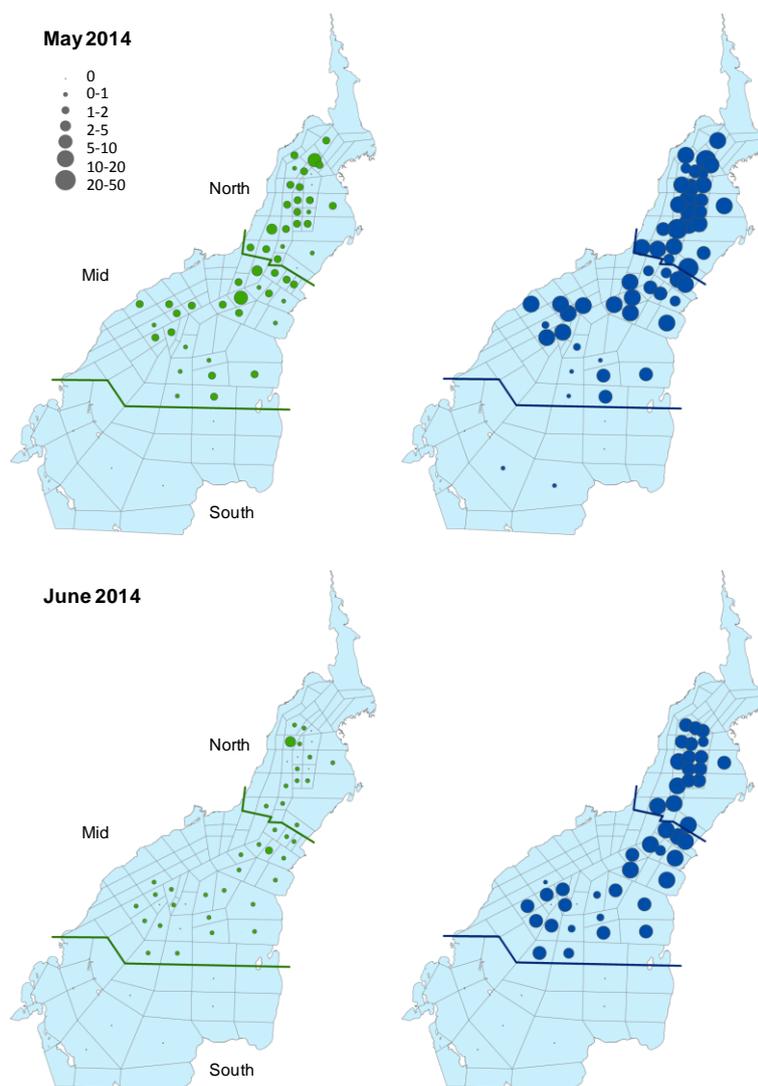


Figure 6.13 (cont.). Catch rates of cuttlefish (number per hour) (*Sepia apama* on the left (green), *S. novaehollandiae* on the right (blue)) determined from fishery-dependent surveys.

6.3.4.4 Population biology

It is well understood that *S. apama* spawn during winter (Chapter 2). This reproductive schedule was clearly evident throughout the biological sampling component of this study where relatively large (>140 mm ML), reproductively mature, Giant Australian Cuttlefish were consistently more abundant during April, May and June (Figure 6.14). Conversely, smaller (<130 mm ML) individuals were more evident during the warmer months (November, December and February). *Sepia novaehollandiae*, however, exhibited an aseasonal reproductive strategy, where relatively high proportions of reproductively mature, or maturing, animals were identified in all of the trawl samples.

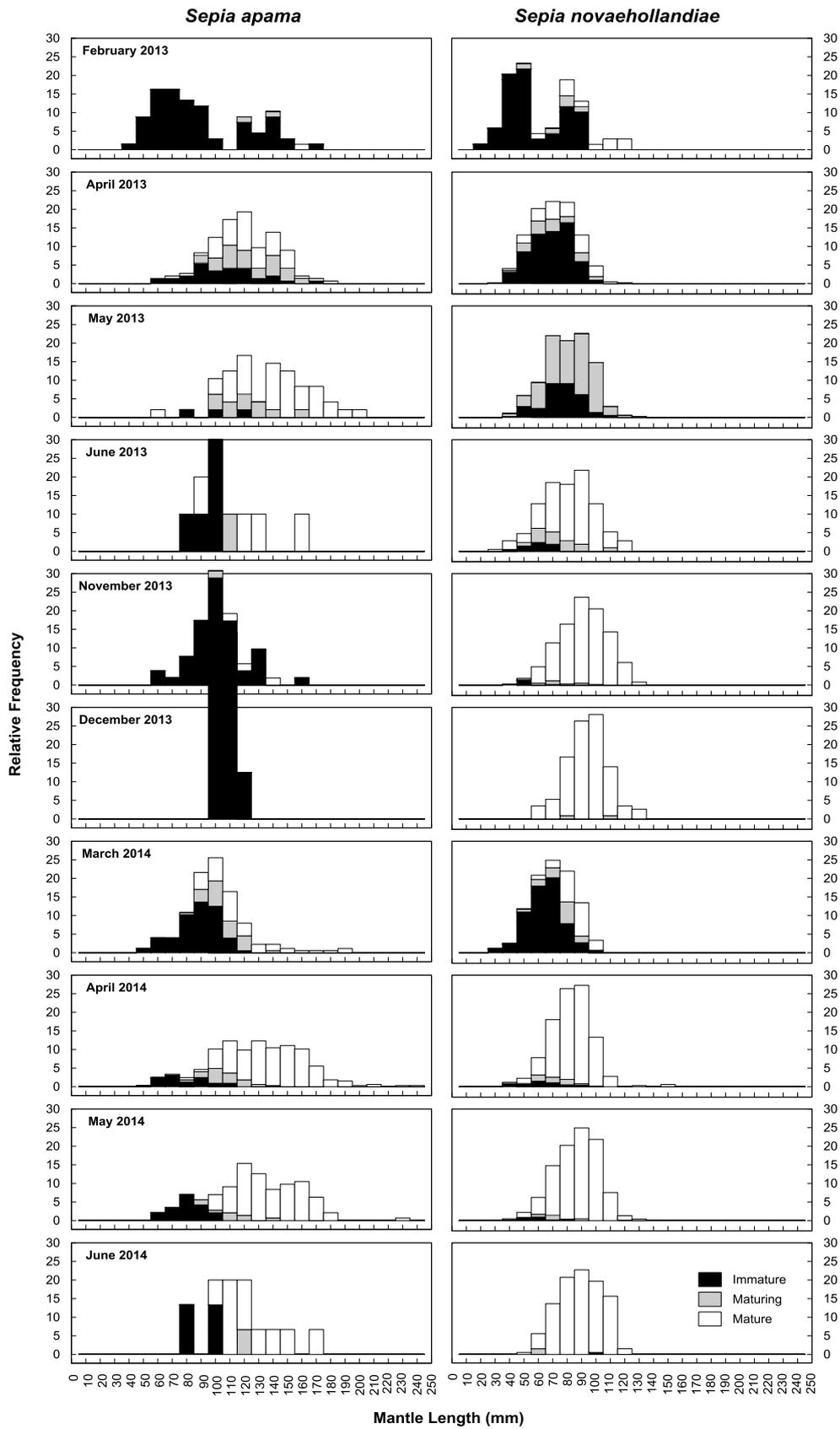


Figure 6.14. Size and sexual maturity composition of all cuttlefish processed from the fishery dependent and independent surveys.

