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Giant Australian cuttlefish in South Australian waters

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Abbreviations

AMOVA	Analysis of molecular variance
ARC	Australian Research Council
BA	Breeding aggregation
BRT	Boosted regression trees
CAP	Canonical analysis of principal coordinates
DAPC	Discriminant analysis of principal components
FRDC	Fisheries Research and Development Corporation
GBS	Genotyping by sequencing
GSV	Gulf St Vincent
ICP-MS	Inductively coupled plasma-mass spectrometry
IUCN	International union for the conservation of nature
LSG	Lower Spencer Gulf
mAFs	Minor allele frequencies
MCMC	Markov Chain Monte Carlo
MDS	Multi dimensional scaling
MFA	Marine fishing area
PCA	Principal component analysis
PERMANOVA	Permutational multivariate analysis of variance
PVA	Population viability analysis
RAD	Restriction site associated DNA
SA	South Australia
SG	Spencer Gulf
SIMPROF	Similarity profile
SNP	Single nucleotide polymorphism
SZ	Sanctuary zone
TRF	Tactical research fund
USG	Upper Spencer Gulf
WOSBF	West of Santos boundary fence

Executive Summary

What the report is about

The iconic giant Australian cuttlefish, *Sepia apama*, is the largest cuttlefish species in the world, and forms an extraordinary breeding aggregation on a small stretch of rock reef in Upper Spencer Gulf (USG), South Australia. This research, coordinated by the University of Adelaide, was undertaken to address key knowledge gaps relating to movement and population structure, as well as factors contributing to population viability and commenced following dramatic declines in abundance at the breeding aggregation location. The research used a range of approaches including statolith and cuttlebone chemistry, next generation nucleotide sequencing, and population viability modelling. Results confirmed that within Spencer Gulf there are two highly resolved genetic clusters with significant levels of divergence and no evidence of recent hybridisation or introgression. The southernmost extent of the northern genetic cluster was -33.828° latitude and the northern most extent of the southern genetic cluster was -33.502° demonstrating some overlap. Our data suggest that these two clusters can be considered two separate species. A spatial age-structured population model was used for scenario testing of different potential threats to the USG cuttlefish population. This model indicated that by-catch due to prawn trawling and cuttlefish harvesting away from the breeding aggregations pose negligible risks to the population, but the population's viability could be compromised by increased mortality of embryos along the aggregation site prior to hatching and increased adult mortality if there is an increase in harvesting at the breeding grounds. Consequently, the continued closure of the aggregation site to cuttlefish harvesting is expected to improve the viability of the northern Spencer Gulf population.

Background

The magnitude of declines in abundance and biomass of the giant Australian cuttlefish along the breeding aggregation location from 2011 to 2013 raised concerns about the sustainability of giant Australian cuttlefish in Spencer Gulf and highlighted the paucity of information relating to the fine-scale population structure and dispersal of this species within USG. Unpublished research investigating the broader-scale population structure suggested that the USG population was genetically isolated from the rest of the State, and may constitute a separate species. This degree of isolation imparts a greater conservation focus on the species and highlights the importance of identifying the ecological/environmental factors that are driving the observed population decline and whether certain life history stages are more vulnerable than others. Given the iconic status of the cuttlefish spawning aggregation and its importance to the national and international community there was a fundamental need to address these clear knowledge gaps.

Objectives

The objectives of the project were to 1) Determine movement throughout the life history and finer scale population structure of the giant Australian cuttlefish in USG; 2) Resolve the systematic status of the USG giant Australian cuttlefish to determine the extent of its geographic boundaries; and 3) Develop an integrated model that assesses and evaluates the response of the USG population to environmental and anthropogenic factors and thereby assess population viability.

Methodology

Trace elements and stable isotopes were analysed in statoliths and cuttlebones of hatchlings to determine if significant differences in chemical signatures occurred among locations. Adult cuttlefish from throughout Spencer Gulf were then analysed to determine if their chemical signatures differed among locations for the core and edge regions and also whether chemical profiles across the statolith suggested different life histories.

A reduced representation genotyping method (genotyping by sequencing; GBS) was used to obtain single nucleotide polymorphism markers for cuttlefish collected in South Australian waters. Genetic clusters were then determined using a range of statistical approaches. The diet of the two genetic clusters of cuttlefish was also assessed using morphological and DNA-based approaches to determine if there was

any eco-phenotypic variation in their feeding ecology, prompted by differences in beak morphology between cuttlefish from Upper and Lower Spencer Gulf found previously.

A population model was developed and used to test different 'scenarios' that represent different combinations of threatening processes, and to evaluate their individual and combined effects on the USG cuttlefish population. Scenarios incorporated commercial harvesting, by-catch from prawn trawlers and desalination discharge.

Results/key findings

While there was spatial variation in chemical signatures among hatchlings collected from different locations along the breeding aggregation the ability to correctly classify samples to their collection location was poor. Given this, no attempt was made to retrospectively trace adults back to their natal region. Instead two approaches were used to investigate whether adult cuttlefish likely originated from a common source population and whether they occupied similar water masses throughout their life history. Results suggested that adults likely originated from the same source population as there was no significant difference in the chemical signature for the core region but a significant difference was found for the edge region of statoliths and cuttlebones. There was some evidence from chemical profiles across the statolith that multiple groups or contingents existed, suggesting that throughout their life history cuttlefish do not necessarily occupy the same water mass.

We observed two highly resolved genetic clusters in Spencer Gulf with significant levels of divergence and no evidence of recent hybridisation or introgression. The ranges of the two clusters overlap at least between -33.502° and -33.828° latitude. The northern population clearly shows a pattern of philopatry for both sexes, with males and females returning to breed at the location of their hatching at the mass breeding aggregation. We presently have no explanation for the mechanism for the philopatric behaviour, other than to note that the southern population does not appear to breed at the mass breeding aggregation. Whether the southern population shows philopatry or simply breeds anywhere within its putative range in lower Spencer Gulf or Gulf St Vincent (GSV) is yet to be established. Differences in the diet of the northern and southern populations of giant Australian cuttlefish in Spencer Gulf can be explained by differences in prey composition along the gulf. Despite observing no evidence to reject the hypothesis that both clusters are opportunistic feeders, a difference in prey availability may be a possible mechanistic explanation for differences in beak morphology between the populations. However, we note that our diet analysis was not able to distinguish variation in prey size, which may be a focus of selective feeding.

A spatial, age-structured population model was developed for the cuttlefish population in Upper Spencer Gulf. Hindcasting the model demonstrated that the population-size trend over the last 15 years could be broadly simulated as a result of harvest adjacent to the aggregation site and sea surface temperature variation affecting the population growth rate. Specifically, hindcast simulations could recreate a decline in population size since the year 2000, minimum population sizes that occurred over the period 2012 to 2013, and an increase in population size over 2014 and 2015. Consequently, there is little need to invoke other causative factors to explain historic variation in the abundance of cuttlefish on the Upper Spencer Gulf breeding grounds in winter. Scenario testing involving different threatening processes indicated that by-catch due to prawn trawling and cuttlefish harvesting away from the breeding aggregations (i.e. outside Marine Fishing Area 21) pose negligible risks to the population. In contrast, the population's viability could be compromised by increased mortality of embryos on the aggregation sites prior to hatching (e.g. due to hypersaline conditions resulting from desalination discharge) and increased adult mortality due to the reinstatement of commercial harvesting adjacent to the breeding grounds (i.e. inside Marine Fishing Area 21). The continued closure of the breeding aggregation area to cuttlefish harvesting is therefore expected to improve the viability of the Upper Spencer Gulf population.

Implications & Recommendations

Molecular observations strongly indicate that gene flow between the northern and southern clusters of giant Australian cuttlefish in Spencer Gulf has ceased, and thus these clusters can be considered two separate species under the biological species concept, which should be treated as separate management units. This implies that the breeding aggregation at Point Lowly is comprised exclusively of northern

cluster individuals and is the only breeding site known for this genetic cluster, highlighting its conservation significance.

The spatial age-structured population model and scenario testing of different threatening processes suggests that increased mortality of embryos or adults could compromise the viability of the population. It is therefore recommended that the closure of the breeding aggregation area to cuttlefish harvesting continues as this will improve the viability of the population.

Keywords

Giant Australian cuttlefish, *Sepia apama*, Point Lowly, Whyalla, Upper Spencer Gulf, South Australia

Introduction

Background and need

Giant Australian cuttlefish, *Sepia apama*, is the largest cuttlefish species in the world, and whilst it is distributed and breeds in waters around the southern coastline of Australia, it forms an extraordinarily large breeding aggregation on a small stretch of rocky reef in Upper Spencer Gulf (USG). Historically this aggregation has consisted of tens of thousands of individuals and is internationally recognised as an iconic natural phenomenon, consequently attracting considerable world-wide media and scientific attention.

The breeding aggregation typically forms in late April/early May, peaks during late May/early June and disperses by early September. At non-breeding times giant Australian cuttlefish (hereafter referred to as cuttlefish) are dispersed and generally solitary, although little is known of their movements when away from the breeding aggregation site. The main cuttlefish aggregation site is spatially limited to a subtidal reef (8 km of coastline, extending 70-130 m offshore, 2-8 m depth) near Point Lowly. Densities as high as 105 cuttlefish per 100 m^2 have been found on the breeding site (Hall and Hanlon 2002). Individuals at the aggregation site are all generally sexually mature. They aggregate here to breed once at the end of their life cycle.

Historically, the population was fished at relatively low levels primarily as bait for the snapper fishery. In the mid 1990s fishing of the breeding aggregation intensified, and there was some concern for the sustainability of the population. At this time, there were also resource allocation issues, including those between the eco-tourism and fishing sectors. Thus, in mid 1998 a renewable seasonal moratorium on taking cuttlefish from the Point Lowly region was imposed, which in 2004 became a year-round closure (Figure 1). In 2012, this closure was extended spatially to encompass the south-eastern side of Point Lowly (Figure 1). The core area of the breeding aggregation therefore has received some conservation protection. In 2013 a temporary closure on targeting and taking of cuttlefish in Upper Spencer Gulf (north of a line between Arno Bay and Wallaroo) was implemented and has been continued each year since. Reported catches in the marine scalefish fishery are considered negligible (<20 tons per year) (Fowler et al. 2014), but cuttlefish are also taken as by-catch in other fisheries.

Abundance and biomass surveys have been carried out in the breeding aggregation area since 1998, although cuttlefish have not been surveyed every year (exceptions: 2002-2004 inclusive, 2006). Abundance of cuttlefish was low in 1998 when significant fishing activity occurred on the breeding aggregation (Hall and Fowler 2003). The following year abundance was estimated at 182 642 (± 34 422) individuals, and remained similar through to 2001, after which time numbers generally decreased through to 2010 (2010 abundance: 106 027 (± 15 379) (Hall and Fowler 2003, Steer and Hall 2005, BHP Billiton 2009, 2011). Dramatic declines occurred in 2011 [60% decrease in abundance from 2010 estimates to 38 373 (± 11 887)], and again in 2012 (Steer et al. 2013) leading the State government to set up a whole of government working group, and the Conservation Council to convene a one day workshop to investigate possible causes. Declines continued in 2013, but 2014 and 2015 saw increases in abundance (Steer 2015, Steer et al. in press).

Biomass estimates have also declined through time (Hall and Fowler 2003, Steer and Hall 2005, BHP Billiton 2009, 2011, Steer et al. 2013). Following the 2011 accelerated decline FRDC funded a TRF project to develop a 'standard' methodology for on-going monitoring and assessment of the cuttlefish and the environment in which they aggregate to spawn, and to develop a preliminary understanding of causes of the observed decline. Habitat suitability modelling work was also commenced through the University of Adelaide and funded by the Australian Research Council (ARC DP1096427). The magnitude of this decline has raised concerns about the sustainability of cuttlefish and highlighted the paucity of information relating to the fine-scale population structure and dispersal of this species

within USG, as most studies have concentrated on the adult component of the population. The dispersal of hatchlings away from the spawning site and residence as sub-adults is currently unknown. Recent research investigating the broader-scale population structure suggested that the USG population was genetically isolated from the rest of the State, and may constitute a separate species (Gillanders and Donnellan, ARC LP0453443). This degree of isolation imparts a greater conservation focus on the species and highlights the importance of identifying the ecological/environmental factors that are driving the observed population decline and whether certain life history stages are more vulnerable than others. Given the iconic status of the cuttlefish spawning aggregation and its importance to the national and international community there is a fundamental need to address these clear knowledge gaps.

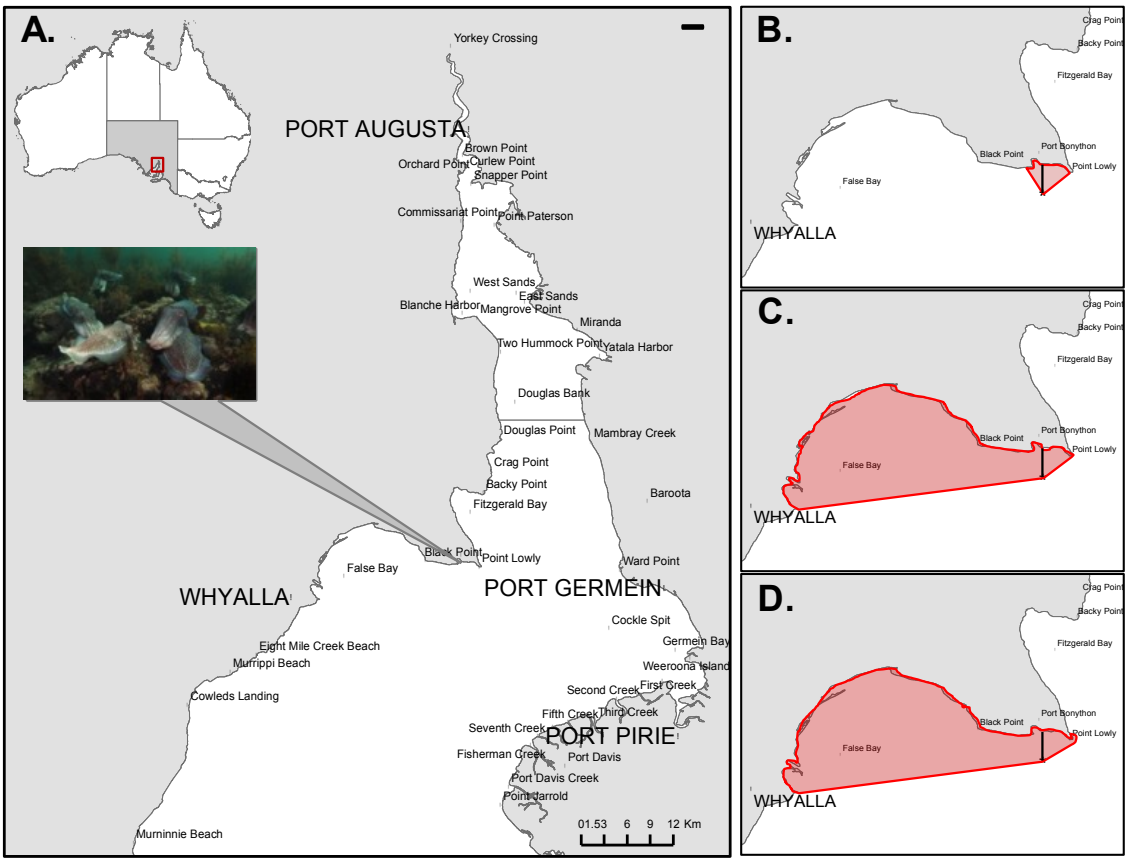


Figure 1. (A) Location of cuttlefish aggregation site in Upper Spencer Gulf, (B) area of the first fishing closure implemented at the beginning of the 1998 spawning season, (C) closure implemented mid-way through the 1998 spawning season following a review, and (D) extension of the closed area to encompass the eastern tip of Point Lowly implemented prior to the 2012 spawning season. Photo credit: Julian Finn, Museum Victoria. From: Steer et al. (2013).

Movement throughout life history and finer scale population structure

The otoliths (ear bones) of fish have been widely used to study movement and life history characteristics. The use of statoliths, analogous calcified structures in marine invertebrates, has also been investigated to understand stock discrimination, assign natal origins and reconstruct environmental histories (Ikeda et al. 1998, Yatsu et al. 1998, Ikeda et al. 2003, Arkhipkin et al. 2004,

Zumholz et al. 2007, Warner et al. 2009). Stable isotopes in cuttlebones have recently been investigated (Dance et al. 2014), but most research has focused on statoliths. These natural chemical tags recorded in calcified structures have some advantages over applied tags such as passive integrated transponder tags, acoustic tags and archival tags, in that the entire life history of the organism is obtained and all organisms are naturally marked (Elsdon et al. 2008).

To use trace elements and stable isotopes (hereafter chemical signatures) within calcified structures to trace movements of individuals among areas and connectivity among populations it is first necessary to check that there are differences in chemical signatures among groups (Elsdon et al. 2008). Thus, animals collected from different areas should have differences in chemical signatures. If differences occur then groups of animals with similar chemical signatures can be linked to determine where and when they moved. Other assumptions include that all possible groups contributing to the group mixture have been characterized and that the marker is stable through time (Elsdon et al. 2008).

Profile analysis of natural chemical signatures can show differences in movement of individuals within a population and identify groups with similar life history behaviours. If relationships between chemical signatures and environmental parameters are unknown then variability in patterns can be determined without the exact movements of the organism (Elsdon et al. 2008). Although such analyses may indicate that groups occupied different environments through their life, it does not necessarily identify the location of the environments.

The specific aims of this section were to: (1) Determine whether cuttlefish return to their natal site along the breeding aggregation, and (2) Determine movement throughout the life history of cuttlefish by analysing elemental chemistry along profiles of the statoliths.

Systematic status of the two cuttlefish populations

A critical issue for the management of cuttlefish in the Upper Spencer Gulf (USG) is whether individuals breeding at the mass breeding aggregation at Point Lowly are drawn from local waters or more broadly from South Australian or southern Australian waters. Molecular analysis with microsatellite makers suggests that animals breeding at Point Lowly are derived primarily from the Upper Spencer Gulf and that the population that breeds at Point Lowly (the northern population) is genetically isolated from populations that breed elsewhere in Spencer Gulf and South Australian waters – the southern population (Gillanders and Donnellan, ARC LP0453443).

Two other lines of evidence point to population structure in South Australian cuttlefish. Firstly, the mass breeding behaviour appears confined to one isolated part of the species range in USG (Rowling 1994, Hall and Hanlon 2002), which could simply reflect limited availability of rocky breeding habitat in that region or could be consistent with a separate population that has a unique breeding behaviour. Secondly, our analysis of morphological variation showed differences in the beak morphology of the two populations which may be related to feeding ecology and/or sexual selection (Gillanders and Donnellan, ARC LP0453443).

The distribution of the two populations overlaps approximately in the lower reaches of the USG where a permanent but spatially seasonally variable salinity gradient has its southern winter limit (O'Connell et al. 2015). It is possible that the gradient has promoted or contributes to the maintenance of population differentiation. Thus we will examine genetic evidence directly (genome wide scan for loci showing differentiation between populations) and eco-phenotypic variation in an important trait - feeding ecology (through diet/prey diversity analysis) – to assess the extent of evolutionary divergence between the two populations and consequent systematic implications.

Typically cephalopods are generalist predators and difficulties with identifying prey are compounded by their macerative feeding style, selective ingestion of prey body parts and rapid digestion rates. These features limit the identification of prey to those that contain diagnostic hard parts, while soft-bodied prey are poorly represented. DNA-based diet analysis methods are particularly useful for

investigating the diet of marine generalists because digested prey can be identified using short DNA sequences from diagnostic gene regions. Prey identification can be achieved irrespective of the degree of maceration and in the absence of diagnostic hard remains improving prey detection rates and taxonomic resolution in dietary studies. However the best outcomes are achieved by a combination of morphological and DNA-based approaches (Braley et al. 2010). Our aim is to quantitatively assess differences in prey diversity between the two SA cuttlefish populations from individuals that have been genotyped to establish their population membership.

Integrated model of population dynamics

Many cuttlefish species are exploited by humans but, of 195 species recorded on the IUCN Red List, 76 % are listed as 'Data Deficient' and only the giant Australian cuttlefish (*Sepia apama*) population from USG is classified as 'Near Threatened' (Jaremovic and Croft 1987, IUCN 2011). During the 1990s it was recognised that hundreds of thousands of cuttlefish assembled to breed during the austral winter in USG, South Australia, forming the densest aggregation known for any cuttlefish species (Steer et al. 2013) (Figure 1, 2).

Cuttlefish are semelparous and breed at either one or two years of age (Hall et al. 2007), primarily on inshore rocky reefs that provide the hard substratum upon which mature females deposit their eggs. Embryonic development in the wild takes approximately 4 months, with juvenile cuttlefish hatching in late winter and early spring (Hall and Fowler 2003, Dupavillon and Gillanders 2009). In Upper Spencer Gulf, the mechanisms by which juveniles disperse away from the aggregation site and then return as mature adults are poorly understood. With a short life cycle, cuttlefish population dynamics are probably strongly influenced by variation in recruitment success resulting from natural environmental stochasticity and anthropogenic stressors (Steer et al. 2013). Laboratory experiments demonstrate that temperature, salinity, and food availability affect egg and/or juvenile development in this species (Hall and Fowler 2003, Dupavillon and Gillanders 2009) (see also temperature and salinity experiments) and cephalopods more generally (Pimentel et al. 2012, Rosa et al. 2012). Human activities have subjected the species to increased mortality pressure resulting from targeted and untargeted commercial and recreational harvesting and incidental by-catch due to prawn trawling (Currie et al. 2009).

Ongoing concerns regarding the viability of the cuttlefish population in USG have necessitated a quantitative assessment of the impact of current human activities and future management strategies. Population viability analysis (PVA) is widely used to assess the extinction risk of vulnerable species, to rank the importance of current threatening processes and to evaluate different management strategies (Akcakaya and Sjogren-Gulve 2000). PVA models must account for the stochastic demographic and environmental processes that contribute to the vulnerability of species or populations (Shaffer 1981) and should be constructed as simply as possible while retaining the capacity to recreate observed patterns and evaluate the full range of threatening processes (Levin 1992, Morris et al. 2002).

This component details the development and application of an age-structured, spatial, stochastic population model for the cuttlefish population in USG. The model was age-structured because different life history stages of cuttlefish (e.g. sessile egg masses, mobile adults) are subject to different threatening processes. We also simulated spatially explicit dispersal and migration because some threats (e.g. by-catch mortality due to prawn trawling) only occur in certain regions. Having developed the model structure, the primary aims of this component were: (1) to 'hindcast' the model (i.e. run simulations representing the last 15-years) and determine whether the population trend can be recreated by known stressors (e.g. harvesting and temperature variation); and (2) to simulate the population trajectory over the next 10 years under different management scenarios. The latter simulations were used to assess the impact of different threatening processes, to rank those threats, and to consider how future changes to marine management might affect the viability of the USG cuttlefish population.

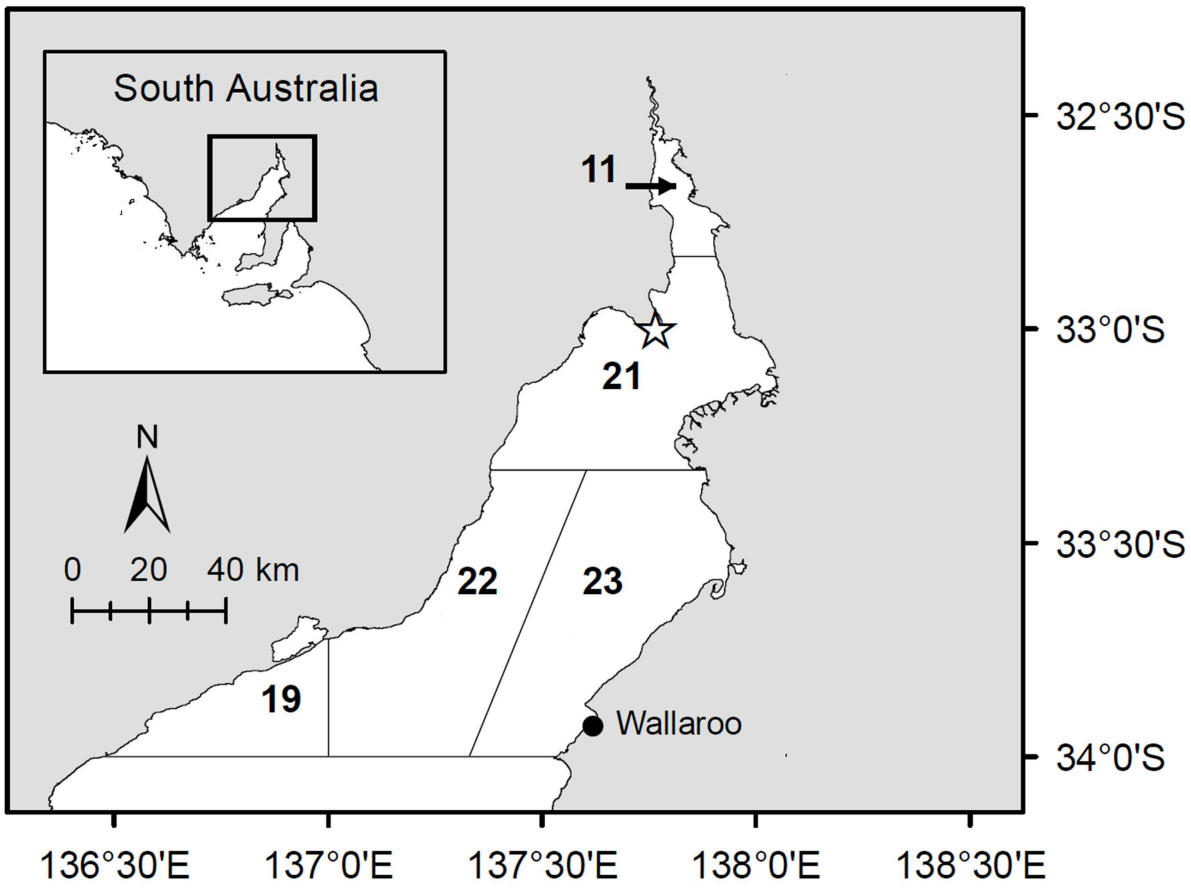


Figure 2. The Upper Spencer Gulf region covered by the spatially explicit PVA model. Shown are five Marine Fishing Areas (MFA; bold numbers) designated for the South Australian Marine Scalefish Fishery which includes cuttlefish harvesting. MFA 21 contains the cuttlefish aggregation sites including the primary breeding grounds near Point Lowly which is marked with a star.

Objectives

Objectives of the project –

1) Determine the movement throughout the life history and finer scale population structure of the giant Australian cuttlefish in Upper Spencer Gulf;

2) Resolve the systematic status of the USG giant Australian cuttlefish to determine the extent of its geographic boundaries;

3) Develop an integrated model that assesses and evaluates the response of the USG population to environmental and anthropogenic factors and thereby assess population viability.

Methods

Movement throughout life history and finer scale population structure

Sample collection

To determine life history movements, cuttlefish were collected during 2012 and 2013 as part of the prawn trawl by-catch. To determine if cuttlefish were returning to their natal site, trace element and isotope analysis was performed on embryo cuttlefish collected from the breeding aggregation, Point Lowly, South Australia, in 2013, and from adults collected from both the breeding aggregation and Spencer Gulf in 2014. Adult cuttlefish were either collected as part of by-catch from prawn trawlers operating within Spencer Gulf, or collected from the breeding aggregation reef at Point Lowly. The statoliths and cuttlebone were removed from each individual, rinsed and allowed to dry.

Trace element analysis

One statolith from each individual was prepared for trace element analysis using a laser ablation – inductively coupled plasma mass spectrometer (ICP-MS). For embryos, statoliths were analysed whole, with a spot laser beam aimed at the lateral dome of the statolith. For adults, statoliths were thinly sectioned and mounted onto glass slides. Profiles were run from the core to the outer edge. Profiles were then quantified, and for 2014 adults a section representing the core and outer edge of the statolith was used for statistical analysis.

Cuttlebones were dissected and dried; embryo cuttlebones were completely homogenised, whereas for adults, a small section from the base (representing conditions at hatching) and outer edge (representing conditions at collection) of the cuttlebone was removed and each individually homogenised. A small amount of each homogenised sample, approximately 1 mg, was weighed, dissolved in 0.153 ml nitric acid for 2 hrs, before 0.847 mL of milli-Q was added and the sample was left for 24 hrs. Finally an additional 4 mL of milli-Q water was added, making the final solution 2% nitric acid. These samples were then run through a solution based ICP-MS.

Elements analysed on both laser ablation and solution ICP-MS included ^{23}Na, ^{24}Mg, ^{43}Ca, ^{44}Ca, ^{88}Sr, ^{115}In, and ^{138}Ba. Indium was used as an internal standard for solution ICPMS and for laser ICPMS to identify when non-statolith material (e.g. resin) was being ablated. Raw count data were processed using the GLITTER software program, and elemental data were expressed as ratios to ^{43}Ca (in mmol.mol^{-1}) to account for fluctuations in ablation yield.

Stable isotope analysis

The second statolith from adult cuttlefish was analysed whole, while a small subset of each of the cuttlebone homogenised samples collected for trace element analysis were used for stable isotope analysis. All samples were measured for δ^{18}O and δ^{13}C. Embryo statoliths were too small for isotope analysis. Because adult statoliths were analysed whole for stable isotopes, isotope values represent an age-integrated signature. The resulting values were used in statistical analyses for comparisons of both the adult core and edge elemental signatures.

Statistical analysis

Data were log(x+1) transformed and converted to Euclidean distance matrices. PERMANOVA was used to determine if differences occurred in the chemical signatures of embryos collected from different sites along the aggregation reef, and to determine if there were differences in the adults

collected from different locations within Spencer Gulf. When significant differences were detected, pairwise tests were performed to determine which sites differed. Canonical analysis of principal coordinates (CAP) was also performed using a leave one out allocation, to determine the ability of the chemical signatures to classify hatchlings back to their collection site. PRIMER v 6.0 with PERMANOVA (www.primer-e.com) was used for these statistical analyses.

Individual element profiles across the statolith were normalized using a spline fit to the data to account for age-related patterns of elemental uptake, using the *dplR* package (Bunn 2010) from the R programming environment (R Development Core Team 2015). Normalised profiles were then decomposed into a set of 13 extracted features, which describe the statistical form of the elemental time series (see Wang et al. 2006). We analysed these 13 extracted features for each of the four elements (52 features per individual) in a hierarchical cluster analysis, with data fitted to an Euclidean distance resemblance matrix. This generated a dendrogram to assess the similarities among individuals based on a similarity profile (SIMPROF) using the *sigclust* package (Huang et al. 2012). SIMPROF used 500 permutations and $p < 0.001$ to assign individual cuttlefish to discrete clusters.

Systematic status of the two cuttlefish populations

Sample collection

A total of 120 specimens of cuttlefish, comprising 73 males and 47 females, were collected between February 2013 and July 2014 from Spencer Gulf (SG) (Figure 3). Of these specimens, 41 were genotyped, 40 were used for dietary analysis and 39 samples were used for both analyses (Appendix A, Table S1). For genotypic analysis we supplemented this collection with 45 samples collected between 1998 and 2006 from SG and Gulf St Vincent (GSV) to give a wider perspective on temporal and geographic variation in allele frequencies (Appendix A, Table S1). Specimens were collected as whole animals either by commercial fishing vessels or by hand; in all cases specimens were frozen (-20°C) on site before storing at -20°C for processing at a later date. Sampling of cuttlefish consisted of (1) taking body length and mass measurements of whole cuttlefish, (2) dissecting cuttlefish to remove the upper and lower beaks and stomachs, and to inspect the gonads to identify sex, and (3) removing a small piece of mantle tissue for DNA analysis. Upper and lower beaks and stomachs were stored at -20°C for later processing, while mantle tissue was stored in 95% ethanol at 4°C.

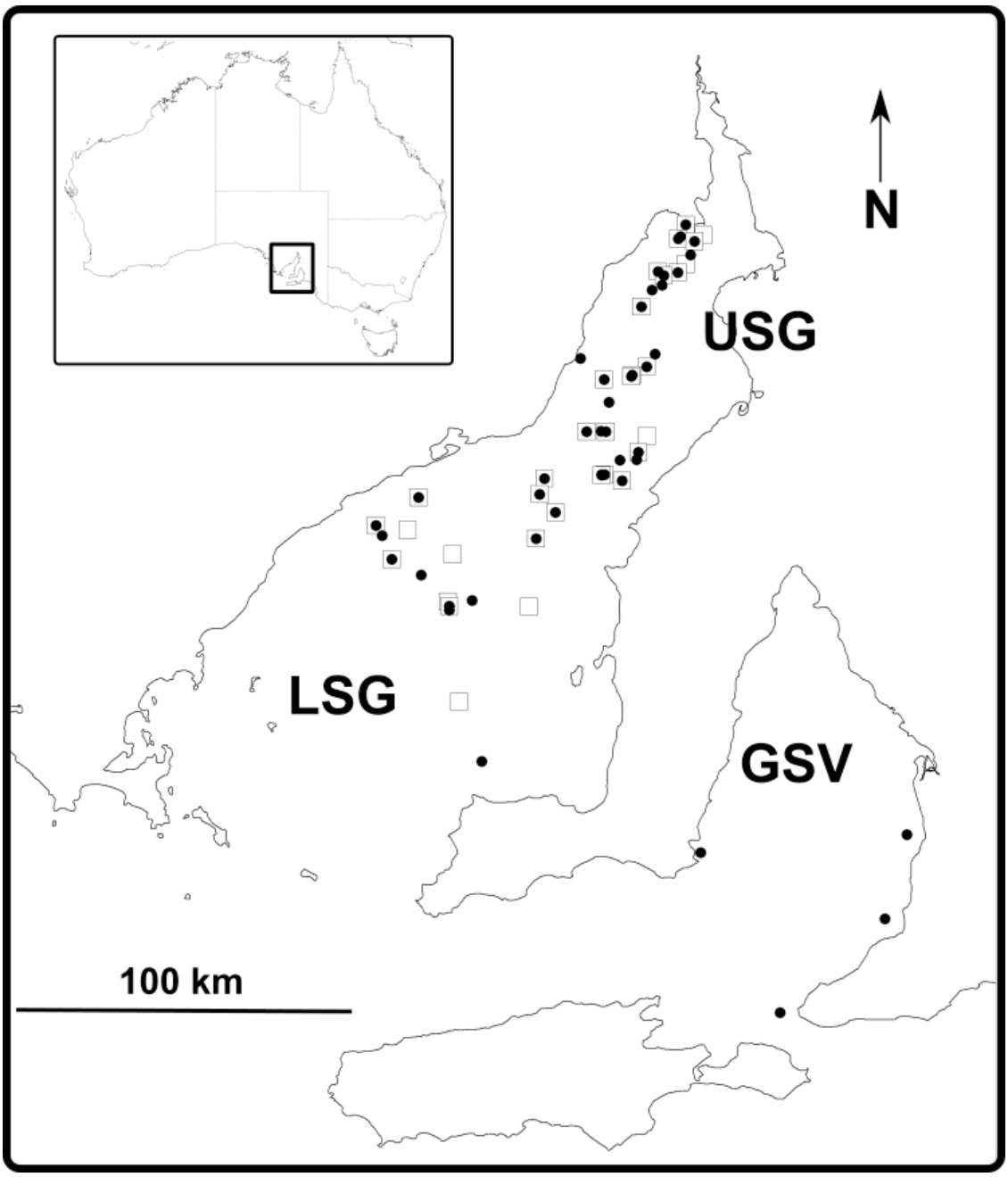


Figure 3. Map of study area and sampling locations. Filled circles indicate locations of samples used for RAD-Seq analysis and open squares indicate locations of samples used for dietary analysis.

Population genomics

DNA extraction, library preparation, & sequencing

We used a reduced representation genotyping method (genotyping by sequencing; GBS) to obtain single nucleotide polymorphism (SNP) markers as described by Elshire et al. (2011), but modified for two restriction enzymes *Pst*I and *Msp*I (Poland et al. 2012). DNA was extracted from 125 samples (Appendix A, Table S1) and normalised to a concentration of 20 ng/μL. We digested each extract individually and ligated adapters, one adaptor contained a unique sequence tag (index) to identify each sample. We amplified fragments ligated with both a *Pst*I and *Msp*I adapter by PCR using specific

Illumina indexed primers. Amplification products from indexed libraries were combined before single-end sequencing on either an Illumina Hi-Seq or Next-Seq DNA sequencer.

We sequenced replicates from independently prepared libraries for 2.5% of samples. Illumina reads were demultiplexed, clustered, and filtered using the UNEAK pipeline in TASSEL v3.0 (Glaubitz et al. 2014). We used a custom Python script to filter calls based on read depth and minor allele frequency, removing SNPs with a read count below 10 or where the minor allele read depth was below 0.25. We recovered 1107 polymorphic, neutral, unlinked SNP loci, and a further 97 loci potentially under selection (of which 88 were under diversifying selection). Our data contained no more than 10% missing data for each locus or individual.

We calculated the genotyping error rate based on mismatches between replicates for loci recovered in both replicates. Minor allele frequency was calculated for each locus to characterise the SNP data and to identify bias introduced by filtering.

Population structure analysis

To identify and characterise genetic clusters we used discriminant analysis of principal components - DAPC (Jombart et al. 2010) implemented in the R package *adegenet* v1.4-2 (Jombart 2008). DAPC yields linear combinations of the original variables (alleles) which maximize differences between groups while minimizing variation within groups. In our DAPC we retained 43 principal components (explaining 50% of total variance), two clusters, and a single discriminant function.

As specimens were sampled across multiple years we examined the contribution of temporal variation to overall genetic variation using a hierarchical analysis of molecular variance (AMOVA) in the program Arlequin v3.11 (Excoffier and Lischer 2010). The variance components were partitioned to reflect (a) variance among the genetic groups identified in the DAPC analysis, (b) variance among years within each genetic group, and (c) variance among individuals within the same year and genetic group. All tests used 10 000 permutations.

We carried out Bayesian clustering analysis implemented in STRUCTURE v2.3.4 (Pritchard et al. 2000, Falush et al. 2007, Hubisz et al. 2009) on all of the samples and in a second analysis we removed GSV samples from the data for Bayesian clustering analysis so that Hardy-Weinberg dynamics of the southern cluster would not be skewed by variation possibly owing to isolation by distance. To infer the most likely number of clusters (K) in each analysis we ran 10 MCMC replicates with a burn-in of 250 000 iterations followed by 250 000 iterations for K=1-4, using the standard admixture model assuming correlated allele frequencies. Posterior probability estimates were averaged over the 10 MCMC replicates. To estimate individual membership to the inferred clusters we ran 10 MCMC replicates with burn-in of 250 000 iterations followed by 250 000 iterations for the most likely value of K (K=2) using the LOCPRIOR admixture model. The LOCPRIOR model is suited to data with weak population structure and utilises prior population information (collection location or genetic group determined from DAPC to reduce the number of combinations of individuals that have to be compared to find clusters that are compliant with Hardy-Weinberg Equilibrium (Hubisz et al. 2009). Estimated memberships to each cluster were averaged over the 10 MCMC replicates.

To further characterise the genetic clusters and divergence we calculated differences in allele frequencies and the fixation index (pairwise F_{ST}) between clusters, and private and fixed allele counts, and heterozygosity within clusters. We calculated the fixation indices, with and without loci under selection, to investigate the role of selection in divergence.

Dietary Analysis

Visual identification of prey

The contents of whole stomachs (see sample collection) were dissected to separate hard remains (e.g. arthropod exoskeletons, cephalopod beaks) for visual identification. The remaining stomach contents were stored in 100% ethanol at 4°C for DNA extraction. Visual identification of hard remains was made where possible before microscopic examination. No otoliths or statoliths were observed in the stomach contents, despite observing other fish and cephalopod remains. All visual identifications were made to the phylum-level. In each case we estimated the minimum number of prey individuals based on counts of hard remains; for cephalopods, upper and lower beaks were counted, and for crustaceans and fish, eyes and other appendages were counted. Where material was present that was not quantifiable but still identifiable, the minimum number of individuals was recorded as one.

Assessment of potential cuttlefish prey and assembly of reference nucleotide sequences

We used extensive prawn trawl data from Spencer Gulf (Currie et al. 2009) to determine potential prey for cuttlefish in Spencer Gulf. With little prior knowledge of cuttlefish diet we assumed conservatively that prey would be restricted to epipelagic or epibenthic species that are present in the gulf and whose adult stages grow no larger than the cuttlefish; this included fish, crustaceans, molluscs, and other invertebrates. We found no hard remains of non-cephalopod molluscs in cuttlefish stomachs so we removed these from our list of candidate prey taxa. We excluded other groups (e.g. Annelida, Nematoda and Platyhelminthes) as these were relatively minor prey items based on stomach content inspection (with the caveat that these groups would have low detectability due to their soft-bodied nature).

For the two molecular markers we employed, we assembled a reference nucleotide sequence database, comprising, in most cases, at least two (where possible) representatives from each genus of potential prey (Appendix, Tables S2 and S3). Where possible, sequences were derived from GenBank; otherwise we sourced tissue samples from SARDI or the Australian Biological Tissue Collection, extracted DNA using either the Gentra Puregen method (see manufacturer's directions) or QIAGEN DNeasy 96 method (see manufacturer's directions), PCR amplified and directly Sanger sequenced the target genes. Primers used in PCR and sequencing the *16S rRNA* gene were 16Sar (CGCCTGTTTATCAAAAACAT) and 16Sbr (CCGGTCTGAACTCAGATCACGT) and for the *18S rRNA* gene were Frag3F (GTTCGATTCCGGAGAGGGA) and 9R (GATCCTTCCGCAGGTTCACCTAC). Polymerase chain reactions were carried out in 25 μL volumes and contained 1× polymerase buffer, 2 mM $MgCl_2$, 0.8 mM each dNTP, 0.2 μM each primer, 0.02 U/L Taq Gold polymerase and approx. 25-50 ng template DNA. The thermal profile used was 94ºC for 11 min, 34 cycles of 94ºC for 45 s, 55ºC for 45s, and 72ºC for 1 min, followed by final extension at 72ºC for 10 min. Sanger sequencing was carried out by AGRF using their PD sequencing service.

DNA extraction & library preparation

To extract DNA from the macerated soft tissue sample preserved in ethanol, we homogenised the suspension by repeated inversion and used a pipette, with the tip cut to allow larger tissue particles through, to transfer the soup to a 1.5 mL flip-cap tube. We condensed the tissue fraction by low speed centrifugation and disposed of the supernatant. This process was repeated until the target amount of tissue was reached; approximately 10-15mg of tissue (1-5 mL of soup) was used in each extraction. We used a DNeasy Mini extraction (QIAGEN). We quantified each DNA sample using Quant-IT™ picogreen®.

Molecular identification of prey

PCR primers were designed with the primer design software (OligoCalc, Kibbe 2007) to amplify short stretches of the *16S rRNA* (mitochondrial) and *18S rRNA* (nuclear) genes based on alignments of our

reference sequences (Appendix, Table S4 and S5). Primer annealing sites were carefully selected to ensure amplification success across the broadest range of taxa (i.e. were placed in highly conserved rRNA stem regions). The amplicon sizes were intended to be short in order to detect degraded prey DNA within predator stomach contents. Before analysing stomach samples, all primers were tested and PCR conditions were optimized using various known templates, to maximize the specificity and reliability of PCR amplification.

We used a multi-marker barcoding approach, incorporating markers targeting the *16S rRNA* and *18S rRNA* genes. The method incorporates dual-barcode paired-end sequencing using custom primers on the Illumina Mi-seq platform, described in detail in Myers et al. (in prep). PCR primers were synthesised with adapters according to the dual-index sequencing strategy for Illumina Miseq developed by Kozich et al (2013).