6.3.5 Harvest Fraction

Given the lack of spatial resolution of the cuttlefish by-catch estimates from the Northern Zone Rock Lobster Fishery and the absence of corresponding data from the Blue Crab Fishery and their low level of Giant Australian Cuttlefish by-catch (Table 6.2) the harvest fraction could only be estimated for the Spencer Gulf Prawn fishery. The Spencer Gulf Prawn Fishery was estimated to harvest between 6.2-9.6% of the spawning population in 2013 and 1.3–6.5% in 2014 (Table 6.5).

Table 6.5. Estimate of the Giant Australian Cuttlefish harvest fraction (%) by the commercial prawn fleet in northern Spencer Gulf.

Month	Pt. Lowly Cuttlefish Abundance	Prawn Fishery Cuttlefish By-catch	Total	Harvest Fraction (%)
May-13	11,067	1,169	12,236	9.6
Jun-13	13,491	894	14,385	6.2
May-14	54,976	3,808	58,784	6.5
Jun-14	57,708	741	58,449	1.3

6.4 Discussion

Preliminary molecular research has indicated that Giant Australian Cuttlefish north of Wallaroo may constitute a separate, genetically distinct population (B. Gillanders and S. Donnellan unpublished data). Given the recent decline of the key spawning aggregation and the species' lack of enduring generations to buffer against unsuccessful breeding seasons, as they breed once and die, the long-term sustainability of the northern Spencer Gulf Giant Australian Cuttlefish population has become a key priority. To achieve this, considerable attention has naturally focused on commercial fisheries that operate in this area as their contact with Giant Australian Cuttlefish is more direct and measurable in-comparison to the less obvious user groups such as recreational fishers or coastal manufacturing industries. Although precautionary management strategies were implemented in March 2013 to prohibit fishers catching Giant Australian Cuttlefish within this area, they were still incidentally captured by three commercial fisheries (Northern Zone Rock Lobster, Spencer Gulf Blue Crab and Spencer Gulf Prawn Fisheries). Of these, the Northern Rock Lobster Fishery posed the least risk as the fleet is confined to the southernmost regions of Spencer Gulf and their estimated cuttlefish by-catch appears negligible.

The Blue Crab and Spencer Gulf Prawn fishing fleets have continued to operate in northern Spencer Gulf throughout the closure. Incidental catches of cuttlefish by Blue Crab fishers have been minor, with annual catch rates rarely exceeding 0.02 individuals per potlift. Given that this fishery operates throughout most of the area, including the deeper gutter sections of the northern gulf, it is likely that this catch rate accounts for both Giant Australian and Nova Cuttlefish. Subsequently, catch rates of Giant Australian Cuttlefish would be proportionately less. The passive gear used by Blue Crab and Rock Lobster pot-based fisheries is also likely to result in higher post release survival of by-catch.

The greatest quantities of cuttlefish by-catch were recorded from the Spencer Gulf Prawn Fishery, with total monthly estimates ranging between 5,275 cuttlefish in December 2013 to 73,176 cuttlefish in May 2014 as calculated for the entire 39 vessel fleet. Estimates were provided for both the fishery-independent and fishery-dependent sampling programs as the respective dynamics of the fishing fleet are considerably different in terms of the area fished and trawl intensity. During fishery-independent surveys the fleet extends further north into areas that are not routinely fished and the duration of individual shots rarely exceeds 30 minutes. Despite these operational differences, it was important to compare the quality of data obtained between the two programs to ascertain whether there were any inherent sampling biases that may have compromised the overall catch estimates. A comparison of the catch composition and mean catch rates for both species of cuttlefish indicated that both sampling programs provided similar results.

The catch composition was consistently dominated (>60%) by Nova Cuttlefish and the largest quantities of Giant Australian Cuttlefish were caught during March, April and May (up to 6,909 individuals in May 2014). This was particularly evident in northern Spencer Gulf, and not unexpected, given that it coincides with the seasonal aggregation of mature animals around Point Lowly. An investigation of the reproductive status of sampled Giant Australian Cuttlefish further confirmed that they were either approaching or were in spawning condition and most likely captured en route to shallow spawning grounds within the area.

The estimated total number of Giant Australian Cuttlefish incidentally caught as by-catch by the commercial fisheries operating in northern Spencer Gulf requires some ecological context to ascertain its level of risk to the population. Determining a 'harvest fraction' typically provides a relatively clear indication of exploitation in general, however, in this case, little is known about the northern Spencer Gulf Giant Australian Cuttlefish population dynamics and, as a consequence, introduces a level of uncertainty. For the purpose of this report, the quantification of Giant Australian Cuttlefish by-catch in the Spencer Gulf Prawn Fishery was assessed in association with the measures of abundance of the spawning population (Chapter 2) to estimate a relative 'harvest fraction' and provide some ecological

context. To achieve this, a number of factors needed to be considered *a priori*. Firstly, comparisons between the estimates of by-catch and the spawning population could only be made during the peak spawning season (May and June) where there was available data. Secondly, it was assumed that all Giant Australian Cuttlefish within northern Spencer Gulf would aggregate around Point Lowly to spawn, and were representative of the entire northern Spencer Gulf population. Thirdly, natural mortality and post-release survival was assumed to be zero. Similarly, potential depletion rates through routine fishing practices were not accounted for. Finally, the residency time and movement of Giant Australian Cuttlefish on and off of the spawning grounds was assumed to be static for each monthly comparison, although acoustic telemetry research has indicated the residency time of spawning cuttlefish is approximately 14-25 days (Payne et al. 2011). These assumptions meant that the estimates of harvest fraction in this study were likely to be positively biased. Based on these assumptions, the comparison of the estimates of by-catch of Giant Australian Cuttlefish from the Northern Zone with the monthly estimates of abundance on the spawning grounds indicated that the Spencer Gulf Prawn Fishery harvested <10% of the spawning population in 2013 and <7% in 2014. These estimates were considerably less than the harvest fraction of 40% that is adopted for most cephalopod fisheries (Beddington et al. 1990). The peak harvest fraction of 9.6% in 2013 did not appear to compromise the subsequent 2014 spawning aggregation as it increased by 325% (Chapter 2).

Quantifying post-release survival rates of cuttlefish is notoriously difficult as mortality can result long after the initial capture event. A small feasibility study carried out in April 2014 aimed to obtain preliminary information on the survivability of Giant Australian Cuttlefish following incidental capture in a prawn trawl net (SARDI unpublished data). This study selected Giant Australian Cuttlefish of varying sizes that appeared to be in good condition and maintained them in onboard holding tanks for 12 hours. The relative condition of each Giant Australian Cuttlefish was assessed at regular intervals. Seven of the eight individuals examined survived the 12 hr observational period and were subsequently released. It was estimated that approximately 5% of cuttlefish caught during a 30 minute trawl were considered to be in 'good' condition and would have the greatest chance of surviving once released, 10-15% displayed signs of life and were unlikely to survive; and 80-85% were dead. The fishery has self-imposed a code of conduct to maximise the post-release survival of Giant Australian Cuttlefish caught in northern Spencer Gulf, by carefully isolating cuttlefish from the catch, maintaining them alive within onboard holding tanks, and releasing them at a time when scavenging predators (i.e. dolphins) are absent. The Spencer Gulf Prawn Fishery has also recently trialed by-catch reduction devices (BRDs) specifically to reduce the capture of cuttlefish and Blue Crabs. The results of these initial trials were promising with two

separate grid-based BRDs reducing the cuttlefish catches by approximately 55% (Kennelly 2014). Further development and broad-scale testing of these BRDs is scheduled for 2015 with the expectation that, if successful, PIRSA Fisheries and Aquaculture and industry would consider their implementation when and where significant Giant Australian Cuttlefish are known to occur (FRDC EOI FN20077).

7 GENERAL DISCUSSION

Five key research priorities for Giant Australian Cuttlefish that were identified by the GCWG were addressed in this report. They were: 1. to undertake a survey that estimates population abundance, habitat condition and water quality at the major spawning location; 2. to explore whether there were alternate pockets of spawning activity within northern Spencer Gulf; 3. to investigate whether spawning Giant Australian Cuttlefish prefer certain den dimensions in which to lay eggs with the expectation that this information would be used to design and develop artificial spawning habitats; 4. to undertake residue testing of Giant Australian Cuttlefish tissues to determine their susceptibility to coastal contaminants; and 5. to quantify the Giant Australian Cuttlefish by-catch from commercial fishing.

7.1 Synthesis

Stock recruitment relationships are typically weak for cephalopods as their short life-span and dynamic life-history compromises our ability to forecast recruitment strength from estimates of spawning biomass (Pierce and Guerra 1994). The recent 325% increase over 12 months in the Point Lowly Giant Australian Cuttlefish spawning population from a considerably depressed population estimate (2013) is a clear example of this disconnection. The relationship between cephalopod life cycles and environmental variability is consistently emphasised within the literature as the underlying mechanism that shapes population size (Boyle and Rodhouse 2005). These variables, however, have the capacity to operate on a variety of scales, some of which are broadly predictable (e.g. peak seasonal patterns), whereas others are unpredictable (e.g. nutrient/pollution pulses, storm events) or perhaps unknown (e.g. seismic activity). Such variation adds considerably more uncertainty to the effective assessment and management of cephalopods in general. Given these uncertainties, protecting known spawning aggregations is the most appropriate precautionary approach, ensuring the maximum supply of eggs is attained to buffer against the unpredictability of the environment.

The unique spawning aggregation at Point Lowly has been effectively protected from fishing since 1999. Expanding the area to encompass northern Spencer Gulf in March 2013 offered greater protection and specifically encompassed the entire range of the northernmost Giant Australian Cuttlefish population which is potentially self-sustaining and genetically distinct (Gillanders and Donnellan unpublished data). Although fishers targeting Giant Australian Cuttlefish were effectively eliminated from the area, the Spencer Gulf Prawn and Blue Crab fisheries still incidentally caught cuttlefish as by-catch. Estimates of total annual catch from the Blue Crab Fishery were negligible, with fishers recording a maximum catch of 2,483 cuttlefish in 2004 at a rate of approximately 0.02 cuttlefish per potlift. Estimated catches

from the prawn fishery were greater (up to 73,176 in May 2014), however, Giant Australian Cuttlefish rarely constituted more than 20% of the total cuttlefish by-catch. Furthermore, the commercial fishing fleet typically targets prawns further south during the winter, inadvertently diverting the fishing pressure away from the key Point Lowly spawning grounds leading into the peak breeding season. Ignoring the potential for post-release survival of Giant Australian Cuttlefish by-catch, natural mortality rates and assuming that all Giant Australian Cuttlefish caught within the northern zone of Spencer Gulf will aggregate at Point Lowly to spawn, the prawn fishery was estimated to harvest up to 9.6% (2013) of the spawning population. The estimated harvest fraction declined to 6.5% in 2014. Given the 2014 spawning population was 325% higher than the previous year, the inverse trend in the prawn fishery's estimated annual harvest fraction suggested that the Spencer Gulf Prawn trawl fleet has not adversely affected the Giant Australian Cuttlefish population in Northern Spencer Gulf.

The relative importance of the Point Lowly spawning population is currently unknown, but is likely to be significant. This was supported by an exploratory survey that found no evidence of spawning activity outside of the spawning grounds, and the absence of spawning on artificial habitats strategically placed in areas where Giant Australian Cuttlefish are known to occur. The lack of optimal spawning habitat throughout northern Spencer Gulf (north of Wallaroo) was apparent in this study. Despite this limitation, it is difficult to eliminate the potential for alternate pockets of spawning activity contributing to the genetically distinct northern population, as the remaining populations that occur further south and throughout the southern coastline of mainland Australia are sustained by more dispersed spawning activity (Rowling 1995). The timing of the exploratory surveys coincided with the lowest estimate of spawning Giant Australian Cuttlefish on record. If the reduced population estimate on the Point Lowly spawning grounds is indicative of northern Spencer Gulf, then locating smaller pockets of spawning activity within the region would be more difficult. Historic accounts of commercial quantities (1–10 t) of cuttlefish being taken from the Wallaroo area during the late 1990s when the population was at its peak (Hall and Fowler 2003), suggests that the area has the capacity to support spawning Giant Australian Cuttlefish and is perhaps dependent on the relative population density of the area. Impending results from a companion study exploring the fine-scale population structure of the potential northern Spencer Gulf sub-population through the combination of molecular and chemical (trace elements and stable isotope) techniques, will provide greater insight into the overall significance of the Point Lowly spawning grounds (Gillanders et al. FRDC 2013/010).