For each marker, we quantified PCR products using Quant-IT™ picogreen®. We pooled PCR products at equal ratios (34 ng each sample) and cleaned up using Agencourt® Ampure®, resuspending at a final volume of 100 µL, so each sample was at a final concentration of approximately 0.34 ng/µL. The completed library was quantified using Tapestation (Agilent Technolgies) and diluted to 2 nmol/L and sequenced with the MiSeq® Reagent Kit v2 (300 cycle).

We used the Mothur pipeline (Schloss et al. 2009) to concatenate, demultiplex, filter, and cluster paired end reads. In our pipeline we allowed for a 1% (16S rDNA) and 1.4% (18S rDNA) sequencing error rate by clustering reads with 2 or less differences. When assigning taxonomic identification to clusters based on our reference sequences, a 90% (16S rDNA) and 80% (18S rDNA) consensus confidence threshold was used – this gave us enough power to consistently assign family-level taxonomic classifications with high confidence. We removed sequences assigned to cuttlefis*h*, and that had identity to sequences that were present in the negative controls. As a final filter to remove erroneous/contaminant reads, we removed sequences that occurred less than 5 times.

Analysis of prey data

We combined molecular data for both the *16S rRNA* and *18S rRNA* genes together with the visual data. If a phylum identified by visual inspection was missing in the molecular dataset, we added it to the data set as an unknown member of that phylum. We calculated proportion of diet at the phylum level, including standard error. For each phylum we examined proportion of diet at the family level. We constructed a presence-absence matrix with which we performed a hierarchical cluster analysis using Ward's cluster method and Squared Euclidian distance measure to produce a dissimilarity matrix in IBM® SPSS® Statistics package version 22. We used the dissimilarity matrix to perform a multidimensional scaling (MDS) analysis in IBM® SPSS® Statistics package v 22.

Temperature and salinity experiments

Egg collection

Two sets of experiments were undertaken on cuttlefish eggs collected from False Bay, Whyalla, South Australia in June/July 2013 and then in May 2014. Eggs collected in May 2014 were used in a temperature spike experiment, and eggs collected in 2013 were used for the temperature × salinity experiment. Eggs were transported to South Australian Research and Development Institute (SARDI) Aquatic Sciences Centre, West Beach or The University of Adelaide in an aerated tank, and transferred into larger holding tanks upon arrival. Eggs were sewn to Styrofoam squares (10 eggs per square) using fishing line to suspend them off the bottom of the tanks, and to mimic natural orientation in the wild. Eggs from individual collection times were randomly assigned to experimental tanks and treatments when more than one collection was required.

Temperature spike experiment

Eggs were held in 40 L tanks with aeration. Weekly water changes were performed to maintain water quality. Temperature and pH was recorded daily, with salinity recorded weekly. Eggs were held at 18°C and experimental conditions consisted of increasing the temperature by 1°C per day until the desired temperature spike was obtained. Temperature spikes were maintained for five days, before temperatures were lowered back to 18°C, decreasing 1°C per day. Temperature spikes were at 2°C intervals, 18°C (control, no change in temperature), 20°C, 22°C, 24°C and 26°C. Once all tanks had been returned to 18°C, temperature was keep constant for 10 days. After this time, eggs were dissected to compare the developmental stage of embryos.

Temperature × Salinity experiment

Experiments consisted of three temperature treatments (16°C, 20°C and 24°C) with three salinity levels (38, 40 and 44 ppt) crossed in an orthogonal design. Three 5 L replicate tanks recirculated water from a common sump (75 L) for each treatment combination, which was aerated and circulated to a heater chillier unit (Teco Seachill) to maintain the desired temperature. Salinity was adjusted using sea salt (Red Sea). Eggs were originally placed into experimental tanks at 15°C, with the correct treatment salinity. Temperature was raised 1°C per day until they reached the desired temperature treatment level. Temperature, salinity, dissolved oxygen and pH were monitored daily, with ammonia monitored weekly. Salinity was maintained via water changes of the common sump. At the beginning of austral spring (September), temperature was increased half a degree each week until an overall 2°C increase was achieved, to coincide with warming of coastal waters during this time of year, and to stimulate hatching.

Besides the above experiments, data were also collated from experiments undertaken by Dupavillon and Gillanders (2009, salinity experiments) and Hall and Fowler (Hall and Fowler 2003, temperature experiments).

Adult collection and acclimation

Adults were collected from False Bay, Whyalla, South Australia and transported to SARDI in aerated tanks. Upon arrival, adults were transferred into individual flow-through conical tanks. Temperature was increased in three of the treatments (16°C, 18°C and 20°C) at a rate of 1°C per day, and kept at field conditions (14°C) for the remaining treatment. All cuttlefish were acclimated at their respective temperature for a minimum of 3 weeks prior to undertaking swimming respirometry.

Swimming respirometry

To investigate the effect of temperature on metabolic rate, cuttlefish were starved for 36 h and then placed in a 90 L Loligo swim chamber respirometer where oxygen levels were recorded. Cuttlefish were initially left to acclimate in the chamber under minimal flow and their resting metabolic rate recorded. Flow was then increased at a number of steps and oxygen consumption recorded. Oxygen levels in the chamber were always maintained at >80% oxygen saturation via a flushing pump which replenished water in the respirometer.

Integrated model of population dynamics

Model overview

The model simulates cuttlefish as they are deposited as eggs at the aggregation sites near Point Lowly, hatch, disperse throughout Upper Spencer Gulf (accounting for harvest and by-catch mortality), aggregate to breed, and die. Using a monthly time-step, the cuttlefish population is simulated spatially

across an arrangement of circa 3 km grid cells representing Upper Spencer Gulf. Over a 10-year time frame, the model can produce a number of outputs such as the mean population size, the mean final population size, the mean population growth rate, the probability of a population decline, and the expected minimum abundance. The model can be used to test different 'scenarios' that represent different combinations of threatening processes, and to evaluate their individual and combined effects on the USG cuttlefish population. The model was coded in the R computing environment (v. 3.1.1, R Development Core Team 2015) and specific details regarding the model parameterisation are provided below and in Table 1.

Table 1. Details of the ensemble model parameterisation used for scenario testing, and of the sensitivity analysis implemented using Latin hypercube sampling. Where no range is given, the parameter remained fixed for all simulations.

Parameter	*Scenario testing*	*Sensitivity Analysis*
Demography		
Background monthly survival rate for embryos (*sE*)	*U*(0.95,1)	*U*(0.95,1)
Background monthly survival rate for subadults and adults (*sA*)	*U*(0.95,1)	*U*(0.95,1)
Probability of maturing at age 1 year (*pMat1*)	*U*(0.5,1)	*U*(0.5,1)
Mean fertility of mature females (*m*; eggs clutch^{-1})	*U*(200,400)	*U*(200,400)
Sex ratio at birth (*sr*; proportion males)	0.5	0.5
Carrying capacity (*K*)	300 000	*U*(100 000,400 000)
Maximum annual population growth rate (λ_{max})	5	*U*(4.25,10)[d]
Minimum annual population growth rate (λ_{min})	0.2	*U*(0.1,0.36)[e]
Theta-logistic parameter (*theta*)[a]	1	*U*(0.5,1.5)
Time to egg hatching (*time2hatch*; months)	4	4
Minimum harvestable age (*ageHarv*; months)	6	6
Dispersal and Migration		
Dispersal exponent (*dispExp*)[b]	5×10^{-5}	*U*(1×10^{-5}, 1×10^{-4})
Migration exponent (*migrExp*)[c]	2	*U*(1,3)
Environmental Stochasticity		
Standard deviation on juvenile survival rate (σ)	0.01	0.01
Anthropogenic threats		
Aggregation harvesting (*aggHarv*, # per annum)	50 000	*U*(0,100 000)
Commercial harvesting elsewhere		
- implemented? (*catchInd*; 0=no, 1=yes)	Scenario testing	0/1
- catch (*catch*, kg per grid cell)	Historical data	Historical data
- conversion (*catchCon*, # per kg)	2.5	2.5
Prawn trawling		
- implemented? (*bycatchInd*; 0=no, 1=yes)	Scenario testing	0/1
- bycatch (*bycatch*; # per grid cell)	Historical data	Historical data
Salinity plumes (aggregation site only)		
- frequency (*salFreq*)	0.2	*U*(0,1)
- effect (multiplier) on embryonic survival (*salEff*)	0.7	*U*(0.5,1)

[a]The parameter *theta* controls the shape of the density-dependent relationship (when theta=1, there is a linear relationship between the population size and the exponential rate of increase).

[b]The parameter *dispExp* controls the speed of dispersal away from the aggregation sites, with larger numbers indicating shorter-range dispersal. The probability of dispersing from one cell to another in one month was assumed to decline exponentially as a function of the distance according to the equation $e^{(-dispExp)x}$, where x is the distance (in metres) between the cell centroids.

[c]The parameter *migrExp* controls the speed with which animals migrate back to the aggregation sites to breed, with larger numbers indicating faster migration.

[d]The lower limit on λ_{max} was set equal to the highest annual population growth rate observed from the empirical aggregation-site abundance data.[e]The upper limit on λ_{min} was set equal to the lowest annual population growth rate observed from the empirical aggregation-site abundance data.

Demography

The model proceeded through a series of demographic steps in the following order:

Initiation. Simulations were seeded with a cohort of mature individuals ready to breed at one of three aggregation sites: Port Bonython, Backy Point, or Whyalla (Figure 4). Mature individuals were split between the three sites based on long-term averages determined from monitoring data (Port Bonython, 95.2 %; Backy Point, 4.2 %, Whyalla, 0.6 %) (Steer 2015). A cohort of 1-year old individuals that were not yet mature was also seeded throughout the Upper Spencer Gulf. The relative proportions of these two cohorts were determined by calculating the stable age distribution of the population from the vital (survival and fertility) rates used for that simulation (see below).

Reproduction. All reproduction (egg deposition) was assumed to take place on June 1 and all reproducing animals died immediately after breeding.

Embryonic stage. Embryos were assumed to develop on the aggregation sites over a period of 4 months, with all hatching occurring on October 1. Embryos were subjected to a background monthly survival rate that was fixed for each simulation.

Juvenile stage. The juvenile stage was assumed to last for 2 months, from hatching on October 1 until December 1. The model assumed that inter-annual variation in recruitment was driven by variation in the monthly survival rate for this stage (see the section ‘Model parameterisation’).

Sub-adult/adult stage. After 6 months, individuals entered the sub-adult/adult stage for which a common monthly survival rate was assumed. Surviving cuttlefish then matured and reproduced at either 1 or 2 years of age.

Demographic stochasticity (i.e. variation in the realised monthly vital rates despite the simulated monthly rates being fixed or variable constants each year) was included by modelling all vital rates as the realisation of binomial sampling distributions and all egg clutch sizes as the realisation of poisson distributions.

Figure 4. Simulated breeding grounds (coloured cells) in Upper Spencer Gulf. Cell shading illustrates the different aggregations sites: white – Whyalla; yellow – Port Bonython; green – Backy Point.

Dispersal and migration

After hatching, cuttlefish juveniles and sub-adults/adults were dispersed stochastically throughout Upper Spencer Gulf, assuming dispersal probabilities between pairs of cells declined exponentially with distance (Table 1, Figure 5). After reaching reproductive maturity, individuals were migrated back to the aggregation sites assuming migration probabilities were inversely proportional to the distance of cells from the aggregation sites.

Threats

Simulations could include any or all of the following threatening processes:

Commercial harvesting on/adjacent to aggregation sites. For hindcasting, annual cuttlefish harvest data for Marine Fishing Area (MFA) 21 were used and assumed to represent catch from the Point Lowly aggregation site just prior to breeding. This assumption is reasonable as when harvesting of cuttlefish occurred the majority were from the breeding aggregation. Simulation of future management strategies could implement any fixed or variable harvest occurring on any of the three simulated breeding grounds. Only sub-adults/adults were vulnerable to harvesting.

Commercial harvesting elsewhere (i.e., outside MFA 21). Hindcasts used the actual cuttlefish harvest for the years 2000 to 2015 for MFAs 11, 19, 22 and 23 (Figure 2). Simulation studies used the mean annual cuttlefish harvest in each MFA for each simulated year. The commercial harvest data includes catch of another cuttlefish species (*Sepia novaehollandiae*), so the models are conservative in that they should not underestimate the impact of commercial fishing outside MFA 21. Only sub-adults/adults were vulnerable to harvesting.

By-catch due to prawn trawling. A recent by-catch survey provided estimates of the number of cuttlefish caught incidentally during trawling, split by month and prawn fishing block (Steer 2015). We calculated the average by-catch of the species for each month and block and used these data for simulation studies. Only sub-adults/adults were vulnerable to trawling. We assumed all by-catch of cuttlefish represented catch from the USG population, which is likely an overestimate since most prawn trawling occurs off Wallaroo at the southernmost range margin of the modelled population (see previous chapter). The simulations are therefore conservative in that they should not underestimate the impact of by-catch from trawling.

Desalination discharge at Point Lowly. If plans for the construction of a desalination plant at Point Lowly were to proceed, the primary aggregation site could be exposed to hypersaline brine discharge.

Although the potential for brine reaching the aggregation site is unlikely (BHP Billiton 2009, but see Kampf et al. 2009), in the laboratory a relatively small increase in seawater salinity to 45 ppt was sufficient to reduce embryonic survival rate of cuttlefish by 30 % (Dupavillon and Gillanders 2009). We therefore modelled desalination discharge as a 30% reduction in embryonic survival that occurred with an arbitrary frequency of once every two years.

Ensemble model development and hindcasting

To determine whether the integrated population model could capture the observed pattern of changing cuttlefish abundance on the aggregation site, we first used the empirical time-series of aggregation-site abundance estimates to derive a relationship between juvenile survival rates and sea surface temperature averaged over the winter and spring, conditional on historical harvesting data and assumptions regarding certain demographic rates. Specifically, we used hierarchical Bayesian MCMC sampling within *JAGS* software (Plummer 2010) to estimate this relationship assuming plausible, fixed values for the embryonic and subadult/adult survival rates and the fertility rate. We then modified these assumptions and refit the relationship, and conducted this process 200 times, resulting in an ensemble of 200 plausible models. We then repeated this entire procedure but this time constrained the relationship between juvenile survival and sea surface temperature such that the cuttlefish population was demographically stable at the mean observed sea surface temperature over winter and spring (17.1 °C). We verified these two model ensembles by using the different parameterisations as inputs to the simulation model which we then hindcast over the period 2000 to 2015, comparing simulated output to the historical time-series. Since these hindcasts captured the general pattern of the empirical data but not the full range of abundance estimates, we also increased the degree of environmental stochasticity, implemented as random annual variation in the juvenile survival rate, until the hindcast simulations could capture the observed variability.

Scenario testing

Although the hindcast models that used fitted parameters could capture the general pattern of changing abundance on the aggregation site, they did not include compensatory density-dependent mechanisms and were therefore unsuitable for considering the effect of deterministic threats such as harvesting. For scenario testing, therefore, we modified the model ensemble by assuming that juvenile cuttlefish survival varied according to a theta-logistic density-dependent relationship:

$$s_{juv} = s_{min} + (s_{max} - s_{min})(1-(N/K)^{\Theta}) + N(0, \sigma)$$

where s_{min} and s_{max} are the juvenile survival rates required to produce the specified minimum and maximum population growth rate, N is the current population size, K is the carrying capacity, Θ controls the shape of the density dependence, and $N(0, \sigma)$ is a Gaussian error term representing unexplained environmental variation (including variation due to temperature) in annual juvenile survival rates.

Using the ensemble of 200 modified simulation models, we assumed a carrying capacity (and starting population size) of 300 000 individuals and tested the following 7 threat scenarios:

(1) No threats.

(2) Commercial harvesting outside Marine Fishing Area 21 (i.e. removed from the aggregation sites).

(3) Commercial harvesting outside Marine Fishing Area 21 and by-catch from prawn trawling.

(4) Commercial harvesting on the Point Lowly aggregation site (50 000 individuals removed per annum).

(5) Desalination discharge at the Point Lowly aggregation site (a 30% reduction in embryonic survival occurring once every two years on average).

(6) Commercial harvesting and desalination discharge at the Point Lowly aggregation site (simulated as detailed above).

(7) All of these threats.

All simulations included environmental stochasticity as 1% standard deviation (i.e. σ= 0.01) on juvenile survival rates applied annually. Although the magnitude of this simulated environmental variation strongly influences the variance of the simulation output, it does not greatly influence comparisons between the different threat scenarios. We recorded the final population at the end of the 10-year management time-frame.

Sensitivity analysis

To evaluate the relative contribution of each input parameter to the final population size simulated after the 10-year management time-frame, we performed a detailed sensitivity analysis on model inputs (Table 1). That is, we generated 1000 new model parameterisations using latin hypercube sampling (Fang et al. 2006) and ran the simulation model for these parameterisations, recording the final population size. We then analysed the sensitivity-analysis output by relating the input variables to simulation output using boosted regression trees (BRT), a flexible machine-learning regression technique that can accommodate non-linear responses and interaction terms (Elith et al. 2008). We fit the BRT with first-order (i.e. two-way) interaction terms only and examined the fitted response curves and the relative contribution metrics for input parameter.

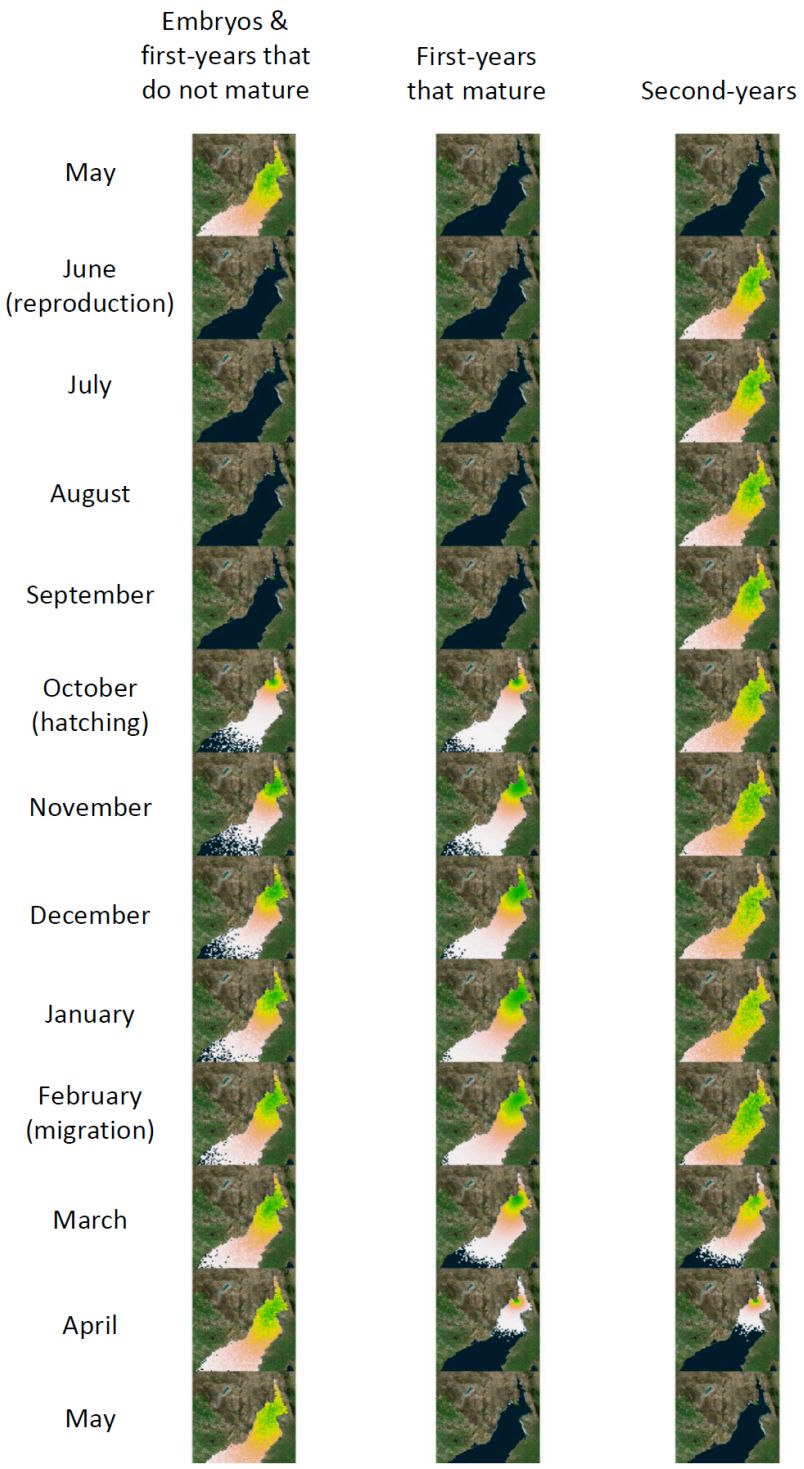


Figure 5. Example screenshots from the spatial population model, illustrating the dispersal and migration of simulated cuttlefish over a 1-year period. Green coloration illustrates the regions of highest cuttlefish density.

Results and Discussion

Movement throughout life history and finer scale population structure

Natal homing

A prerequisite for determining if cuttlefish return to their natal site is to first demonstrate that there are spatial differences in chemical signatures among different locations. Significant differences were found among locations for trace elements in statoliths, trace elements and stable isotopes in cuttlebones, and for statolith and cuttlebone data combined (Table 2) in hatchlings. Despite significant differences in chemical signatures there was a relatively poor ability to classify these hatchlings back to their collection location (CAP analysis: statoliths 41.3% correctly reclassified; cuttlebones 51.5% correct; statoliths and cuttlebones combined 46.2% correct). There was significant overlap in chemical signatures among locations (Figure 6), which helps explain why the classification success was relatively poor. Such poor classification success may in part be due to the small spatial scale over which hatchlings were collected. Most samples were collected from a 8 km stretch of reef at Point Lowly with a further sample collected just north of Point Lowly at Fitzgerald Bay. Despite extensive exploration, samples of eggs and hatchlings from other areas (e.g. Point Riley on the eastern side of Spencer Gulf) were not found. Given the poor classification success it was not possible to retrospectively trace adult cuttlefish to their natal site along the Point Lowly reef.

A further approach focusing on adult cuttlefish was then undertaken. The core representing the early life history and the edge representing recent life history of adult cuttlefish from three areas of Spencer Gulf were examined to determine if they differed. All datasets comprised both trace element and stable isotope data. The statolith, cuttlebone and combined statolith and cuttlebone datasets all showed no significant difference among locations for the core region but a significant difference among locations for the edge region (Table 2). These data suggest that cuttlefish may have a common source population but cannot be used to determine the actual location individuals came from. Differences for the edge data are as expected based on collection locations across a gradient of temperature and salinity in Spencer Gulf.

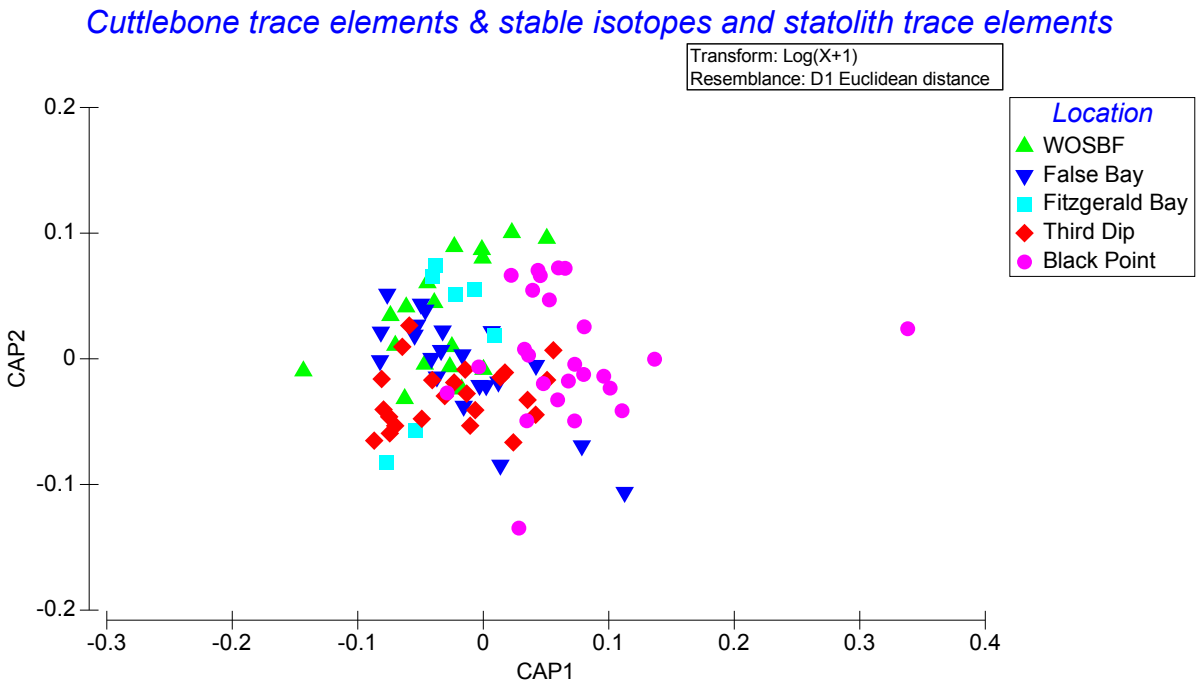


Figure 6. CAP analysis for the chemical signature (trace elements and stable isotopes) from the cuttlebones of embryo cuttlefish collected from the aggregation reef, Point Lowly, Whyalla, in 2013. Samples were collected from five locations.

Table 2. Permutational MANOVA results for hatchling and adult core (natal) and edge statolith, cuttlebone and combined statolith and cuttlebone data testing for differences among locations.

Structure	Life history	df	F	P
Statolith	Hatchling	4, 104	4.338	0.002
	Adult core (natal)	2, 52	1.944	0.099
	Adult edge	2, 52	4.288	0.001
Cuttlebone	Hatchling	4, 92	5.957	0.001
	Adult core (natal)	2, 54	0.814	0.492
	Adult edge	2, 54	8.549	0.001
Statolith & cuttlebone combined	Hatchling	4, 88	5.310	0.001
	Adult core (natal)	2, 51	0.994	0.385
	Adult edge	2, 51	7.871	0.001

Profiles along statoliths

Analysis of similarities among individual statolith profiles for cuttlefish from the 2012/2013 cohort suggested that there were four groups or contingents (Figure 7). Each cluster contained cuttlefish from all regions (Upper Spencer Gulf, contact zone, Lower Spencer Gulf) with the exception of the smallest cluster in which no cuttlefish from Upper Spencer Gulf occurred. All four cuttlefish in this cluster were collected in November 2012. All other clusters contained cuttlefish collected at all sampling times. The largest proportion of cuttlefish from all regions occurred in the cluster with most individuals. Thus, while the data suggested there were four contingents the potential discriminating variables (region, month, sex, and size) did not appear to influence cluster membership. This analysis suggests that cuttlefish have moved through variable environments throughout their life.

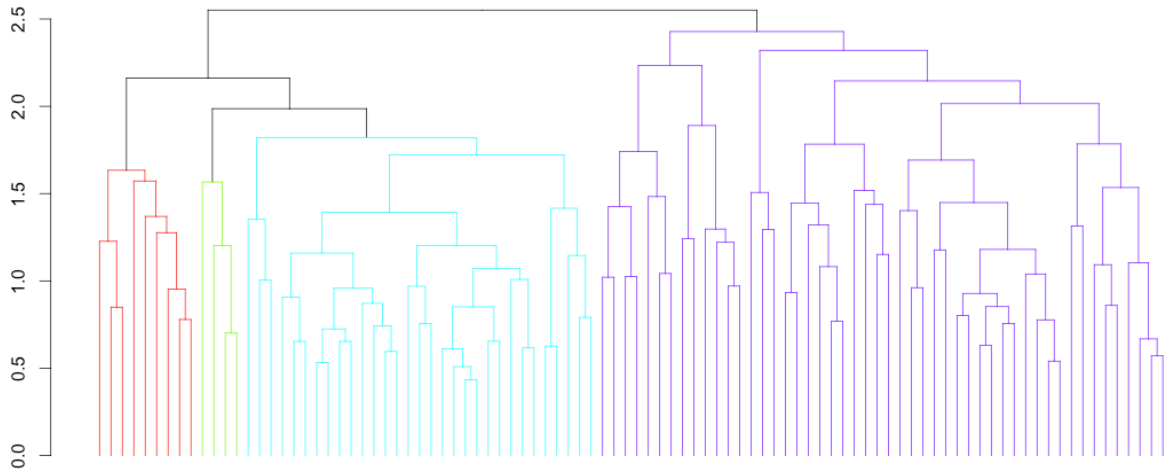


Figure 7. Cluster analysis of extracted features for profiles across cuttlefish statoliths.

Systematic status of the two cuttlefish populations

Previous studies suggest no difference in sex ratio; however, the specimens we collected were at a ratio of 1.6:1 (M:F) (Appendix A, Table S1). Closer examination showed a skew in sex ratio only during breeding months and only at breeding aggregation locations. During breeding most male cuttlefish aggregate *en masse* at breeding grounds and remain there for longer than the females (Hall and Hanlon 2002, Payne et al. 2011). This behaviour results in a male skew in sex ratio at these locations during this period, and the male biased sex ratio in our data is most likely a reflection of this.

Population genomics

A total of 1107 loci were genotyped with an error rate of <1%. A histogram of the distribution of minor allele frequencies (mAFs) (Figure 8) shows that 1092 loci (98.6%) of the 1107 loci had mAF >0.1, indicating that 98.6% of the loci had relatively high information content.

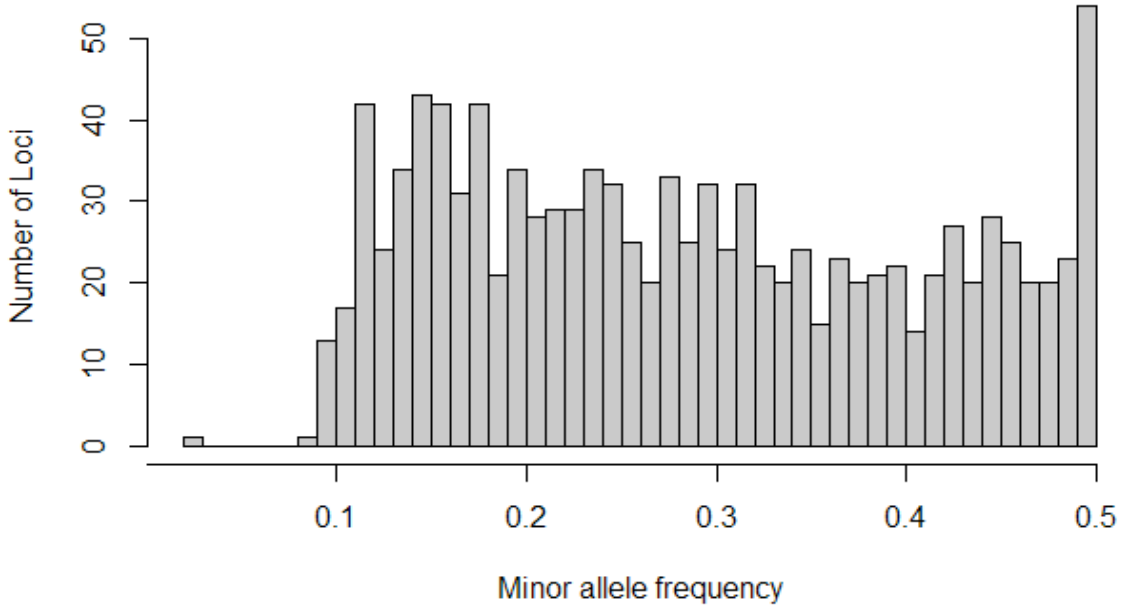


Figure 8. Distribution of minor allele frequencies (mAFs) across 1107 loci.

The DAPC analysis identified two major clusters of individuals that correlate with geography; one cluster comprised only specimens from upper Spencer Gulf (hereafter referred to as the northern cluster), while the other comprised all specimens from lower Spencer Gulf and Gulf St. Vincent, and some specimens from upper Spencer Gulf (hereafter referred to as the southern cluster) (Figure 9A). The underlying principal components of the DAPC also show clear and robust separation of the USG and LSG clusters on the first principal component (PC) (Figure 9B), which accounts for 85% of the total variance explained by the retained principal components.

Consistent with the observed correlation between genetic variation and geography, AMOVA showed the contribution of geographic variation was significant ($P<0.001$) with 1.0% variation explained by sampling location. The contribution of temporal variation was negligible with only 0.1% variation ($P=0.126$) explained by difference in sampling period. A further 1.9% variation ($P<0.001$) among individuals that could not be explained by temporal or spatial variation points to a more important factor contributing to genetic variation.

STRUCTURE estimated the logarithm of probability of the data for K=1-4 as maximal for K=2 (Figure 10A), supported by delta K (Figure 10B). This is congruent with the number of clusters identified by DAPC. Individual assignments to respective clusters were strong, showing no evidence of genetic admixture (Figure 11). Fifty-eight individuals were assigned with 0.86 or greater (mean = 0.97) membership to the northern cluster and 52 individuals were assigned with 0.92 or greater (mean = 0.98) membership to the southern cluster. Therefore, memberships were weakest in the northern cluster, which could reflect a signal of historic asymmetric introgression into the northern cluster or a founder effect, both likely scenarios in this system. Observed heterozygosity was slightly higher than expected in the northern cluster (mean Ho = 0.360 ± 0.004 SE; mean He=0.355 ± 0.004 SE), while it was slightly lower than expected in the southern cluster (mean Ho = 0.357 ± 0.004 SE; mean He=0.366 ± 0.004 SE). This may be expected under a scenario of historic asymmetric introgression into the northern cluster, but is less likely under a founder effect scenario (Chakraborty and Nei 1977).

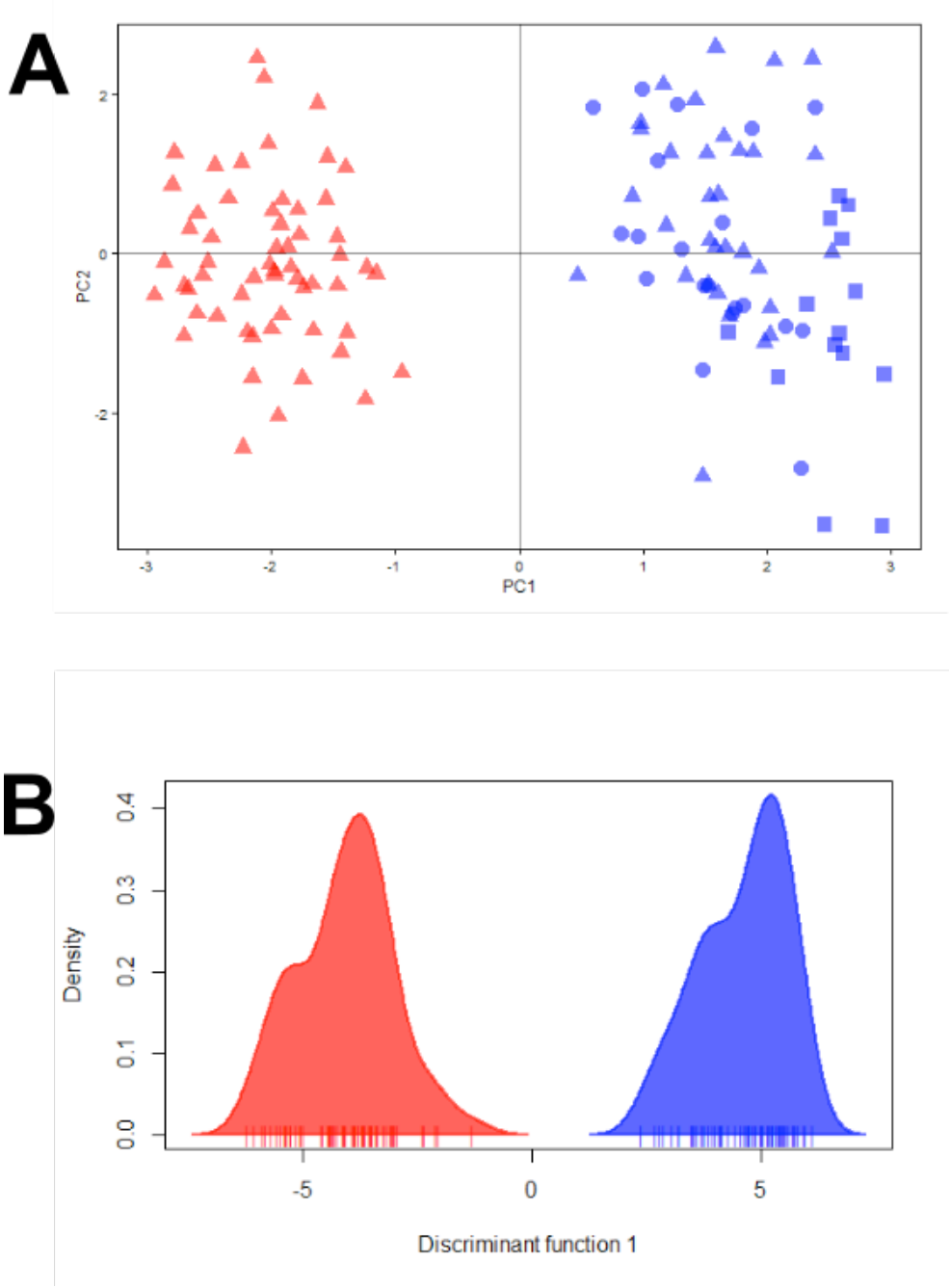


Figure 9. DAPC Analysis. A) PCA ordination of genetic distances in cuttlefish across Spencer Gulf and Gulf St Vincent. Shape indicates geographic location [triangle = upper Spencer Gulf (above -33.9178° latitude), circle = lower Spencer Gulf (below -33.9178° latitude), square = Gulf St Vincent]. B) Densities of individuals on the single discriminant function output from the DAPC analysis. Each vertical coloured line represents an individual. Colour indicates genetic cluster: red = northern cluster, blue = southern cluster.

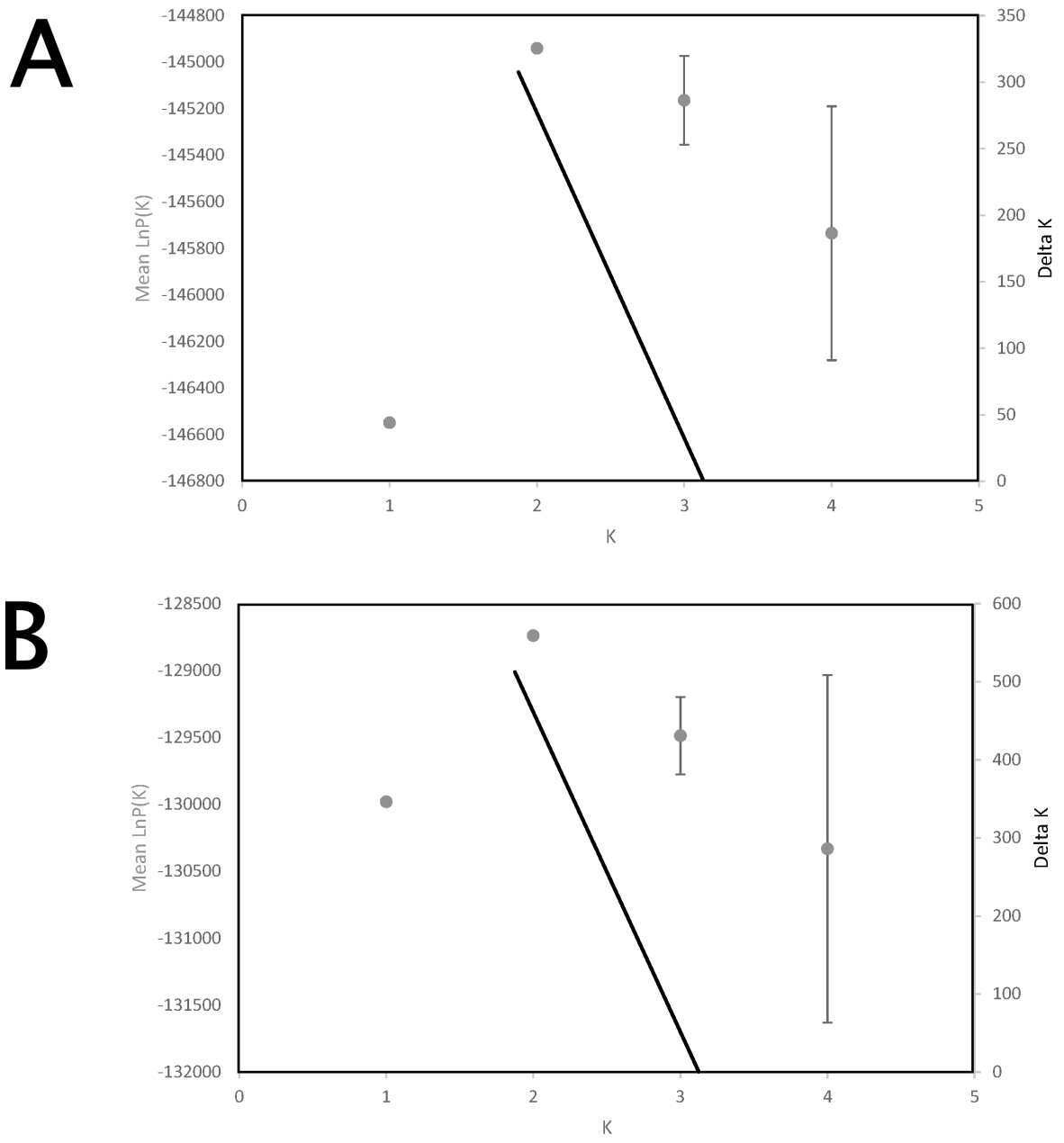


Figure 10. Inference of *K* using the Bayesian clustering software STRUCTURE; Ln probability of data (mean ± SD) (left hand axis) and delta *K* (right hand axis and solid line) for *K* = 1-4 using the standard admixture model over 10 MCMC replicates for samples A) from Spencer Gulf and Gulf St Vincent, and B) Spencer Gulf only.

Aligning individual memberships in latitudinal order shows the range of the two genetic clusters clearly overlap between -33.502° and -33.828° latitude (Appendix A, Table S1, Figures 11, 12); no northern cluster individuals were observed further south than -33.828° latitude and no southern cluster individuals were observed further north than -33.502° latitude. Importantly no southern cluster individuals were collected from the mass breeding aggregation. Further sampling around the zone of overlap at different times across the year may be necessary to further resolve the zone of overlap between the two clusters, but sampling at the breeding aggregation at least was sufficiently large to suggest it is unlikely that southern cluster individuals participate in the aggregation.

The fixation index (F_{ST}) indicates that neutral genetic differentiation between clusters identified by DAPC was 2.7%, indicating significant divergence. Including loci under selection, genetic differentiation between clusters was 4.7%, suggesting strong divergent selection between the clusters has been prevalent in this system. Seven percent of loci showed no difference in allele frequency between the clusters, while 36% of loci showed a difference in allele frequencies >0.1 (Figure 13), indicating that a large proportion of loci contribute to the observed divergence between clusters. Difference in allele frequencies ranged from 0.00 to 0.36 (mean=0.09), hence no loci had fixed differences between clusters, and there were also no loci with fixed alleles in either population, consistent with the hypothesis that this divergence is relatively young in evolutionary time.