Unlike the obvious and direct effects of fishing, there are numerous environmental processes that have the potential to indirectly affect Giant Australian Cuttlefish population dynamics. For example, discharges from coastal industries (i.e. aquaculture activity, wastewater release, contaminated run-off) can promote localised eutrophication and/or pollution and, if inappropriately timed, has the potential to compromise embryonic development and subsequent recruitment (Pierce et al. 2010). Tracing back these potential drivers and disentangling their flow-on ecological effects is complex and challenging, particularly as the spawning ground is situated in a highly industrialised area and is consequently exposed to a variety of inputs (see Steer et al. 2013). This study assessed the local water quality and habitat characteristics over the past two years and screened Giant Australian Cuttlefish within the area for heavy metals to determine whether any anthropogenic influences could be detected. Ambient water chemistry properties appeared relatively consistent throughout the surveys, however, changes in the density of the opportunistic alga *Hincksia sordida* increased from sparse coverage (<20%) in 2013 to a maximum of 70% in 2014 at key spawning sites. Drawing a definitive link between this increased coverage and nutrient input is difficult as the alga also proliferates with increasing temperature. Given its sparse coverage in 2013, *H. sordida* appeared to have a negligible effect on embryonic development as the subsequent 2014 recruiting population was relatively successful. The timing of its 2014 bloom and subsequent effect on embryo development is yet to be determined as the resultant population is expected to aggregate in the forthcoming winter (2015).

A significant regional difference in metal burden was detected in Giant Australian Cuttlefish, with the relative concentration of many metals (i.e. Cd, Zn, Pb, Au, Cu) more pronounced in animals collected from the Point Lowly spawning grounds compared to those collected further south (Wallaroo). This finding was not surprising given the long history of metal contamination in northern Spencer Gulf (Gaylard 2014). Despite this, however, the observed concentrations were comparable to other cuttlefish species (Miramand et al. 2006; Pereira et al. 2009), suggesting that they were not likely to exceed the physiological tolerance of cephalopods. Cephalopods typically detoxify metals through the digestive gland (Bustamante et al. 2002a), and this study confirmed this organ constituted >90% of the animal's total metal burden, whereas the metal concentration in the mantle (edible portion) was well within food safety standards. The limits of physical tolerance of the Giant Australian Cuttlefish are not known, but given no clear association was found between the recent decline in the population and reported levels of anthropogenic discharges of heavy metals from 1994 to 2012 (Steer et al. 2013), they do not appear to be currently adversely affected by metal contamination within northern Spencer Gulf.

The temperature at which embryonic development takes place has been consistently identified as the most important influence of developmental rates and the timing of hatching (Boyle et al. 2001; Boletzky 2003). Similarly, the timing of hatching with favourable environmental conditions is essential for successful growth and survival, and is commonly referred to as the 'match and mis-match' concept (Cushing 1982). Species, like Giant Australian Cuttlefish, that exhibit a distinct breeding season risk recruitment failure should hatching not coincide with optimal conditions (Boyle and Rodhouse 2005). An investigation of the daily average temperature over an estimated 120 day embryo development period, has, so far, provided the strongest signal in explaining the recent inter-annual variation in both abundance and biomass of the Point Lowly spawning population. Although important, the temperature regime during the early life history is not the exclusive determinate of favourable conditions, other factors such as predatory/prey abundance, and water quality are likely to contribute in shaping the population. A synthesis of the findings of this report has indicated that the 2013 cohort that lead to a relatively successful 2014 spawning aggregation, not only encountered warming temperatures during the early critical stages of development, but also developed through an environment relatively devoid of *Hinckia sordida*, experienced low fishing pressure from commercial fisheries operating within the area, and consisted of a population that appears tolerant to current levels of metal contamination. The relative influence of the predator and prey abundance within northern Spencer Gulf, however, remains a key knowledge gap and is an avenue of research that is currently being investigated (Gillanders et al. FRDC 2013/011).

Although the recent increase in the spawning population was positive, it only represented 32% of the peak observed in 1999. The lack of spawning activity on the eastern end of the Point Lowly Peninsula and on the strategically deployed artificial substrate within northern Spencer Gulf also indicated that the population density was not large enough to spill out of the traditional spawning sites located west of Stony Point. It is still unknown whether the peak estimate of ~180,000 animals in 1999 was a result of an extraordinary population increase, or was indicative of a natural population size that has persisted through time, and until the scale of the population dynamics is understood management should remain cautiously optimistic about the recent increase.

7.2 Implications

This project addressed a number of broad Giant Australian Cuttlefish related issues that were identified by South Australia's cross-government and community Giant Cuttlefish Working Group in association with a concurrent research program that is more specifically focused on deciphering the fine-scale structure of the northern Spencer Gulf population (Gillanders et al. FRDC 2013/010). Our project reaffirmed the relative importance of the

Point Lowly spawning grounds as an essential component of the northern Spencer Gulf Giant Australian Cuttlefish population as there was no evidence of spawning activity outside of the area. An assessment of the relative impacts of recent levels of commercial fishing pressure and the rates of bio-accumulation of heavy metals within the area does not appear to have had a detrimental effect on the spawning population. It must be emphasised, however, that these results should not preclude the fishing and local manufacturing industries from adopting precautionary practices that consider the future conservational value of the spawning population.

The estimates of commercial by-catch of cuttlefish provided in this report have been calculated through the most rigorous means possible and have provided a detailed evaluation of the fishing pressure within Spencer Gulf. This information can be used by the Spencer Gulf and West Coast Prawn Fishers Association to inform future harvest strategies that minimise their ecological impact. Similarly, PIRSA Fisheries and Aquaculture can refer to these estimates to assess the relative value of the current broad-scale closure of the Northern Spencer Gulf and in the development of future management strategies.

There is currently no evidence to suggest that habitat loss has contributed to the decline in Giant Australian Cuttlefish abundance. Extensive habitat surveys carried out by SARDI and BHP Billiton over the past three spawning seasons has provided no clear indication that the spawning habitat has been structurally compromised. The deployment of artificial spawning habitat is unlikely to significantly promote the recovery of the population to the levels that were observed in the late 1990s. The effectiveness and relative ecological value of the artificial dens used in this study in mitigating habitat loss is unknown as none of the structures supported spawning animals during the 2014 spawning season. They have, however, been left *in situ* and will be assessed again throughout next year's spawning period.

The recent increase in the spawning aggregation at Point Lowly highlights the importance of maintaining an on-going, annual, monitoring program to provide a greater understanding of the natural dynamics of the population. Although welcomed, the Giant Cuttlefish Working Group and associated stakeholders are remaining cautiously optimistic about the recent increase and are anticipating the findings of the companion study that seeks to provide a greater understanding of how Giant Australian Cuttlefish from northern Spencer Gulf utilise the Point Lowly spawning grounds, and determine the viability of the population through simulated exposure to various environmental drivers (Gillanders et al. FRDC 2013/010).

7.3 Recommendations

Given the uncertainties regarding the underlying process that contribute to shaping the population dynamics of Giant Australian Cuttlefish, protecting the known spawning aggregation is the most appropriate precautionary approach to ensure the maximum supply of eggs is attained to buffer against the unpredictability of the environment. The broader-scale protection of the northern Spencer Gulf sub-population from targeted fishing is also a practical strategy, particularly when the population is at a low level. Continued collection of cuttlefish by-catch data through established fishery-independent programs in the Spencer Gulf Prawn and Blue Crab fisheries would also add value in the on-going assessment of the relative impact of these fisheries. Relying on fishery-independent programs within the prawn fishery would streamline the process as this study indicated that it was a relatively accurate representation of the fishery-dependent data.

An on-going monitoring program that assesses the spawning population, particularly in relation to the future expansion of coastal industries and planned infrastructure within the area, would contribute to our understanding of the population's capacity to fluctuate over short and long-term time scales. The standardisation of a monitoring program (see Steer et al. 2013) provides an opportunity for other government and non-government agencies to undertake their own surveys or collaborate together (as successfully undertaken by BHP Billiton) and ensure the continuity of the data. With the appropriate training and expert supervision it may also be possible to enlist qualified volunteers (i.e. citizen scientists) to contribute to data collection through recreational dive clubs, and community or school groups. Enlisting diverse groups to undertake the surveys, however, raises issues around quality control and assurance of the collected data. Ensuring that divers are appropriately trained or accompanied by experts who have contributed to the surveys in the past would ensure greater scientific rigor in data collection and result in meaningful estimates of Giant Australian Cuttlefish abundance and biomass. Appropriately archiving habitat images would also facilitate audits, or re-analysis, if required to investigate data integrity. Similarly, the EPA could be used for the on-going analysis of water samples to ensure that the appropriate systems and practices were in place for the delivery of high quality environmental data.

7.3.1 Further Development

Although the artificial reefs did not promote spawning activity in their respective areas, it remains unclear whether these structures can be effectively used to mitigate habitat loss. It is likely that the reduced Giant Australian Cuttlefish population within northern Spencer Gulf lowered the chance of these structures being used as spawning substrate, particularly in

areas where habitat was limited. All artificial reefs have been left *in situ* and will be reassessed during the 2015 spawning season.

The Spencer Gulf Prawn Fishery has recently trialed by-catch reduction devices (BRDs) specifically to reduce the capture of cuttlefish species and Blue Crabs. The results of these initial trials were promising with two separate grid-based BRDs reducing the cuttlefish catches by approximately 55% (Kennelly 2014). Further development and broad-scale testing of these BRDs is scheduled for 2015 with the expectation that, if successful, PIRSA Fisheries and Aquaculture and industry would consider their implementation when and where significant Giant Australian Cuttlefish are known to occur (FRDC EOI FN20077).

7.4 Extension and Adoption

The details of this project, including its aims and objectives; progress; interim results; and final results were disseminated through regular Giant Cuttlefish Working Group meetings and with key stakeholders and fishery managers (i.e. PIRSA, Spencer Gulf and West Coast Prawn Fishermen's Association, Marine Fishers Association, BHP Billiton, Conservation Council of South Australia, Reefwatch).

A synopsis of this project (and other related projects) was presented at the Australian Society for Fish Biology (ASFB) joint conference in Hamilton, New Zealand in August 2013. The presentation was entitled: "What's going on with South Australia's Giant Australian Cuttlefish spawning aggregation". Similar public presentations were made at SARDI's Open Day, SARDI's internal seminar series, and at PIRSA's Fishwatch volunteer Annual General Meeting.

Annual updates of this project have also been provided through PIRSA's website on a specifically constructed 'Cuttlefish Update' web-page (www.pir.sa.gov.au/cuttlefish). There has also been cabinet submissions and widespread media (i.e. print, radio, television and internet) dissemination coordinated through the Giant Cuttlefish Working Group, PIRSA communications and FRDC.

7.5 Project coverage

- 4 May 2013: 'Cuttlefish numbers' - Radio.
- 14 May 2013: 'University launches study into Giant Australian Cuttlefish' - Whyalla News.
- 22 May 2013: 'Cuttlefish numbers' - Radio.
- 30 May 2013: 'Cuttlefish numbers plummet' - Whyalla News.
- 19 June 2013; 'Cuttlefish Research' - Radio.
- 21 June 2013: 'Cuttlefish funding research' - Radio.
- 25 June 2013: '\$50,000 boost for cuttlefish' - Whyalla News.
- 2 July 2013: 'More funding for cuttlefish' - Yorke Peninsula Country Times.
- 22 August 2013: 'Cuttlefish breeding Upper Spencer Gulf' - Radio.
- 5 September 2013: 'Fears mount for cuttlefish as gulf numbers plunge' - The Advertiser.
- 5 September 2013: 'Cuttlefish numbers study' - Radio.
- 16 September 2013: 'Research projects shed light on cuttlefish' - Whyalla News.
- 26 September 2013: 'Lifecycle of cuttlefish' Scope- Network Ten (episode 02/162)
- 7 October 2013: 'Cuttlefish lose out Point Bonython' - The Advertiser.
- 24 December 2013: 'Cuttlefish decline not linked to prawn fishers' - Port Lincoln Times.
- 9 January 2014: 'Prawn fishing not killing cuttlefish' - Whyalla News.
- 19 February 2014: 'Cuttlefish Adelaide Fringe' - Radio,
- 12 March 2014: 'Spencer Gulf research' - Radio.
- 28 March 2014: 'Cuttlefish protection continues' - Radio.
- 3 April 2014: 'Plight of cuttlefish' - Radio,
- 6 May 2014: 'Cuttlefish Point Lowly' - Radio.
- 20 May 2014: 'Cuttlefish Upper Spencer Gulf breeding season' - Radio.
- 21 May 2014: 'Cuttlefish arrive early Point Lowly breeding season' - Radio.
- 21 May 2014: 'Cuttlefish breeding' - Radio.
- May-June 2014: 'Plight of the Giants' Australian Geographic issue 120.
- 25 July 2014: 'Cuttlefish populations Port Bonython' - Radio.
- 5 September 2014: 'Scientists baffled by return of Giant Cuttlefish' - The World Today.
- 5 September 2014: 'Cuttlefish aggregation finishing' - Radio.