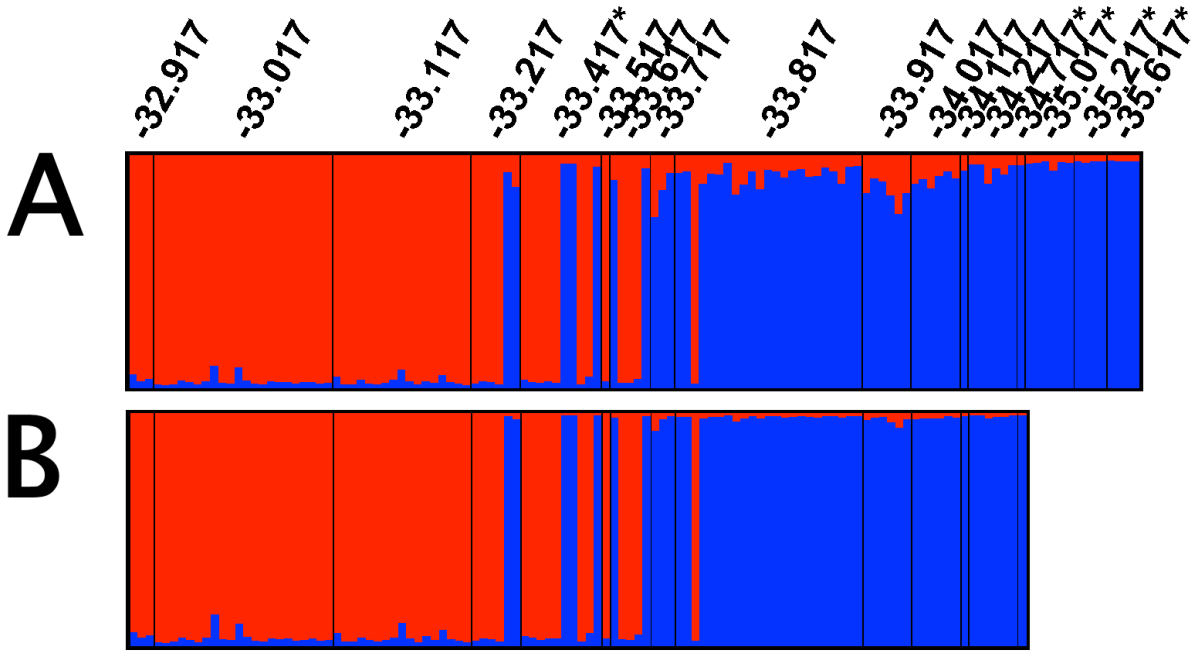


Figure 11. Bar plot of individual memberships estimated by the Bayesian clustering software STRUCTURE using the LOCPRIOR model for *K*=2 with 10 iterations for samples A) from Spencer Gulf and Gulf St Vincent and B) Spencer Gulf only. Samples are sorted from left (north) to right (south) by latitude in descending order; each section represents an area of the gulf spanning 0.1 decimal degrees (~11km); each latitude figure shows the most northern point in the area represented by the section. * indicates a gap between a section and its northern neighbour, i.e. discontinuous sections. Colour indicates genetic cluster: red = northern cluster, blue = southern cluster.

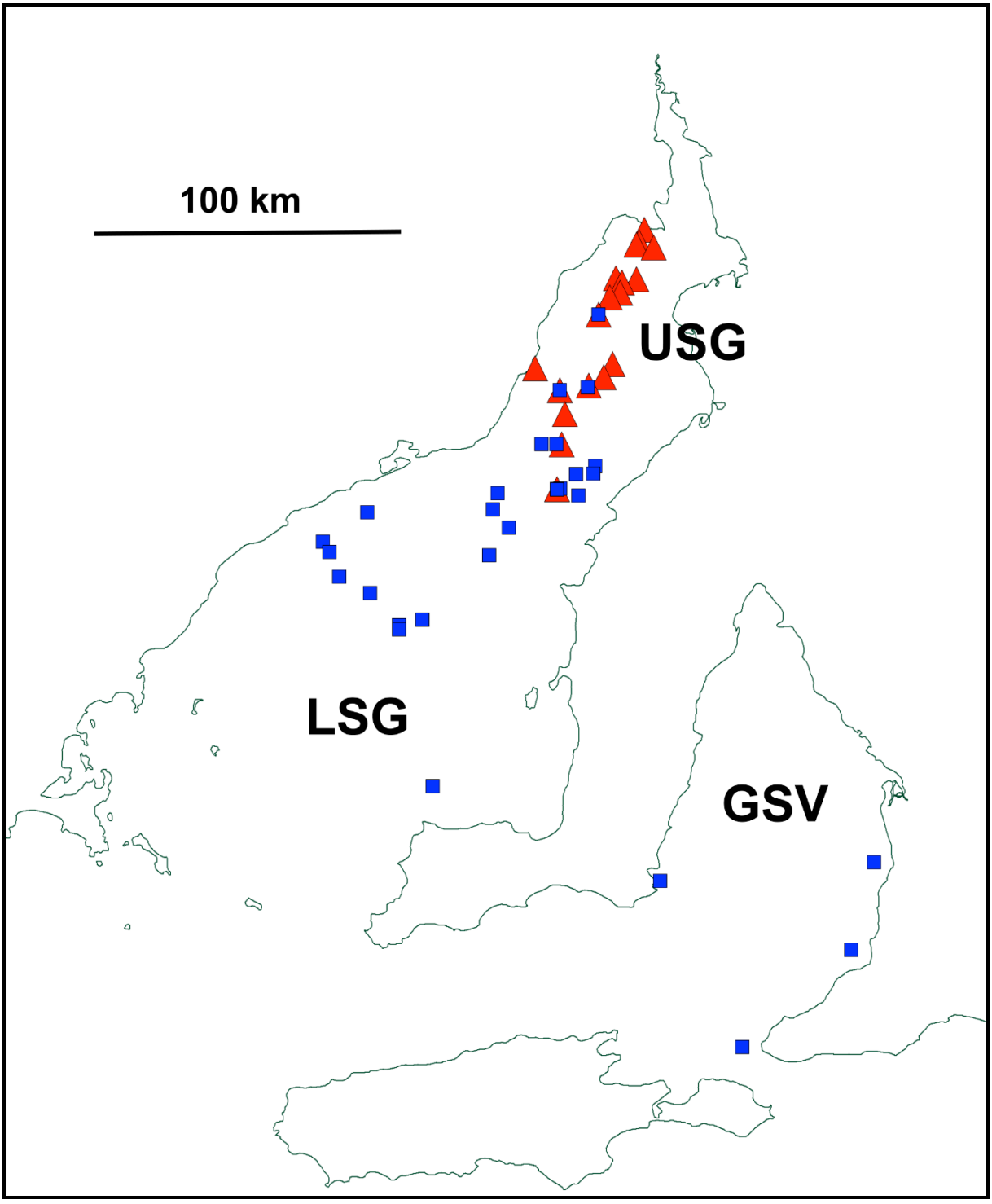


Figure 12. Map of the central South Australian coast showing sampling locations and the presence of genetic clusters. Colour and symbol indicates genetic cluster: red triangle = northern cluster, blue square = southern cluster.

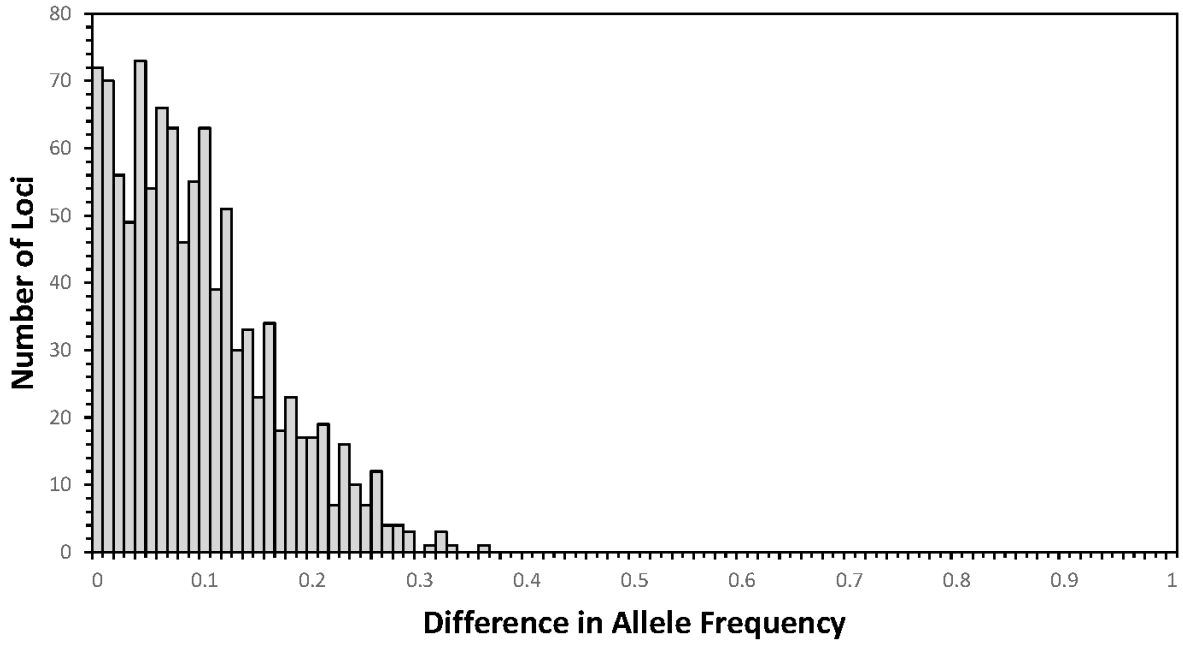


Figure 13. Distribution of differences in allele frequencies between genetic clusters across loci.

Dietary Analysis

Ninety-one of the 101 stomachs (90.1%) had contents visually identifiable. Forty-six stomach DNA extracts (45.5%) amplified successfully for *16S rRNA*, and 83 stomach DNA extracts (82.2%) amplified successfully for *18S rRNA*. Four samples that amplified successfully (1 using *16S rRNA* and 3 using *18S rRNA*) had contents that could not be identified visually. Forty-two samples (41.6%) had *16S rRNA* sequence recovered, of which 29 samples (28.7%) had molecular identifications made after pipeline processing. Seventy-five samples (74.3%) had *18S rRNA* sequence recovered, of which 26 samples (25.7%) had molecular identifications made after pipeline processing. Molecular identification was made for 35 samples (34.7%) in total, with 10 samples having identifications made using both *16S rRNA* and *18S rRNA*. This identifies two bottlenecks in the process for *16S rRNA* and one for *18S rRNA*, where samples with potential prey sequences were removed at a high frequency; (1) failure to amplify, restricted to *16S rRNA* only, and (2) failure to yield molecular identification, shared by both *16S rRNA* and *18S rRNA*. The large discrepancy between sequence recovery and molecular identification can be explained by a high frequency of samples having only DNA sequence of high similarity to the host cuttlefish recovered, which was filtered out during pipeline processing. Samples had an average of 61 324 reads, so we infer that coverage was sufficient in most cases to detect prey DNA, if it was present, and conclude that samples removed during processing after amplification had no prey DNA. It is likely the prey target sequence in these samples was too degraded.

Only 3 (12%) fish, 5 (42%) cephalopods, and 6 (12%) crustaceans were unable to be identified using molecular techniques. The higher fail rate in cephalopods is most likely a result of sharing sequence similarity with the host. Despite having observable differences between the sequences of cuttlefish and, e.g. squid and octopus, in both marker regions, it was apparently not sufficient in many cases to be retained after clustering at 5% similarity. Four molecular identifications matched families that were unlikely to comprise cuttlefish prey; 2 matched Dasyatidae, and 1 matched each of Sphyraenidae and Calliostomatidae. It is possible that juveniles of these families may be prey to cuttlefish, but it seems more likely that scavenging prey such as crustaceans (the most abundant taxonomic group observed in

our data) fed on carcasses of individuals belonging to these families shortly before being preyed upon by our cuttlefish specimens. Therefore, we removed these identifications from our analyses.

We identified an average of 2.15 prey items per individual. We observed 22 unique family-level taxa; 10 fish, 3 cephalopods, and 9 crustaceans. The most dominant taxonomic group in terms of numerical abundance were crustaceans (an average of 1.17±0.14 observed per sample; 58±8% of all observations; Figure 14, Table 3). Alpheidae, Pandalidae, and unidentified crustaceans were the most dominant crustacean families (6.9±1.2% of all observations; Figure 15A, Table 4). Octopodidae and unidentified cephalopods were the most dominant cephalopod families (5.6±1.1% of all observations; Figure 15B, Table 4). Pinguipedidae was the most dominant fish family observed (11.1±1.5% of all observations; Figure 15C, Table 4), and also the most dominant family of any taxonomic group.

Considering diets within genetic clusters, cephalopods comprised the smallest proportion of cuttlefish prey in both the northern and southern clusters (Figure 14), most likely explained by the much higher fail rate of the metabarcoding method to detect cephalopods (almost 3× higher than for fish and crustaceans). In the southern cluster, crustaceans comprised a significantly larger proportion of cuttlefish prey than fish and cephalopods, while in the northern cluster, crustaceans and fish comprised an equivalent proportion of cuttlefish prey, larger than that of cephalopods.

Table 3. Comparison of total per cent numerical abundance of prey species found in the diet of cuttlefish. 'Morphology' indicates the results obtained using morphological analysis of prey remains, *16S rRNA* and *18S rRNA* indicate the results obtained through the application of the two molecular markers, and 'Combined' indicates the result obtained by the combination of the approaches.

Group	**Morphology**	***16S rRNA***	***18S rRNA***	**Combined**
Crustacean	0.76	0.37	0.89	0.58
Cephalopod	0.13	0.15	0.03	0.11
Fish	0.11	0.49	0.09	0.31

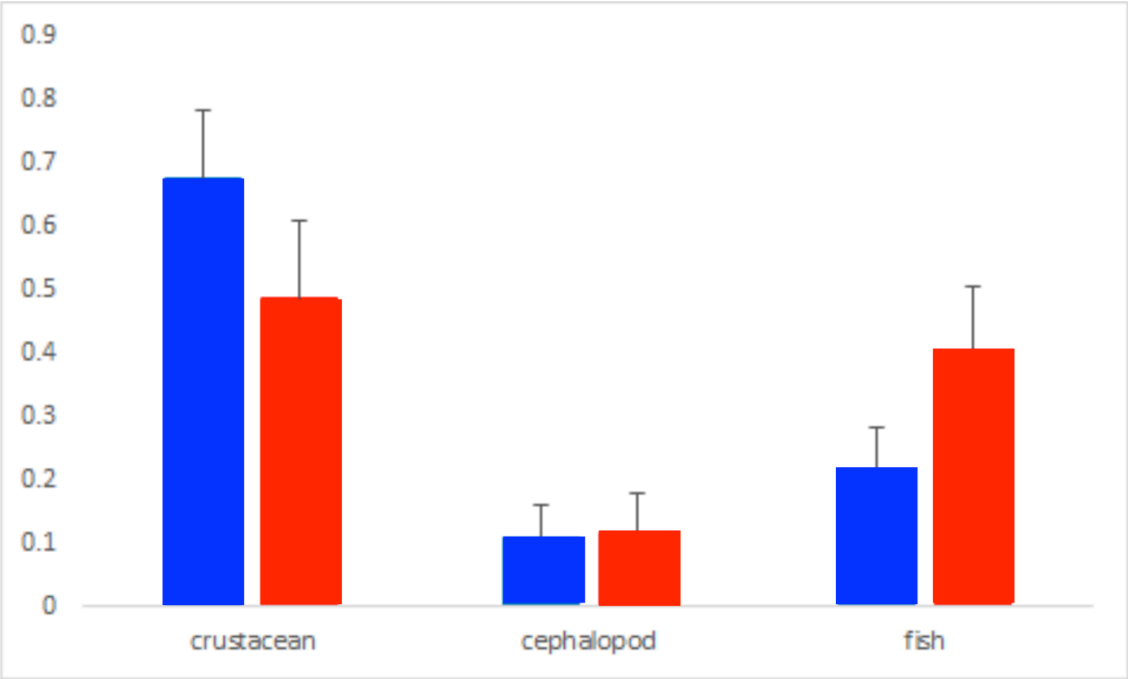


Figure 14. Proportional composition of diet at a phylum-level by genetic cluster. Colour indicates genetic cluster: red = northern, blue = southern.

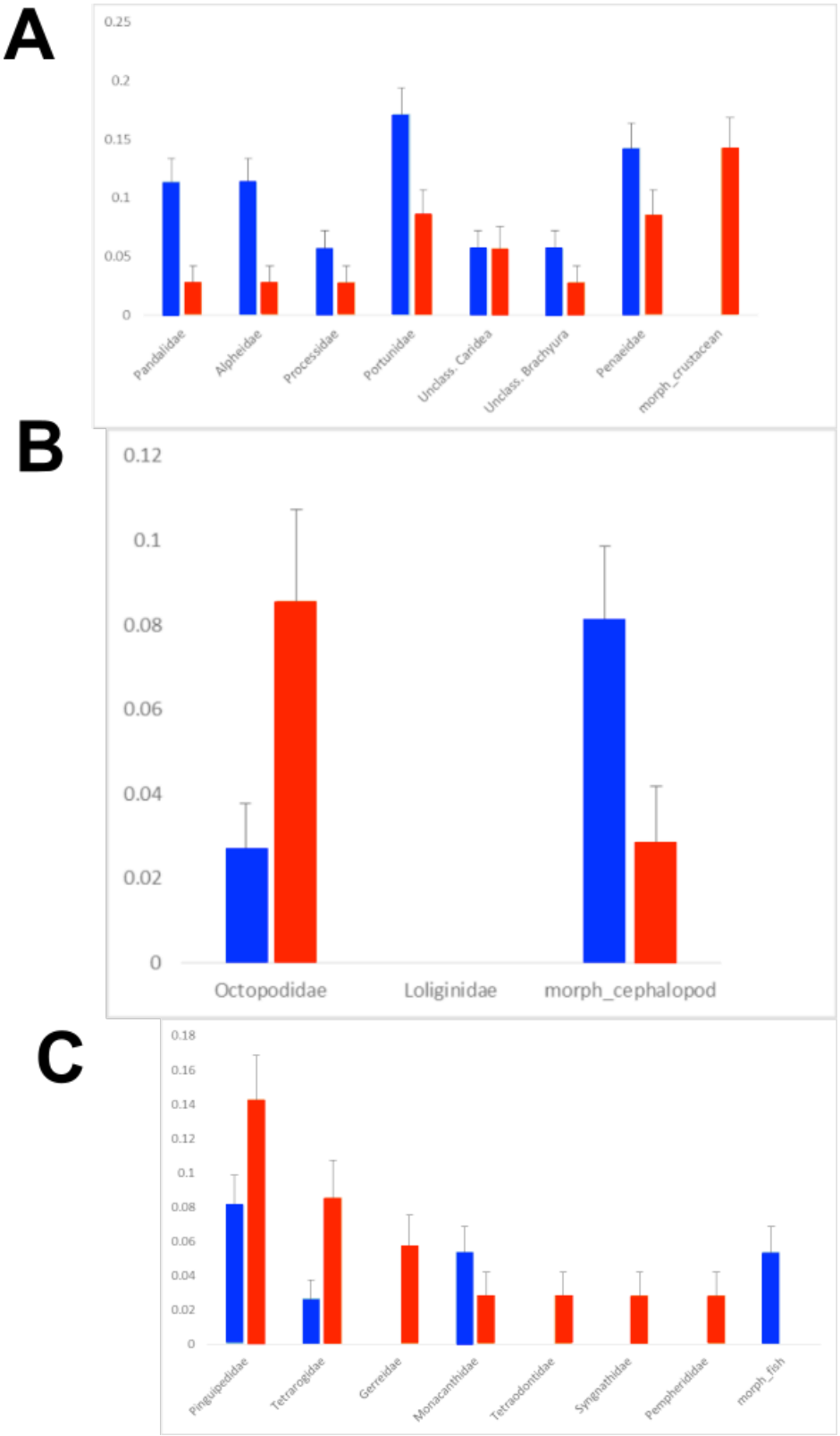


Figure 15. Proportional composition of components of diet at a family-level by genetic cluster (with standard error bars) for A) crustaceans; B) cephalopods and C) fish. Colour indicates genetic cluster: red = northern, blue = southern.

Table 4. Comparison of total per cent numerical abundance of prey species found in the diet of giant Australian cuttlefish. *16S rRNA* and *18S rRNA* indicate the results obtained through the application of molecular techniques, and 'Combined' indicates the result obtained by the combination of the approaches.

Group	Family	*16S rRNA*	*18S rRNA*	combined
Crustacean	Alpheidae	-	14.3	6.6
Crustacean	Pandalidae	2.4	17.1	9.1
Crustacean	Penaeiade	24.4	-	1.2
Crustacean	Pilumnidae	2.4	-	1.2
Cephalopod	Loliginidae	2.4	-	1.2
Crustacean	Portunidae	7.3	28.6	16.7
Crustacean	Processidae	-	8.6	3.9
Crustacean	Unclassified Caridea	-	11.4	5.3
Crustacean	Unclassified Brachyura	-	8.6	3.9
Cephalopod	Octopodidae	12.2	2.9	7.3
Fish	Gempylidae	-	2.9	1.3
Fish	Gerreidae	7.3	-	3.6
Fish	Monacanthidae	7.3	-	3.6
Fish	Mullidae	2.4	-	1.2
Fish	Pempheridídae	2.4	-	1.2
Fish	Pinguipedidae	19.5	-	9.6
Fish	Syngnathidae	-	2.9	1.3
Fish	Tetrarogidae	9.8	-	4.8
Fish	Unclassified Actinopterygii	-	2.9	1.3

Comparing prey proportions among genetic clusters, we observe that fish comprised a significantly smaller proportion of cuttlefish prey in the southern cluster than the northern cluster, balanced by a larger proportion of crustacean prey in the southern cluster. When considering only distance between diet of individual cuttlefish, individuals grouped weakly based on genetic cluster (Figure 16), suggesting that a significant proportion of the variation among populations is attributable to the sum of individuals from each population, rather than restricted to a few individuals from each population. A difference of this nature could be explained either by opportunistic feeding behaviour coupled with differences in prey availability, or selective feeding behaviour reinforced by genetics. Sepiids are generally known as opportunistic feeders (Mqoqi et al. 2007). If cuttlefish in Spencer Gulf are opportunistic feeders then there must be some variation in prey availability. Indeed, there is a difference in diversity across the gulf that correlates with latitude, which is linked to latitudinal differences in trawl intensity (Currie et al. 2009). Upper Spencer Gulf is more heavily trawled than Lower Spencer Gulf, reducing the overall diversity and abundance of potential cuttlefish prey, especially prawn and other crustacean prey which are selectively harvested in trawling (Currie et al. 2009). These data are congruent with the hypothesis that both genetic clusters of cuttlefish in Spencer Gulf are opportunistic feeders, with their respective diets determined by prey availability that has likely been shaped by differences in trawling intensities across the gulf.