8 References

- Anderson, M, R Gorley and R Clarke, 2008, *Permanova+ for Primer: Guide to Software and Statistical Methods*. Plymouth, UK., Primer-E Ltd.
- Arup. 2013. Port Bonython bulk commodities export facility. EPBC 2012/6336: Preliminary Documentation Report.
- BHP Billiton. 2009. Olympic Dam Expansion Draft Environmental Impact Statement.
- Beddington, JR, AA Rosberg, JA Crombie and GP Kirkwood. 1990. Stock assessment and the provision of management advice for the short-fin squid fishery in Falkland Island waters. *Fisheries Research* **43**: 351-365.
- Boletzky, SV. 2003. Biology of early life stages in cephalopod molluscs. *Advances in Marine Biology* **44**: 143-200.
- Boucaud-Camou, E and M Yim, 1980, 'Fine structure and function of the digestive cell of *Sepia officinalis* (Mollusca: Cephalopoda)', *Journal of Zoology*, **191**,(1): 89-105.
- Boucher-Rodoni, R and E Boucaud-Camou, 1987, 'Fine structure and absorption of ferritin in the digestive organs of *Loligo vulgaris* and *L. Forbesi* (Cephalopoda, Teuthoidea)', *Journal of Morphology*, **193**,(2): 173-184.
- Boyle, PR, L Noble, AM Emery, S Craig KD Black and J Overnell. 2001. Development and hatching in cephalopod eggs. A model system for partitioning environmental and genetic effects on development. In Atkinson, D and M Thorndyke (eds). *Environment and Animal Development. Genes, Life Histories and Plasticity*, 251-267. Oxford, BIOS Scientific.
- Boyle, P and P Rodhouse. 2005. *Cephalopods: Ecology and Fisheries*. Blackwell Science, London. 451 pp.
- Brock, DJ, PJ Hawthorne, TM Ward and AJ Linnane. 2007. Two monitoring methods that assess species composition and spatio-temporal trends in by-catch from an important temperate rock lobster (*Jasus edwardsii*) fishery. *Marine and Freshwater Research*, **58**: 273-285.
- Bryan, G, M Waldichuk, R Pentreath and A Darracott. 1979. Bioaccumulation of Marine Pollutants [and Discussion], *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, **286**,(1015): 483-505.
- Bryars, S. 2003. An inventory of important coastal fisheries habitats in South Australia. Fish Habitat Program, Primary Industries and Resources South Australia.
- Bustamante, P, M Bertrand, E Boucaud-Camou and P Miramand. 2006. Subcellular distribution of Ag, Cd, Co, Cu, Fe, Mn, Pb, and Zn in the digestive gland of the common cuttlefish *Sepia officinalis*. *Journal of Shellfish Research*, **25**,(3): 987-993.
- Bustamante, P, RP Cosson, I Gallien, F Caurant and P Miramand. 2002a. Cadmium detoxification processes in the digestive gland of cephalopods in relation to accumulated cadmium concentrations. *Marine Environmental Research*, **53**: 227-241.
- Bustamante, P, J-L Teyssié, B Danis, SW Fowler, P Miramand, O Cotret and M Warnau. 2004. Uptake, transfer and distribution of silver and cobalt in tissues of the common cuttlefish *Sepia officinalis* at different stages of its life cycle. *Marine Ecology Progress Series*, **269**: 185-195.
- Bustamante, P, J-L Teyssie, AJ Fowler, O Cotret, B Danis, P Miramand and M Warnau. 2002b. Biokinetics of zinc and cadmium accumulation and depuration at different stages in the life cycle of the cuttlefish *Sepia officinalis*. *Marine Ecology Progress Series*, **231**: 167-177.
- Campbell, SJ. 2001. Ammonium requirements of fast-growing ephemeral macroalgae in a nutrient enriched embayment (Port Phillip Bay, Australia). *Marine Ecology Progress Series*, **209**: 99-107.
- Carrick, N. 1997. A preliminary assessment of the by-catch from the Spencer Gulf Prawn Fishery. South Australian Fisheries Assessment Series 97/02.

- Chouvelon, T, J Spitz, Y Chereil, F Caurant, R Sirmel, P Mèndez-Fernandez and P Bustamante. 2011. Inter-specific and ontogenic-related differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and Hg and Cd concentrations of cephalopods. *Marine Ecology Progress Series*, **433**: 107-120.
- Commonwealth of Australia. 2013. Australia New Zealand Food Standards Code - Standard 1.4.1 - Contaminants and Natural Toxicants, Australian Government, Canberra, F2013C00140: 8.
- Corbin, T and S Wade. 2004. Heavy metal concentrations in razorfish (*Pinna bicolor*) and sediments across the northern Spencer Gulf, Environment Protection Authority.
- Currie, DR, GE Hooper and TM Ward. 2007. Blue Crab (*Portunus pelagicus*) Fishery 2005/06. Stock Assessment Report to PIRSA Fisheries. South Australian Research and Development Institute (Aquatic Sciences). RD 03/0274-4. SARDI Research Report Series No. 204
- Currie, DR, CD Dixon, SD Roberts, GE Hooper, SJ Sorokin and TM Ward. 2009. Fishery-independent by-catch survey to inform risk assessment of the Spencer Gulf Prawn Trawl Fishery. Report to PIRSA Fisheries. South Australian Research and Development Institute (Aquatic Sciences). Adelaide. SARDI Publication No. F2009/000369-1. SARDI Research Report Series No. 390.
- Cushing, DH. 1982. *Climate and Fisheries*. London, Academic Press.
- Depledge, M and P Rainbow. 1990. Models of regulation and accumulation of trace metals in marine invertebrates. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*, **97**,(1): 1-7.
- Dixon, CD, I Svane and TM Ward (2005) Monitoring and assessment of by-catch and by-product species of the Spencer Gulf Prawn Fishery. Report to PIRSA. SARDI Aquatic Sciences Publication No. RD04/0249. SARDI Research Report Series No. 102.
- Dixon, CD, J Raptis, D Gorman, S Roberts, G Hooper, N Bicknell, S Sorokin, R Newman, C Noell, T Benediktsson, J Saint and W Hill. 2013. A collaborative approach to novel by-catch research for rapid development, extension and adoption into a commercial trawl fishery. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2012/000250-1. SARDI Research Report Series No. 643. 47pp.
- Ferguson, J. 1983. Concentrations and Speciation of Lead, Zinc and Cadmium in Seawater-like Smelter Effluent and Adjacent Marine Environments, Port Pirie, South Australia. *Australian Journal of Marine & Freshwater Research*, **34**: 375-385.
- Finger, J and J Smith. 1987. .Molecular association of Cu, Zn, Cd and ^{210}Po in the digestive gland of the squid *Nototodarus gouldi*. *Marine Biology*, **95**,(1): 87-91.
- Forsythe, JW. 1993. A working hypothesis of how seasonal temperature change may impact the field growth of young cephalopods. In.T. Okutani, RK O'Dor, T Kubodera (eds) '*Recent Advances in Cephalopod Fisheries*'. Tokai University Press, Tokyo, Japan. Pp: 133-143.
- Fowler, AJ, R McGarvey, MA Steer and JE Feenstra. 2014. The South Australian marine scalefish status report - 2013/14. Report to PIRSA Fisheries and Aquaculture, South Australian Research and Development Institute (Aquatic Sciences), F2007/000565-9. SARDI Research Report Series Number 816.: 44 pp.
- Gaylard, S. 2014. Marine Pollution in Spencer Gulf, South Australia, B Gillanders, S Madigan, S Murray-Jones, S Shepherd and D Wiltshire (Eds), Ch: 26, *A Natural History of Spencer Gulf*, Royal Society of South Australia., Adelaide.
- Gaylard, S, S Thomas and M Nelson. 2011. An Assessment of the Current Status of Bioavailable Metal Contamination Across South Australia Using Translocated Mussels *Mytilus Galloprovincialis*. *Transactions of the Royal Society of South Australia*, **135**,(1): 39-54.
- Gillanders, B and N Payne. 2014. Giant Australian cuttlefish. In B Gillanders, S Madigan, S Murray-Jones, S Shepherd and D Wiltshire (Eds), Ch: 21, *Natural History of Spencer Gulf*, Royal Society of South Australia, Adelaide: 288-301.
- Gillanders, BM, S Donnellan, D Fordham, T Prowse, K Rowling and MA Steer. (In progress) Giant Australian Cuttlefish in South Australian Waters. FRDC Project 2013/010