The opportunistic feeding behaviour of cuttlefish can be used to reveal relative abundances of taxa at different locations. Our data show that the crustacean families Alpheidae and Pandalidae comprise a far lower proportion of cuttlefish diet in the northern cluster than the southern cluster, suggesting that these families of crustaceans are less common in Upper Spencer Gulf. The reason for this is unclear, but it is possible these crustaceans may be more sensitive to trawling than Penaeidea, the primary

target of trawling, or conditions in the north may be unsuitable to support large populations of these crustaceans as seen in the south.

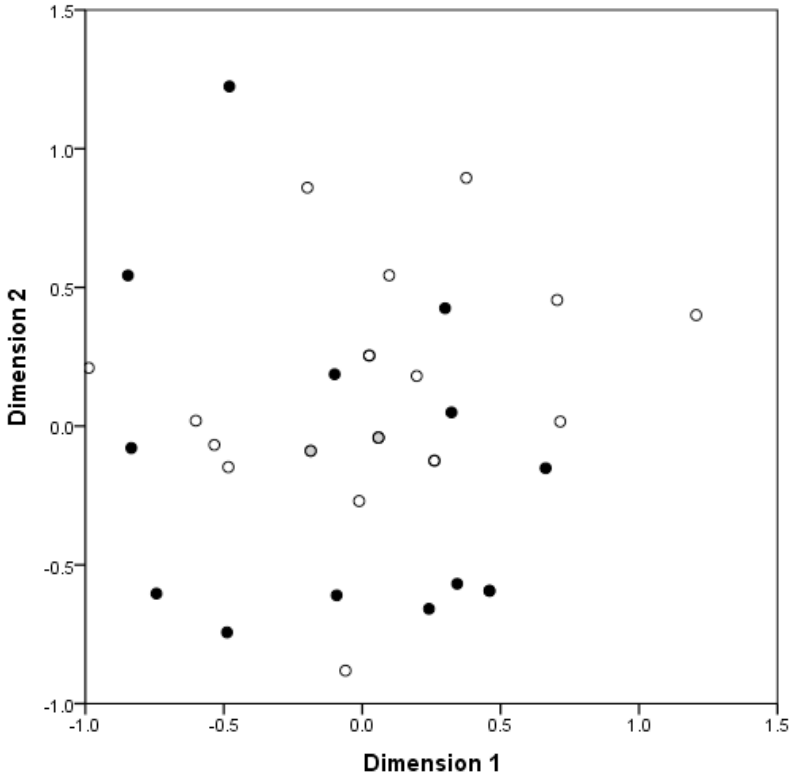


Figure 16. MDS plot summarising distances between the diets of cuttlefish from northern cluster (black filled circles) and southern cluster (open circles); grey filled circles represent points occupied by cuttlefish from both clusters.

Temperature and salinity experiments

Temperature spike experiment

Results showed that above 20°C there was a decrease in the number of live cuttlefish. Although similar numbers of cuttlefish began developing, there was increased mortality at higher temperatures. Thus spikes of temperature above 20°C may negatively impact development of embryos.

Temperature x salinity

At ambient salinity there was only a decrease in survival at the highest temperature (24°C), whereas at the highest salinities (>48 ppt) no eggs survived through to hatching (Figure 17). At mid level salinities (44-45 ppt) there was a decrease in survival from 14°C through to 22°C. At the current temperature and salinity levels embryo survival is maximum, but with potential increases in either salinity or temperature embryo survival may be compromised.

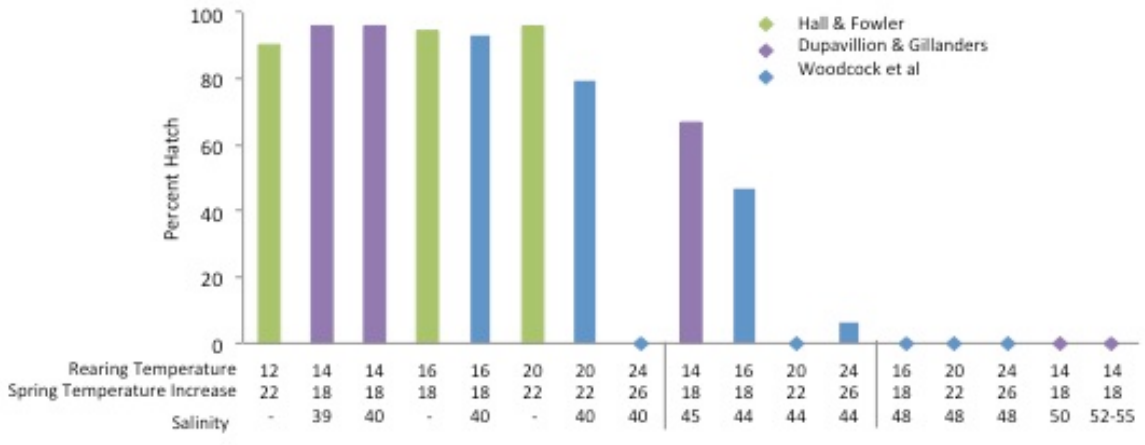


Figure 17. Percent of embryos successfully hatching under different temperature and salinity treatments. Shown are initial rearing temperatures as well as the temperature eggs were raised to in spring, and salinity. The studies are Hall and Fowler (2003), Dupavillon and Gillanders (2008) and Woodcock et al. (this study).

Swimming respirometry

Standard and maximum metabolic rate both increased with increasing temperature (Figure 18). However, maximum metabolic rate at 20°C showed large variation. These results suggest that adult cuttlefish can tolerate all temperatures when resting but that 20°C is likely at their upper limit when they are actively moving. Absolute aerobic scope (the difference between maximum and resting metabolic rate) increased with temperature but there was overlap in standard errors for 18°C and 20°C treatments suggesting that the optimal temperature for adult cuttlefish was around 18-20°C. An increase in temperature above 20°C would be expected to see an onset in loss of performance.

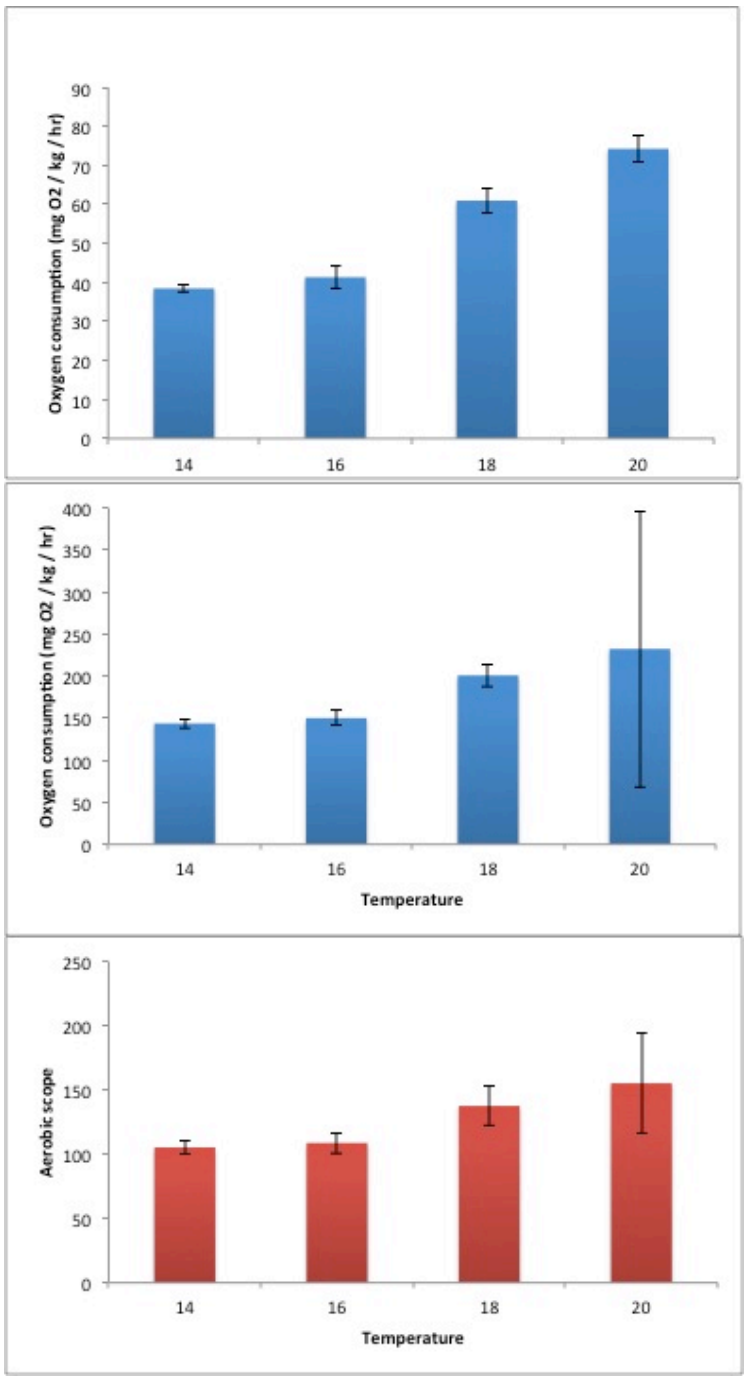


Figure 18. Resting (top figure) and maximum (middle figure) metabolic rate of adult cuttlefish at different temperatures based on flume respirometry. Absolute aerobic scope is shown in the bottom figure.

Integrated model of population dynamics

Ensemble model development and hindcasting

Using the empirical data, we estimated a positive relationship between sea surface temperature and the survival rate of juvenile cuttlefish. This relationship was robust to assumptions about the other vital rates required to fit this response curve (Figure 19). When the model ensemble that simulated these temperature effects and historical harvesting pressure was used for hindcasting, the simulated time-

series recreated some important patterns including a decline in population size since the year 2000, minimum population sizes that occurred over the period 2012 to 2013, and increase in population size over 2014 and 2015 (Figure 20a). However, these hindcasts substantially overestimated the cuttlefish population size in the year 2001 because mean sea surface temperature in the preceding winter/spring was unusually warm (18.1 °C).

Assuming a demographically stable cuttlefish population at the mean observed sea surface temperature over winter and spring, a second model ensemble produced simulated population time-series that recreated the general pattern of abundance on the aggregation site over time but could not capture all the empirical variability (Figure 20b). However, simulating additional environmental stochasticity (a 1 % standard deviation on juvenile survival rates applied annually) was sufficient to bring the empirical aggregation-site abundances within the 95% confidence intervals derived from hindcast simulations (Figure 20b). These results illustrate how, for a short-lived species with high fertility such as cuttlefish, tiny variations in juvenile survival can have large population-level consequences.

In summary, hindcast cuttlefish simulation models that incorporated harvesting and temperature variation appear to capture the decline in aggregation-site abundance since the year 2000. These results indicate that the historical decline could largely have resulted from known anthropogenic and environmental factors.

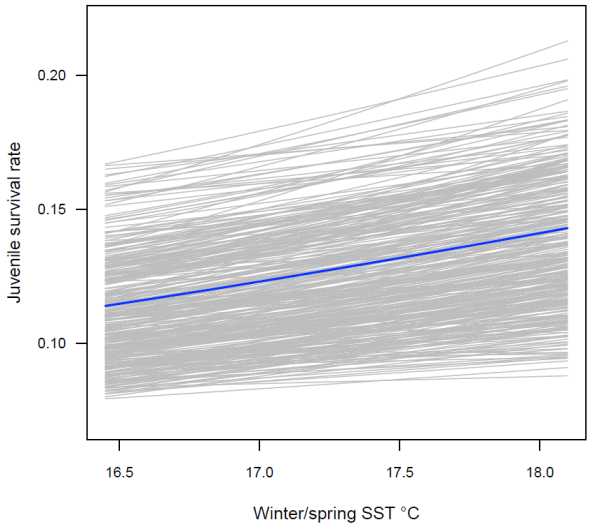


Figure 19. The estimated effect of sea surface temperature (SST) on the juvenile survival rate of *S.apama*, conditional on assumptions regarding other vital rates. The blue line illustrates the mean relationship estimated, whilst the grey lines represent variation in the fitted relationship due to differing vital rate assumptions.

Scenario testing

Scenario testing involving different threatening processes and the density-dependent ensemble of models indicated that cuttlefish harvesting away from the breeding aggregations (i.e., outside Marine Fishing Area 21) and by-catch due to prawn trawling pose negligible risks to the population (Figure 21). Compared to simulations with no threats imposed, implementing these threats did not substantially affect the mean final population size simulated over the 10-year management time-frame (no threats: 280 165 individuals; with harvesting outside MFA 21: 271 844 individuals; with bycatch: 269 687 individuals). In contrast, the mean final population size could be compromised by increased adult mortality due to the reinstatement of commercial harvesting adjacent to the breeding grounds (with cuttlefish harvesting within MFA 21: 53 188 individuals), or increased mortality of embryos on the aggregation sites prior to hatching (with hypersaline conditions resulting from desalination

discharge: 179 138 individuals). The continued closure of Upper Spencer Gulf to commercial cuttlefish harvesting is therefore expected to reduce the vulnerability of the cuttlefish population.

When both aggregation-site harvesting and the impact of desalination discharge on embryos was modelled, the mean final population size declined by more than 95 % (to 2422 individuals) and extinctions occurred in 50 % of simulations. When all threats were implemented, all simulated populations went extinct within the 10-year time-frame. These results indicate how the combined effect of multiple stressors could compromise the viability of the Upper Spencer Gulf cuttlefish population.

Sensitivity analysis

The sensitivity analysis confirmed that the key threatening process for the Upper Spencer Gulf cuttlefish population is direct harvesting on the aggregation site. Our BRT analysis of the sensitivity-analysis output demonstrated that the relative contribution of this parameter was great (45.5 %; Figures 22a & 22b). Other influential parameters were those contributing to the strength of the density-dependent response, including the capacity and maximum population growth rate, as well as the effect of hypersaline discharge on the embryonic survival rate (Figure 22b). Once again, however, the inclusion of harvesting away from the aggregation site (outside MFA 21) or bycatch mortality due to prawn trawling had little impact on the simulation output (Figure 22a). The latter results held even when the parameters governing dispersal and migration were modified to permit higher densities of cuttlefish reaching the southern regions of Upper Spencer Gulf.

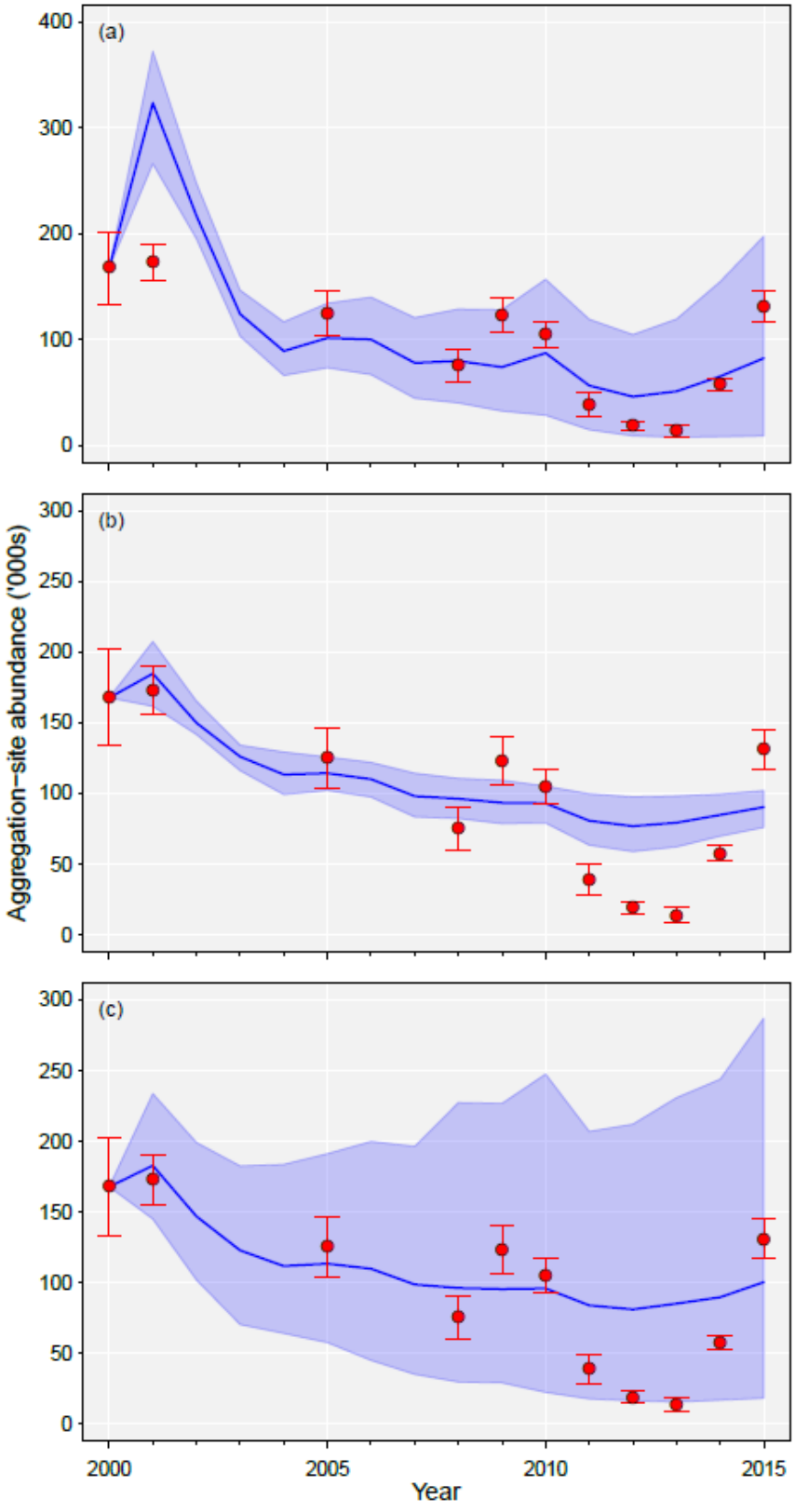


Figure 20. The performance of hindcast simulations (mean ± 95 % confidence intervals; blue lines/shading) compared to the empirical estimates of abundance on the aggregation site (mean ± 95 % confidence intervals; red points/error bars). (a) The ensemble of simulations accounting for harvesting and a fitted relationship between juvenile survival and sea surface temperature (SST). (b) The ensemble of simulations that included harvesting and the constrained relationship between juvenile survival and SST. (c) The same model as in (b) but with additional environmental stochasticity in the form of a 1 % standard deviation on the juvenile survival rate.

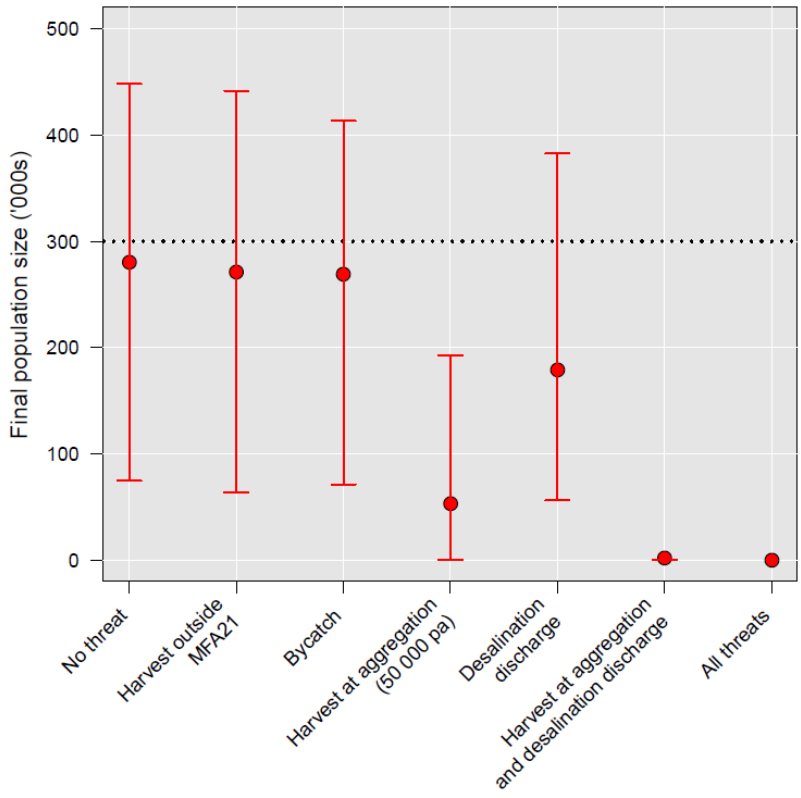


Figure 21. Final population size (mean ± 95 % confidence intervals) after 10 years, derived from 200 stochastic simulations for each of the seven different threat scenarios tested. The horizontal dotted line indicates the assumed carrying capacity of 300 000 individuals. Note that when all threats were applied, all simulations conducted resulted in extinctions.

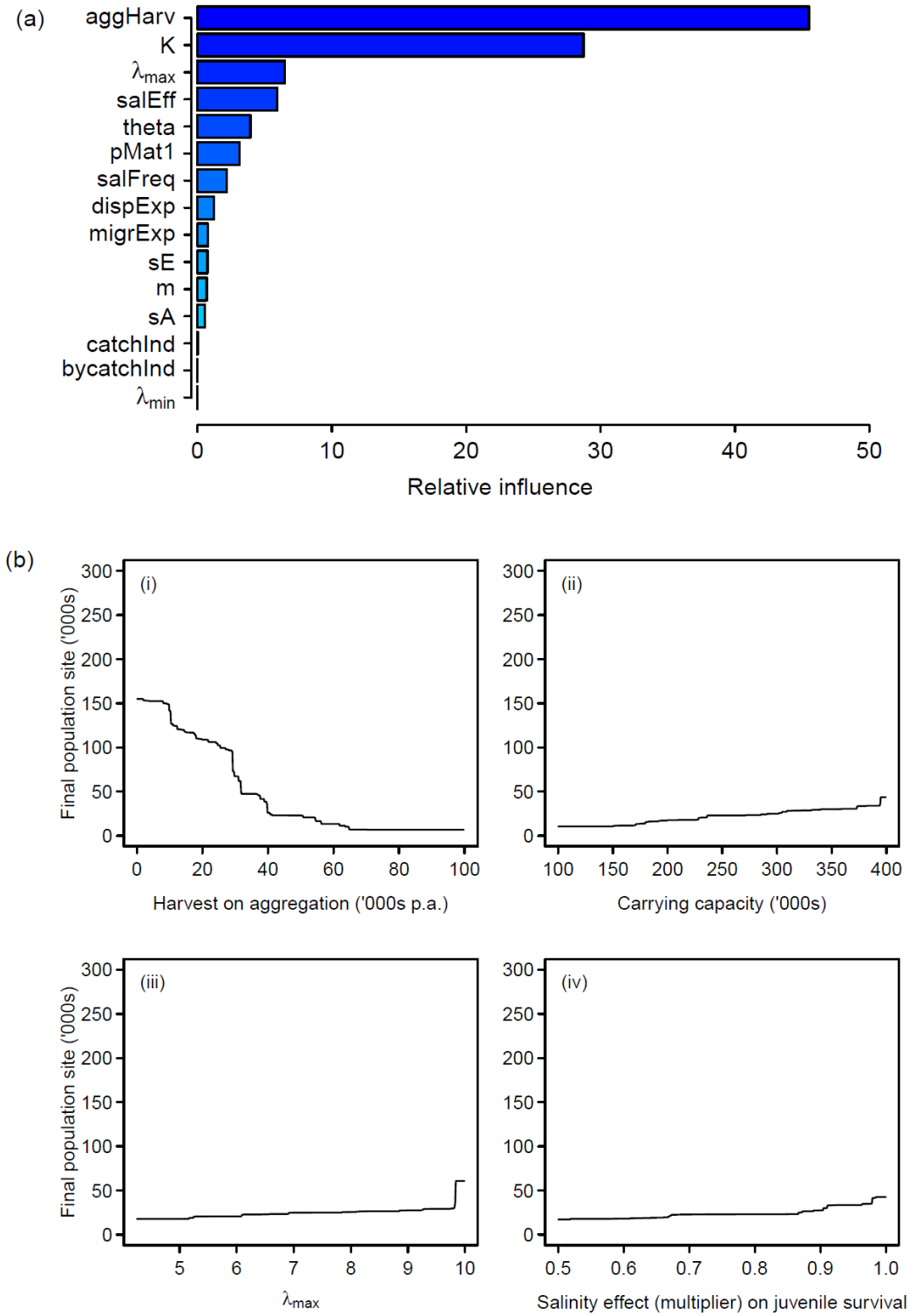


Figure 22. Results of the sensitivity-analysis comparing the influence of different simulation inputs on the final population size after 10 years. (a) Relative influence of each input parameter, as determined by the boosted regression tree (BRT) analysis of the sensitivity-analysis output. Parameter abbreviations are provided in Table 1. (b) Partial effects plots from the BRT analysis showing the final simulation population size as a function of: (i) the intensity of aggregation-site harvesting; (ii) the carrying capacity, (iii) the maximum population growth rate; and (d) the effect of hypersaline discharge on the embryonic survival rate. Note that, for each of these partial effects plots, all unplotted parameters were set at the mean value tested in the sensitivity analysis.

Conclusions

Movement throughout life history

Spatial variation in chemical signatures among hatchlings collected from different locations along the breeding aggregation was found, but the ability to correctly classify samples to their collection location was poor. Given this, no attempt was made to retrospectively trace adults back to their natal region. Instead two approaches were used to investigate whether adult cuttlefish likely originated from a common source population and whether they occupied similar water masses throughout their life history. Results suggested that adults likely originated from the same source population. There was some evidence from chemical profiles across the statolith that multiple groups or contingents existed suggesting that throughout their life history cuttlefish do not necessarily occupy the same water mass.

Systematic status of the two populations

We observed two highly resolved genetic clusters in Spencer Gulf with significant levels of divergence and no evidence of recent hybridisation or introgression. The ranges of the two clusters overlap at least between -33.502° and -33.828° latitude, confirming that the observed divergence is not a signal of isolation by distance. These observations strongly indicate that gene flow between the northern and southern clusters of cuttlefish in Spencer Gulf has ceased, and thus these clusters can be considered two separate species under the biological species concept, and should be treated as separate management units. This implies that the breeding aggregation at Point Lowly is comprised exclusively of northern cluster individuals and is the only breeding site known for this genetic cluster, highlighting its conservation significance. The northern population clearly shows a pattern of philopatry for both sexes, with males and females returning to breed at the location of their hatching at the mass breeding aggregation. We presently have no explanation for the mechanism for the philopatric behaviour, other than to note that the southern population does not appear to breed at the mass breeding aggregation. Whether the southern population shows philopatry or simply breeds anywhere within its putative range in lower Spencer Gulf or GSV is yet to be established.

Differences in the diet of the northern and southern populations of cuttlefish in Spencer Gulf can be explained by differences in prey composition along the gulf. Despite observing no evidence to reject the hypothesis that both clusters are opportunistic feeders, a difference in prey availability may be a possible mechanistic explanation for differences in beak morphology between the populations. However, we note that our diet analysis was not able to distinguish variation in prey size, which may be a focus of selective feeding.

Integrated model of population dynamics

A spatial, age-structured, stochastic model was developed for the cuttlefish population in Upper Spencer Gulf to assess the viability of the population in the face of current and future threatening processes. Hindcasting the model demonstrated that population-size variation over the last 15 years could be broadly recreated as a result of harvest adjacent to the aggregation site and sea surface temperature variation affecting the population growth rate. These results indicate that the historical decline could largely have resulted from known anthropogenic and environmental factors. Consequently, there is little need to invoke other causative factors to explain historic variation in the abundance of cuttlefish on the Upper Spencer Gulf breeding grounds in winter.

Scenario testing involving different threatening processes indicated that by-catch due to prawn trawling and cuttlefish harvesting away from the breeding aggregations (i.e. outside Marine Fishing Area 21) pose negligible risks to the population. Although approximately 20 000 cuttlefish in Spencer Gulf could be lost to bycatch per annum (Steer 2015), the mostly heavily trawled regions are located

towards the southernmost limit of the Upper Spencer Gulf population and so many individuals caught by trawling are likely to be from the genetically differentiated southern cluster (this report). In contrast, the population's viability could be compromised by increased mortality of embryos on the aggregation site prior to hatching (e.g. due to hypersaline conditions resulting from desalination discharge) and increased adult mortality due to the reinstatement of commercial harvesting adjacent to the breeding grounds (i.e., inside Marine Fishing Area 21). Sensitivity analysis identified direct harvesting on the aggregation site as the single greatest threat to the Upper Spencer Gulf population. Consequently, the continued closure of the aggregation area to cuttlefish harvesting is expected to improve the viability of the northern Spencer Gulf population.

Implications & Recommendations

Our research has confirmed the importance of the Upper Spencer Gulf population of giant Australian cuttlefish. Molecular genetic data have shown that this population is restricted to Upper Spencer Gulf (north of -33.828° latitude). A further population also occurs in Spencer Gulf but was not found on the breeding aggregation at Point Lowly. The southern genetic cluster was found as far north as -33.502 latitude and also occurs in Gulf St Vincent. Molecular observations strongly indicate that gene flow between the northern and southern clusters of giant Australian cuttlefish in Spencer Gulf has ceased, and thus these clusters can be considered two separate species under the biological species concept. They should therefore be treated as separate management units.

The breeding aggregation at Point Lowly exclusively comprises northern cluster individuals and is the only breeding site known for this genetic cluster. Steer (2015) and the RedMap and Feril or In Peril citizen science programs also failed to find other areas where the northern cluster individuals may be breeding. These results highlight the conservation significance of the Point Lowly area. The small rocky reef area where the cuttlefish aggregate to breed is part of the Upper Spencer Gulf marine park, where there are multiple zones. The breeding aggregation area forms part of several Special Purpose Area's including for harbour activities and shore based recreational line fishing and there are small Sanctuary Zone's (SZ) adjacent to the shore at Black Point (SZ-5) and further offshore (SZ-6). In addition, the area also includes the Cephalopod Fishing Closure Area where the taking of squid, cuttlefish and octopus is prohibited. Thus, there is considerable protection provided to cuttlefish when they are on the breeding aggregation.

Once the cuttlefish hatch and move away from the breeding aggregation rocky reef area they are currently protected through a temporary closure on the targeting and taking of cuttlefish which applies to all waters north of -33.928° latitude. This protection is in place until at least 2016. This closure is important given the relatively small area (~6,466 km^2) that the northern cluster occurs in and the iconic status of this population which attracts international media attention and provides ecotourism opportunities for the Whyalla region.

The spatial age-structured population model and scenario testing of different threatening processes indicated that by-catch due to prawn trawling and cuttlefish harvesting away from the breeding aggregation (i.e. outside Marine Fishing Area 21) pose negligible risks to the population when viewed independently of other activities. The population viability could however be compromised by increased mortality of embryos on the aggregation site prior to hatching (e.g. due to hypersaline conditions resulting from desalination discharge if this brine were to reach the embryos) and increased adult mortality due to the reinstatement of commercial harvesting adjacent to the breeding grounds (i.e., inside Marine Fishing Area 21). It is also worth noting that when multiple threats (e.g. aggregation-site harvesting of cuttlefish and the impact of desalination discharge on embryos) were modelled they interacted to produce a negative population-level response that was greater than the sum of their parts. The continued closure of breeding aggregation area to cuttlefish harvesting is therefore expected to improve the viability of the northern Spencer Gulf population.

This population model can be used to test other potential threatening processes provided data are available for model parameters. If additional threats or combinations of threats are identified then further scenario testing could be undertaken as cumulative threatening processes may impact the viability of the northern Spencer Gulf population

Extension and Adoption

Updates on this project were disseminated through regular Giant Cuttlefish Working Group meetings and via the project Steering Committee. Both groups comprised key managers from PIRSA and DEWNR, and the working group had additional stakeholders including Environment Protection Authority, Department of State Development, Department of Planning Transport and Infrastructure, SA Tourism, SA Water, Whyalla City Council and the Conservation Council. The Spencer Gulf Ecosystem Development Initiative Board were also updated – this group comprised industry representatives including BHP Billiton, Santos, Arrium, Flinders Ports, as well as a community, fisheries and aquaculture representative.

A half day symposia was held at SARDI Aquatic Sciences (20 November 2015) where outcomes of all projects on cuttlefish were communicated to scientists, managers and other interested stakeholders (see Appendix B). In addition, results of this project have been communicated at a number of symposia/seminars including:

- Cephalopod International Advisory Council symposia, Hakodate, Japan, 6-14 November, 2015
- Academy of Technological Sciences and Engineering, SA Regional tour dinner, Whyalla, 17 October 2015
- Royal Society of South Australia seminar series, Adelaide, 13 August 2015
- International Otolith Symposia, Majorca, Spain, 20-24 October 2014
- Natural Resources Management Science Conference, Adelaide, 15-16 April 2014
- Australian Marine Science Association Annual Conference, Gold Coast, Queensland, 7-11 July 2013

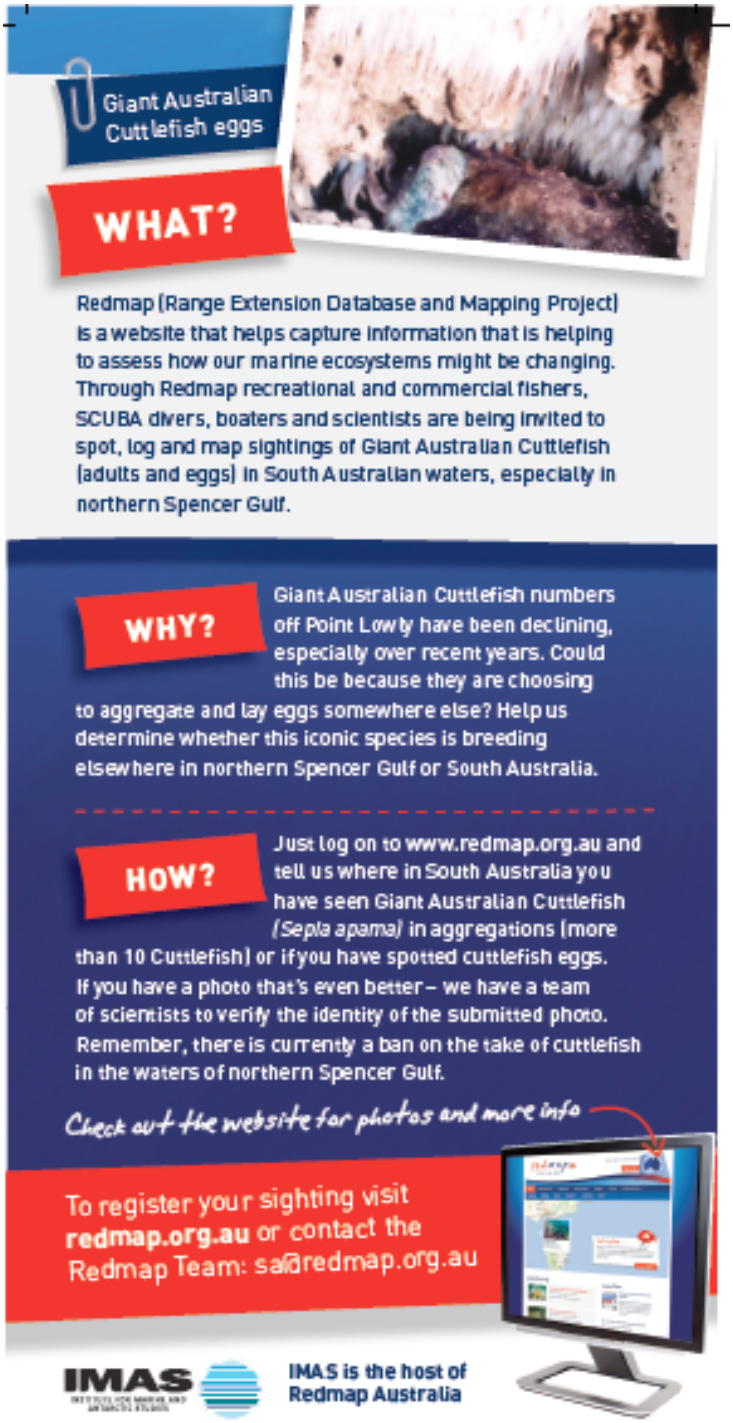
A brochure was also developed (see project materials developed) to engage citizen scientists to report potential aggregations of cuttlefish. This involved interactions with other stakeholders and the media (e.g. Public called on to help with cuttlefish research, University of Adelaide news and events, 3 May 2013). A presentation was provided to Fishcare Volunteers (November 2013) and the brochure was available at community events attended by Fishcare Volunteers.

Annual updates were provided through PIRSA's website on a dedicated "Cuttlefish update" webpage.

The project was communicated to the broader public through the Gillanders Aquatic Ecology Lab website: http://www.gillanderslab.org

Project Materials Developed

In order to determine if cuttlefish were aggregating elsewhere to breed we used two approaches to engage citizen scientists. The first was through the Conservation Council's Reef Watch program Feril or In Peril, where sightings of marine pests and species of conservation concern can be reported. The second approach was through RedMap (Range Extension Database and Mapping Project) where we developed a brochure to encourage citizen scientists to report if they saw groups of 10 or more cuttlefish in South Australian waters. Neither of these citizen science projects nor an extensive search of other reef habitat in Upper Spencer Gulf (Steer 2015) reported significant numbers of cuttlefish that would suggest they had moved elsewhere to breed.



The following journal article was the result of research partly funded via this FRDC project:

Prowse TAA, BM Gillanders, BW Brook, AJ Fowler, KC Hall, MA Steer, C Mellin, N Clisby, JA Tanner, TM Ward, DA Fordham. 2015. Evidence for a broad-scale decline in giant Australian cuttlefish (*Sepia apama*) abundance from non-targeted survey data. *Marine and Freshwater Research* 66: 692-700.

The journal article can be accessed at the following website:
http://www.publish.csiro.au/paper/MF14081.htm

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Appendices

Appendix A. Supplementary tables

Table S1. Specimens examined, genetic cluster identity and collection information. * in Genetic Cluster column indicates that cluster membership was inferred from collection location.

Sample ID	Region	RAD	DIET	Sex	Collection Date	Declat	Declong	Genetic Cluster
140714_5	USG	Y	-	M	14/07/2014	-32.997	137.720	northern
140714_6	USG	Y	-	M	14/07/2014	-32.997	137.720	northern
140714_7	USG	Y	-	M	14/07/2014	-32.997	137.720	northern
140715_14	USG	-	Y	M	15/07/2014	-32.997	137.720	northern*
140715_15	USG	-	Y	F	15/07/2014	-32.997	137.720	northern*
140715_19	USG	-	Y	M	15/07/2014	-32.997	137.720	northern*
140715_6	USG	-	Y	M	15/07/2014	-32.997	137.720	northern*
140718_1	USG	-	Y	M	15/07/2014	-32.997	137.720	northern*
130409_715	USG	-	Y	F	9/04/2013	-33.031	137.778	northern*
C91	USG	Y	-	-	01/05/2000	-33.036	137.702	northern
C94	USG	Y	-	-	01/05/2000	-33.036	137.702	northern
C98	USG	Y	-	-	01/05/2000	-33.036	137.702	northern
C100	USG	Y	-	-	01/05/2000	-33.036	137.702	northern
C101	USG	Y	-	-	01/05/2000	-33.036	137.702	northern
C113	USG	Y	-	-	01/05/2000	-33.036	137.702	northern
C120	USG	Y	-	-	01/05/2000	-33.036	137.702	northern
P185	USG	Y	-	-	01/04/2006	-33.036	137.702	northern
SG27	USG	Y	-	-	01/04/2005	-33.036	137.702	northern
WS7	USG	Y	-	-	01/04/2005	-33.036	137.702	northern
W38	USG	Y	-	-	01/05/1998	-33.036	137.702	northern
W40	USG	Y	-	-	01/05/1998	-33.036	137.702	northern
W44	USG	Y	-	-	01/05/1998	-33.036	137.702	northern
W55	USG	Y	-	-	01/05/1998	-33.036	137.702	northern
W63	USG	Y	-	-	01/05/1998	-33.036	137.702	northern
WS12	USG	Y	-	-	01/04/2005	-33.036	137.702	northern
130208_9	USG	Y	Y	M	8/02/2013	-33.044	137.694	northern
130208_14	USG	Y	Y	F	8/02/2013	-33.053	137.749	northern
130208_15	USG	Y	Y	M	8/02/2013	-33.053	137.749	northern
130208_16	USG	Y	Y	M	8/02/2013	-33.053	137.749	northern
130208_17	USG	Y	Y	F	8/02/2013	-33.053	137.749	northern
P187	USG	Y	-	M	1/04/2006	-33.098	137.736	northern
130209_83	USG	-	Y	F	9/02/2013	-33.128	137.720	northern*
130209_84	USG	-	Y	M	9/02/2013	-33.128	137.720	northern*
130209_85	USG	-	Y	F	9/02/2013	-33.128	137.720	northern*
130209_86	USG	-	Y	M	9/02/2013	-33.128	137.720	northern*
140228_46	USG	Y	Y	M	28/02/2014	-33.153	137.628	northern
140228_47	USG	Y	Y	M	28/02/2014	-33.153	137.628	northern
140228_49	USG	Y	Y	M	28/02/2014	-33.153	137.628	northern
140228_51	USG	Y	Y	F	28/02/2014	-33.153	137.628	northern

140228_48	USG	Y	-	M	28/02/2014	-33.153	137.628	northern
130208_55	USG	Y	Y	M	8/02/2013	-33.156	137.694	northern
130409_211	USG	Y	-	F	9/04/2013	-33.156	137.694	northern
130409_212	USG	Y	-	M	9/04/2013	-33.156	137.694	northern
130409_214	USG	Y	-	F	9/04/2013	-33.156	137.694	northern
130208_54	USG	Y	-	M	8/02/2013	-33.156	137.694	northern
131102_655	USG	-	Y	M	2/11/2013	-33.156	137.694	northern*
130208_30	USG	Y	Y	M	8/02/2013	-33.166	137.647	northern
130409_368	USG	Y	-	F	9/04/2013	-33.166	137.647	northern
130409_371	USG	Y	-	M	9/04/2013	-33.166	137.647	northern
130409_372	USG	Y	-	F	9/04/2013	-33.166	137.647	northern
130409_373	USG	Y	-	M	9/04/2013	-33.166	137.647	northern
130409_365	USG	-	Y	F	9/04/2013	-33.166	137.647	northern*
130409_367	USG	-	Y	M	9/04/2013	-33.166	137.647	northern*
130208_29	USG	-	Y	M	8/02/2013	-33.166	137.647	northern*
130409_26	USG	Y	-	F	9/04/2013	-33.198	137.641	northern
130208_23	USG	Y	-	F	8/02/2013	-33.214	137.608	northern
130410_653	USG	Y	Y	M	10/04/2013	-33.269	137.572	northern
130410_654	USG	Y	Y	M	10/04/2013	-33.269	137.572	northern
130410_665	USG	Y	Y	F	10/04/2013	-33.269	137.572	northern
130410_667	USG	Y	Y	F	10/04/2013	-33.269	137.572	northern
130208_11	USG	Y	Y	M	8/02/2013	-33.269	137.572	northern
130410_652	USG	Y	-	M	10/04/2013	-33.269	137.572	northern
130208_12	USG	Y	-	F	8/02/2013	-33.269	137.572	northern
130208_33	USG	Y	-	M	8/02/2013	-33.427	137.618	northern
PP3	USG	Y	-	-		-33.441	137.370	northern
PP4	USG	Y	-	-		-33.441	137.370	northern
130208_19	USG	Y	Y	M	8/02/2013	-33.469	137.589	northern
130208_21	USG	-	Y	F	8/02/2013	-33.469	137.589	northern*
130510_456	USG	Y	-	M	10/05/2013	-33.497	137.542	northern
130510_455	USG	-	Y	F	10/05/2013	-33.497	137.542	northern*
140501_87	USG	Y	Y	M	1/05/2014	-33.502	137.538	southern
140501_86	USG	Y	-	F	1/05/2014	-33.502	137.538	southern
140501_88	USG	-	Y	F	1/05/2014	-33.502	137.538	northern*
140301_84	USG	Y	Y	M	1/03/2014	-33.512	137.448	northern
140301_85	USG	Y	Y	M	1/03/2014	-33.512	137.448	southern
140301_87	USG	Y	Y	M	1/03/2014	-33.512	137.448	northern
130409_324	USG	Y	-	M	9/04/2013	-33.588	137.465	northern
130506_50	USG	Y	-	M	6/05/2013	-33.684	137.439	southern
130410_623	USG	Y	Y	F	10/04/2013	-33.685	137.454	northern
130410_621	USG	Y	-	F	10/04/2013	-33.685	137.454	northern
130410_624	USG	Y	-	M	10/04/2013	-33.685	137.454	northern
130410_622	USG	-	Y	F	10/04/2013	-33.685	137.454	northern*
130507_13	USG	Y	Y	M	7/05/2013	-33.685	137.390	southern
130410_209	USG	-	Y	F	10/04/2013	-33.700	137.591	southern*
130508_313	USG	Y	Y	F	8/05/2013	-33.754	137.562	southern
130410_258	USG	Y	-	M	10/04/2013	-33.779	137.557	southern
130506_114	USG	Y	-	F	6/05/2013	-33.780	137.502	southern
130507_105	USG	Y	Y	M	7/05/2013	-33.827	137.450	southern