- Hall, K and AJ Fowler. 2003. *The fisheries biology of the cuttlefish Sepia apama Gray, in South Australian waters. Final report to FRDC (Project No. 98/151)*. Adelaide, SARDI Aquatic Sciences 289pp.
- Hanlon RT and JB Messenger. 1996. *Cephalopod Behaviour*. Cambridge University Press, UK. 232 pp.
- Jereb, P and CFE Roper. 2005. Cephalopods of the world. An annotated illustrated catalogue of cephalopod species known to date. Volume 1. Chambered nautilus and sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and Spirulidae). FAO species Catalogue for Fishery Purposes. No. 4, Vol. 1. Rome, FAO. 262 pp.
- Kennelly, SJ. 2014. Bycatch reduction devices (BRDs) to reduce the incidental catch of cuttlefish in the Spencer Gulf Prawn Fishery. Final FRDC Report. 2013/052. 47 pp.
- Kassahn, KS, SC Donnellan, AJ Fowler, KC Hall, M Adams and PW Shaw. 2003. Molecular and morphological analyses of the cuttlefish *Sepia apama* indicate a complex population structure. *Marine Biology*, **143**,(5): 947-962.
- Lacoue-Labarthe, T, M Warnau, F Oberhänsli, J-L Teyssié and P Bustamante. 2009. Bioaccumulation of inorganic Hg by the juvenile cuttlefish *Sepia officinalis* exposed to 203Hg radiolabelled seawater and food. *Aquatic Biology*, **6**: 91-98.
- Linnane, A. and R McGarvey. 2014. Tactical Research Fund: Assessing the feasibility of spatial management in the South Australian Northern Zone Rock Lobster (*Jasus edwardsii*) fishery. Final Report to the Fisheries Research and Development Corporation. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2013/000460-1. SARDI Research Report Series No. 790. 44pp.
- Linnane, A., R McGarvey, J Feenstra, and M Hoare. 2014. Northern Zone Rock Lobster (*Jasus edwardsii*) Fishery 2012/13. Fishery assessment report to PIRSA Fisheries and Aquaculture South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2007/000320-8. SARDI Research Report Series No. 797. 75pp.
- Lipinski, MR. 1979. Universal maturity scale for commercially important squids (Cephalopoda: Teuthoidea). The results of maturity classification for *Illex illecebrosus* (Le Seur 1821) population for years 1973-1977. ICNAF, Res. Doc. 79/11/38 Ser. No. 5364.
- Lu, CC. 1998. A synopsis of Sepiidae in Australian waters. In. Voss NA, M Vecchione, RB Toll and M Sweeney (eds.) *Systematics and Biogeography of Cephalopods* Vol. 1. Smithsonian Contributions to Zoology. Number 586.
- Miller, DJ, ARM Wright, and PG Fairweather. 2014. Benthic habitat mapping in Spencer Gulf. B Gillanders, S Madigan, S Murray-Jones, S Shepherd and D Wiltshire (Eds), Ch: 6, *Natural History of Spencer Gulf*. Royal Society of South Australia, Adelaide: 80-91.
- Miramand, P, P Bustamante, D Bentley and N Kouéta. 2006. Variation of heavy metal concentrations (Ag, Cd, Co, Cu, Fe, Pb, V, and Zn) during the life cycle of the common cuttlefish *Sepia officinalis*. *Science of the Total Environment*, **361**,(1): 132-143.
- Nabhitabhata J. 2014. *Sepia pharaonis*. Inglesias, J, L Fuentes and R Villanueva (eds). *Cephalopod Culture*. Springer, New York. 97-112.
- National Measurement Institute. 2011. NMI Method Description Summary: NT2_46 Food Summary, Australia, National Measurement Institute: 1pp.
- Noell, CJ, CL Beckmann, and GE Hooper. 2014. Blue Crab (*Portunus armatus*) Fishery 2012/13. Fishery Assessment Report to PIRSA Fisheries and Aquaculture. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2007/000729-10. SARDI Research Report Series No. 757. 77pp.
- Olsen, AM. 1983. Fisheries Research Paper: Heavy Metal Concentrations of Fish, Other Aquatic Biota, River Murray and South Australian Aquatic Environments. Department of Fisheries South Australia: 1-117.

- Orlich, S. 2000. Kruskal-Wallis Multiple Comparisons with a MINITAB Macro Dunn's Test. PDF Document Minitab, Inc. <http://www.minitab.com/support/macros/default.aspx>.
- Payne, NL, BM Gillanders, and JM Semmens. 2011. Breeding durations as estimators of adult sex ratios and population size. *Oecologia*, **165**: 341-347.
- Pereira, P, J Raimundo, C Vale and E Kadar. 2009. Metal concentrations in digestive gland and mantle of *Sepia officinalis* from two coastal lagoons of Portugal. *Science of the Total Environment*, **407**,(3): 1080-1088.
- Pierce, GJ and A Guerra. 1994. Stock assessment methods used for cephalopod fisheries. *Fisheries Research* **21**: 255-286.
- Pierce GJ, L Allcock, I Bruno, P Bustamante, A Gonzales, A Guerra, P Jereb, E Lefkaditou, S Malham, A Moreno, J Pereira, U Piatkowski, M Rasero, P Sanchez, M Gegona Santos, M Santurtun, S Seixas, I Sobrino, and R Villanueva. 2010. Cephalopod biology and fisheries in Europe. *ICES Cooperative Research Report No. 303*, 175 pp.
- Prowse, TAA, BM Gillanders, BW Brook, AJ Fowler, KC Hall, MA Steer, C Mellin, N Clisby, JE Tanner, CD Dixon, T Ward, SC Donnellan and DA Fordham. (in press). Bayesian hierarchical models confirm broad-scale declines in giant cuttlefish (*Sepia apama*) density in northern Spencer Gulf, South Australia. *Marine and Freshwater Research*.
- Raimundo, J, P Pereira, C Vale and M Caetano. 2005. Fe, Zn, Cu and Cd concentrations in the digestive gland and muscle tissues of *Octopus vulgaris* and *Sepia officinalis* from two coastal areas in Portugal. *Ciencias Marinas*, **31**: 243-251.
- Rainbow, P. 2002. Trace metal concentrations in aquatic invertebrates: why and so what? *Environmental Pollution*, **120**: 497-507.
- Rainbow, PS. 1990. The Significance of Accumulated Heavy Metal Concentrations in Marine Organisms, PS Rainbow (Eds), Ch, *Heavy metal levels in marine invertebrates*. CRC Press Ltd., U.S.A., 120: 1-13.
- Rainbow, PS. 1993. The significance of trace metal concentrations in marine invertebrates. *Ecotoxicology of metals in invertebrates*, **23**.
- Rowling KP. 1995. Den ecology and behavioural interactions of the Australian giant cuttlefish *Sepia apama*. Honours Thesis, Flinders University of South Australia.
- Rjeibi, M, M Metian, T Hajji, T Guyot, BR Chaouacha-Chékir and P Bustamante. (in press) Interspecific and geographical variations of trace metal concentrations in cephalopods from Tunisian waters. *Environmental monitoring and assessment*, DOI 10.1007/s10661-014-3656-2.
- Saunders, RJ, 2009. The reproductive biology and recruitment dynamics of snapper, *Chrysophrys auratus*. PhD Thesis. School of Earth and Environmental Sciences, University of Adelaide, Australia. Pp 154.
- Steer, MA and K Hall. 2005. Estimated abundance and biomass of the unique spawning aggregation of the giant Australian cuttlefish (*Sepia apama*) in northern Spencer Gulf, South Australia. Report to the Coastal Protection Branch, Department of Environment and Heritage, South Australia. South Australian Research and Development Institute (Aquatic Sciences) Adelaide, RD 05/0012-1.
- Steer, MA, S Gaylard and M Loo. 2013. Monitoring the relative abundance and biomass of South Australia's Giant Cuttlefish breeding population. Final report for the Fisheries Research and Development Corporation. South Australian Research and Development Institute (Aquatic Sciences), Adelaide, SARDI Publication No. F2013/000074-1. SARDI Research Report Series No 684: 103pp.
- Steer, MA, AJ Fowler, TM Ward, GK Jones and S Mayfield. 2014. The history of commercial fisheries of Spencer Gulf. B Gillanders, S Madigan, S Murray-Jones, S Shepherd and D Wiltshire (Eds), Ch: 23, *Natural History of Spencer Gulf*, Royal Society of South Australia, Adelaide: 318-328.

- Svane, I, K Rodda, and P Thomas. 2007. Prawn fishery by-catch and discards: marine ecosystem analysis - population effects. FRDC Final Report Project 2003/023. SARDI Publication No. RD03/0132. SARDI Research Report Series No 199: 403pp.
- Sykes, AV, P Domingues and JP Andrade. 2014. *Sepia officinalis*. Inglesias, J, L Fuentes and R Villanueva (eds). *Cephalopod Culture*. Springer, New York.97-112.
- Tanaka, T, YHayashi and M Ishizawa. 1983. Subcellular distribution and binding of heavy metals in the untreated liver of the squid; comparison with data from the livers of cadmium and silver exposed rats. *Experientia*, **39**, 746–748.
- Viarengo, A and JA Nott. 1993. Mechanisms of heavy metal cation homeostasis in marine invertebrates. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*, **104**,(3): 355-372.
- Walker, DJ, R Clemente, A Roig, and MP Bernal. 2003. The effects of soil amendments on heavy metal bioavailability in two contaminated Mediterranean soils. *Environmental Pollution* **122**, 303–312.
- Ward, T, R Correll and R Anderson. 1986a. Distribution of cadmium, lead and zinc amongst the marine sediments, seagrasses and fauna, and the selection of sentinel accumulators, near a lead smelter in South Australia. *Marine and Freshwater Research*, **37**,(5): 567-585.
- Ward, T and P Young. 1981. Effects of Sediment Trace Metals and Particle Size on the Community Structure of Epibenthic Seagrass Fauna Near a Lead Smelter, South Australia. *Marine Ecology Progress Series*, **9**,(2): 137-146.
- Ward, TJ. 1984. Role of acute metal toxicity in structuring seagrass fauna near a lead smelter. *Marine Ecology Progress Series*, **17**: 117-124.
- Ward, TJ, RL Correll and RB Anderson. 1986. Distribution of Cadmium, Lead and Zinc Amongst the Marine Sediments, Seagrasses and Fauna, and the Selection of Sentinel Accumulators, Near a Lead Smelter in South Australia. *Australian Journal of Marine & Freshwater Research*, **37**,(567-585).
- Ward, TJ and PA Hutchings. 1996. Effects of trace metals on infaunal species composition in polluted intertidal and subtidal marine sediments near a lead smelter, Spencer Gulf, South Australia. *Marine Ecology Progress Series*, **135**: 123-135.
- Ward, TJ, LJ Warren, and DJ Swaine. 1982. Effects of heavy metals on aquatic life. Commonwealth Scientific and Industrial Research Organization, Commonwealth Scientific and Industrial Research Organization: 1-61.
- Ziedler, W and HK Norris. 1989. Squid, cuttlefish and octopuses (Class Cephalopoda). pp 789-822 In Shepherd SA and IM Thomas (eds.) *Marine Invertebrates of South Australia* Part II, Adelaide. South Australian Government Print Division.

9 Appendix

9.1 Staff Involved

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