130507_106	USG	Y	-	F	7/05/2013	-33.827	137.450	southern
SG4	USG	Y	-	-	Jan/Feb 05	-33.828	137.439	southern
SG2	USG	Y	-	-	Jan/Feb 05	-33.828	137.439	southern
P110	USG	Y	-	-	01/10/2005	-33.828	137.439	southern
SG49	USG	Y	-	-	01/08/2005	-33.828	137.439	southern
WA5	USG	Y	-	-	01/04/2005	-33.828	137.439	southern
WA16	USG	Y	-	-	01/04/2005	-33.828	137.439	northern
WA1	USG	Y	-	-	01/04/2005	-33.828	137.439	southern
SG17	USG	Y	-	-	01/04/2005	-33.828	137.439	southern
MB5	USG	Y	-	-	01/04/2005	-33.828	137.439	southern
SG15	USG	Y	-	-	01/02/2005	-33.828	137.439	southern
SG12	USG	Y	-	-	01/02/2005	-33.828	137.439	southern
SZ12	USG	Y	-	-	03/04/2004	-33.828	137.439	southern
130508_67	USG	Y	Y	F	8/05/2013	-33.829	137.439	southern
130508_69	USG	Y	Y	M	8/05/2013	-33.829	137.439	southern
130410_433	USG	Y	Y	M	10/04/2013	-33.841	137.250	southern
130410_434	USG	Y	-	M	10/04/2013	-33.841	137.250	southern
130507_201	USG	Y	Y	M	7/05/2013	-33.848	137.508	southern
130410_17	USG	Y	Y	F	10/04/2013	-33.893	137.234	southern
130409_360	USG	Y	Y	F	9/04/2013	-33.903	136.832	southern
130409_358	USG	Y	-	M	9/04/2013	-33.903	136.832	southern
130409_359	USG	Y	-	F	9/04/2013	-33.903	136.832	southern
130410_582	LSG	Y	Y	M	10/04/2013	-33.953	137.286	southern
130410_587	LSG	Y	Y	F	10/04/2013	-33.953	137.286	southern
130410_581	LSG	Y	-	M	10/04/2013	-33.953	137.286	southern
130410_583	LSG	Y	-	M	10/04/2013	-33.953	137.286	southern
130410_588	LSG	Y	-	M	10/04/2013	-33.953	137.286	southern
130208_76	LSG	Y	Y	M	8/02/2013	-33.997	136.691	southern
130409_679	LSG	-	Y	F	9/04/2013	-34.012	136.796	southern*
130409_680	LSG	-	Y	M	9/04/2013	-34.012	136.796	southern*
130409_681	LSG	-	Y	M	9/04/2013	-34.012	136.796	southern*
130409_682	LSG	-	Y	F	9/04/2013	-34.012	136.796	southern*
130409_685	LSG	-	Y	M	9/04/2013	-34.012	136.796	southern*
130409_687	LSG	-	Y	F	9/04/2013	-34.012	136.796	southern*
130409_688	LSG	-	Y	M	9/04/2013	-34.012	136.796	southern*
130409_689	LSG	-	Y	M	9/04/2013	-34.012	136.796	southern*
130514_336	LSG	Y	-	M	14/05/2013	-34.030	136.712	southern
140426_210	LSG	Y	Y	M	26/04/2014	-34.040	137.222	southern
140426_215	LSG	Y	Y	F	26/04/2014	-34.040	137.222	southern
140426_216	LSG	Y	Y	F	26/04/2014	-34.040	137.222	southern
140426_207	LSG	-	Y	M	26/04/2014	-34.040	137.222	southern*
140426_209	LSG	-	Y	M	26/04/2014	-34.040	137.222	southern*
140426_212	LSG	-	Y	F	26/04/2014	-34.040	137.222	southern*
140426_214	LSG	-	Y	F	26/04/2014	-34.040	137.222	southern*
140426_217	LSG	-	Y	M	26/04/2014	-34.040	137.222	southern*
130209_101	LSG	-	Y	F	9/02/2013	-34.093	136.946	southern*
130209_102	LSG	-	Y	M	9/02/2013	-34.093	136.946	southern*
130209_99	LSG	-	Y	M	9/02/2013	-34.093	136.946	southern*
130517_88	LSG	Y	Y	F	17/05/2013	-34.110	136.743	southern

130517_89	LSG	Y	-	F	17/05/2013	-34.110	136.743	southern
130208_36	LSG	Y	-	M	8/02/2013	-34.161	136.841	southern
130515_1	LSG	Y	-	F	15/05/2013	-34.247	137.009	southern
131102_103	LSG	-	Y	M	2/11/2013	-34.251	136.929	southern*
130514_38	LSG	Y	Y	F	14/05/2013	-34.265	136.934	southern
130514_37	LSG	Y	-	M	14/05/2013	-34.265	136.934	southern
130514_40	LSG	Y	-	M	14/05/2013	-34.265	136.934	southern
130514_39	LSG	-	Y	F	14/05/2013	-34.265	136.934	southern*
140403_6	LSG	-	Y	M	3/04/2014	-34.267	137.200	southern*
140403_7	LSG	-	Y	M	3/04/2014	-34.267	137.200	southern*
130516_54	LSG	Y	-	M	16/05/2013	-34.279	136.934	southern
130516_55	LSG	Y	-	M	16/05/2013	-34.279	136.934	southern
131202_26	LSG	-	Y	M	2/12/2013	-34.583	136.967	southern*
130410_3	LSG	Y	-	F	10/04/2013	-34.781	137.043	southern
C9	GSV	Y	-	-	Feb/Mar 1998	-35.025	138.455	southern
C7	GSV	Y	-	-	Feb/Mar 1998	-35.025	138.455	southern
C6	GSV	Y	-	-	02/03/1998	-35.025	138.455	southern
C19	GSV	Y	-	-	02/03/1998	-35.305	138.381	southern
C20	GSV	Y	-	-	02/03/1998	-35.305	138.381	southern
M28	GSV	Y	-	-	01/01/2005	-35.305	138.381	southern
M66	GSV	Y	-	-	Feb/March 05	-35.305	138.381	southern
M76	GSV	Y	-	-	May/June 2005	-35.085	137.770	southern
M81	GSV	Y	-	-	May/June 2005	-35.617	138.033	southern
P299	GSV	Y	-	-	Aug/Sep 06	-35.617	138.033	southern
P309	GSV	Y	-	-	Aug/Sep 06	-35.617	138.033	southern
P317	GSV	Y	-	-	Aug/Sep 06	-35.617	138.033	southern
SV19	GSV	Y	-	-	01/03/2005	-35.085	137.770	southern
SV16	GSV	Y	-	-	01/03/2005	-35.085	137.770	southern

Table S2. Reference sequence database for *16S rRNA* gene.

Genus	**species**	**Taxonomic Group**	**Accession Number**	**Gene**
Acanthaluteres	*spilomelanurus*	Fish	KR153514	16S
Acanthaluteres	*vittiger*	Fish	EU848434.1	16S
Alpheus	*lottini*	Crustacean	KR153529	16S
Alpheus	*villosus*	Crustacean	KR153535	16S
Ammotretis	*rostratus*	Fish	EU848450.1	16S
Aptychotrema	*vincentiana*	Fish	KR153504	16S
Aracana	*aurita*	Fish	KR153509	16S
Aracana	*ornata*	Fish	AY679662.1	16S
Arripis	*truttaceus*	Fish	KR153522	16S
Arripis	*truttacea*	Fish	HM755836.1	16S
Arripis	*trutta*	Fish	HM755838.1	16S
Arripis	*georgianus*	Fish	HM755839.1	16S
Asymbolus	*vincenti*	Fish	KR153524	16S
Aulopus	*purpurissatus*	Fish	AF049732.1	16S

Austrolabrus	*maculatus*	Fish	AY279680.1	16S
Belosquila	*laevis*	Crustacean	KR153534	16S
Brachaluteres	*jacksonianus*	Fish	AY679651.1	16S
Callorhinchus	*milii*	Fish	JN703267.1	16S
Centroberyx	*affinis*	Crustacean	KR153488	16S
Centroberyx	*lineatus?*	Crustacean	KR153519	16S
Cheilodactylus	*nigripes*	Crustacean	KR153503	16S
Chelmonops	*curiosus*	Crustacean	KR153501	16S
Cnidoglanis	*macrocephalus*	Fish	KR153526	16S
Conger	*myriaster*	Fish	AB617702.1	16S
Conger	*japonicus*	Fish	KF681818.1	16S
Conger	*oceanicus*	Fish	AJ244815.1	16S
Conger	*oceanicus*	Fish	AJ244814.1	16S
Contusus	species	Fish	KR153533	16S
Contusus	species	Fish	KR153536	16S
Cristiceps	*australis*	Fish	KR153502	16S
Cynoglossus	*broadhursti*	Fish	KR153527	16S
Dasyatis	*brevicaudata*	Fish	EU848428.1	16S
Diodon	*nicthemurus*	Fish	KR153496	16S
Diodora	*listeri*	Mollusc	HM771540.1	16S
Diodora	*lineata*	Mollusc	HM771542.1	16S
Diodora	*ticaonica*	Mollusc	HM771543.1	16S
Dosinia	*victoriae*	Mollusc	DQ459271.1	16S
Engraulis	*australis*	Fish	GQ412304.1	16S
Engraulis	*australis*	Fish	GQ365274.1	16S
Enoplosus	*armatus*	Fish	AP006008.1	16S
Enoplosus	*armatus*	Fish	DQ532873.1	16S
Equichlamys	*bifrons*	Mollusc	HM561992.1	16S
Equichlamys	*bifrons*	Mollusc	HM561996.1	16S
Eubalichthys	*mosaicus*	Fish	AP009217.1	16S
Eubalichthys	*mosaicus*	Fish	NC_011953.1	16S
Foetorepus	*calauropomus*	Fish	KR153498	16S
Genypterus	*tigerinus*	Fish	KR153516	16S
Glyptauchen	*panduratus*	Fish	KR153495	16S
Gonorynchus	*greyi*	Fish	AB054134.1	16S
Gonorynchus	*greyi*	Fish	NC_004702.1	16S
Gymnapistes	*marmoratus*	Fish	AY538994.2	16S
Heteroclinus	species	Fish	KR153500	16S
Heterodontus	*portusjacksoni*	Fish	EU848461.1	16S
Hippocampus	*abdominalis*	Fish	AF355013.1	16S
Histiophryne	*cryptacanthus*	Fish	GQ981542.1	16S
Histiophryne	*cryptacanthus*	Fish	FJ219606.1	16S
Histiophryne	*cryptacanthus*	Fish	JN835577.1	16S
Hyperlophus	*vittatus*	Fish	EU848444.1	16S
Hyperlophus	*vittatus*	Fish	EU552752.1	16S

Hyperlophus	*vittatus*	Fish	EU552751.1	16S
Hypselognathus	*rostratus*	Fish	AF355020.1	16S
Ibacus	*peronii*	Crustacean	JN701700.1	16S
Ibacus	*peronii*	Crustacean	JN701699.1	16S
Kanekonia	*queenslandica*	Fish	KR153494	16S
Kathetostoma	*laeve*	Fish	KR153507	16S
Lamarckdromia	*globosa*	Crustacean	KR153530	16S
Lepas	*anserifera*	Crustacean	GU993686.1	16S
Lepas	*anserifera*	Crustacean	GU993685.1	16S
Lepidotrigla	*papilio*	Fish	EU848437.1	16S
Lepidotrigla	*spinosa*	Fish	AY539001.2	16S
Leviprora	*inops*	Fish	KR153537	16S
Lophonectes	*gallus*	Fish	EU848459.1	16S
Maxillicosta	*scabriceps*	Fish	KR153499	16S
Melicertus	*latisulcatus*	Crustacean	EF589710.1	16S
Melicertus	*latisulcatus*	Crustacean	JF338895.1	16S
Melicertus	*latisulcatus*	Crustacean	EF589710.1	16S
Metapenaeopsis	*provocatoria*	Crustacean	AF105048.1	16S
Metapenaeopsis	*palmensis*	Crustacean	AF105046.1	16S
Metapenaeopsis	*liui*	Crustacean	AF105044.1	16S
Metapenaeopsis	*lamellata*	Crustacean	AF105043.1	16S
Metapenaeopsis	*commensalis*	Crustacean	AF105042.1	16S
Metapenaeopsis	*barbata*	Crustacean	AF105041.1	16S
Meuschenia	*scaber*	Fish	EU848462.1	16S
Mimachlamys	*asperrima*	Mollusc	HM540085.1	16S
Mimachlamys	*asperrima*	Mollusc	JF339126.1	16S
Mustelus	*antarcticus*	Fish	EU848452.1	16S
Myliobatis	*australis*	Fish	EU848472.1	16S
Naxia	*aries*	Crustacean	KR153531	16S
Nelusetta	*ayraudi*	Fish	NC_011921.1	16S
Nelusetta	*ayraudi*	Fish	AY679654.1	16S
Nemadactylus	*valenciennesi*	Fish	EU848449.1	16S
Neoodax	*balteatus*	Fish	AY279760.1	16S
Neoplatycephalus	*richardsoni*	Fish	EU848464.1	16S
Neosebastes	*bougainvilllii*	Fish	KR153497	16S
Neosebastes	*pandus*	Fish	DQ532917.1	16S
Nototodarus	*gouldi*	Mollusc	GQ365351.1	16S
Nototodarus	*gouldi*	Mollusc	GQ365352.1	16S
Octopus	*australis*	Mollusc	KR153532	16S
Octopus	*pallidus*	Mollusc	AJ252754.1	16S
Octopus	*berrima*	Mollusc	AY545105.1	16S
Octopus	*kaurna*	Mollusc	AY545106.1	16S
Odax	*acroptilus*	Fish	AY279761.1	16S
Omegophora	*armilla*	Fish	NC_015349.1	16S
Omegophora	*armilla*	Fish	AP011936.1	16S

Oplegnathus	*woodwardi*	Fish	DQ532924.1	16S
Orectolobus	*maculatus*	Fish	EU848431.1	16S
Ostrea	*angasi*	Mollusc	AF540594.1	16S
Ostrea	*angasi*	Mollusc	AF052063.1	16S
Pagrus	*auratus*	Fish	AF247424.1	16S
Pandalus	*montagui*	Crustacean	EU868698.1	16S
Pandalus	*latirostris*	Crustacean	AB244633.1	16S
Parapercis	*haackei*	Crustacean	KR153523	16S
Parapercis	*ramsayi*	Fish	AY539067.2	16S
Parapriacanthus	*elongatus*	Fish	GQ412299.1	16S
Parascyllium	*ferrogineum*	Fish	KR153489	16S
Parazanclistius	*hutchinsi*	Fish	KR153487	16S
Parequula	*melbournensis*	Fish	GQ412292.1	16S
Parequula	*melbournensis*	Fish	EU848467.1	16S
Pecten	*fumatus*	Mollusc	JF339109.1	16S
Pecten	*fumatus*	Mollusc	JF339110.1	16S
Pelates	*octolineatus*	Fish	GU205407.1	16S
Pempheris	*multiradiata?*	Fish	KR153506	16S
Pempheris	species	Fish	KR153508	16S
Penaeus	*latisulcatus*	Crustacean	KR153528	16S
Pentaceropsis	*recurvirostris*	Fish	KR153505	16S
Phycodurus	*eques*	Fish	GU182926.1	16S
Phycodurus	*eques*	Fish	GU182927.1	16S
Phyllophryne	*scortea*	Fish	GQ981556.1	16S
Phyllophryne	*scortea*	Fish	GQ981557.1	16S
Phyllopteryx	*taeniolatus*	Fish	AF355027.1	16S
Phyllopteryx	*taeniolatus*	Fish	AF355028.1	16S
Pictilabrus	*laticlavius*	Fish	AY279736.1	16S
Pilumnus	*sayi*	Crustacean	GU144435.1	16S
Pilumnus	*vespertilio*	Crustacean	FJ548952.1	16S
Pinna	*bicolor*	Mollusc	JN182779.1	16S
Platycephalus	*speculator*	Fish	KR153520	16S
Polyspina	*piosae*	Fish	NC_015339.1	16S
Polyspina	*piosae*	Fish	AP011913.1	16S
Portunus	*pelagicus*	Crustacean	DQ388052.1	16S
Portunus	*pelagicus*	Crustacean	FJ152161.1	16S
Pristiophorus	*cirratus*	Fish	KR153490	16S
Pristiophorus	*nudipinnis*	Fish	AY147885.1	16S
Pseudocaranx	*wrighti*	Fish	GQ412291.1	16S
Pseudophycis	*bachus*	Fish	EU848440.1	16S
Pseudorhombus	*jenynsii*	Fish	KR153521	16S
Pterygotrigla	*polyommata*	Fish	KR153515	16S
Repomucenus	*calcaratus*	Fish	KR153512	16S
Rhycherus	*filamentosus*	Fish	GQ981552.1	16S
Rhycherus	*filamentosus*	Fish	GQ981553.1	16S

Sardinops	*neopilchardus*	Fish	EU848443.1	16S
Scobinichthys	*granulatus*	Fish	AP009228.1	16S
Scobinichthys	*granulatus*	Fish	NC_011926.1	16S
Sepia	*apama*	Mollusc	AB675086.1	16S
Sepia	*apama*	Mollusc	AY616977.1	16S
Sepia	*apama*	Mollusc	NC_022466.1	16S
Sepia	*officinalis*	Mollusc	AB193804.1	16S
Sepiadarium	*austrinum*	Mollusc	AY616976.1	16S
Sepioloidea	*lineolata*	Mollusc	AY616975.1	16S
Sepioteuthis	*australis*	Mollusc	AB675085.1	16S
Seriolella	*brama*	Fish	AB205417.1	16S
Seriolella	*brama*	Fish	EU848466.1	16S
Seriolella	*brama*	Fish	HQ592207.1	16S
Sillaginodes	*punctatus*	Fish	EU848447.1	16S
Sillago	*bassensis*	Fish	GQ412300.1	16S
Siphonognathus	*argyrophanes*	Fish	AY279765.1	16S
Sphyraena	*novaehollandiae*	Fish	KR153517	16S
Sphyraena	*obtusata*	Fish	KR153518	16S
Spratelloides	*robustus*	Fish	GQ412293.1	16S
Spratelloides	*robustus*	Fish	EU552786.1	16S
Squalus	*megalops*	Fish	GU130625.1	16S
Squatina	*australis*	Fish	FN431819.1	16S
Squatina	*australis*	Fish	FN431818.1	16S
Squatina	*australis*	Fish	FN431817.1	16S
Stigmatopora	*argus*	Fish	JN662994.1	16S
Stigmatopora	*argus*	Fish	AF355014.1	16S
Stigmatopora	*argus*	Fish	JN663000.1	16S
Stigmatopora	*argus*	Fish	JN663004.1	16S
Sutorectus	*tentaculatus*	Fish	KR153525	16S
Tetractenos	*glaber*	Fish	NC_015347.1	16S
Tetractenos	*glaber*	Fish	AB742037.1	16S
Tetractenos	*glaber*	Fish	AY679673.1	16S
Thamnaconus	*degeni*	Fish	KR153513	16S
Thysanophrys	*cirronasus*	Fish	KR153511	16S
Torquigener	*pleurogramma*	Fish	NC_015367.1	16S
Torquigener	*pleurogramma*	Fish	AY679674.1	16S
Trachichthyidae	*environmental*	Fish	GQ365275.1	16S
Trachurus	*novaezelandiae*	Fish	DQ660425.1	16S
Trachurus	*novaezelandiae*	Fish	DQ660424.1	16S
Trichomya	*hirsuta*	Mollusc	GQ472163.1	16S
Upeneichthys	*vlamingii*	Fish	EU848456.1	16S
Urolophus	*cruciatus*	Fish	EU848454.1	16S
Vincentia	*conspersa*	Fish	KR153491	16S
Vincentia	*badia*	Fish	KR153492	16S
Vincentia	*conspersa*	Fish	KR153493	16S

Zebrias	*scalaris*	Fish	KR153510	16S

Table S3. Reference sequence database for *18S rRNA* gene.

Genus	species	Taxonomic Group	Accession Number	Gene
Acanthaluteres	*spilomelanurus*	Fish	KM588849	18S
Acanthaluteres	*vittiger*	Fish	KM588859	18S
Alpheus	*packardii*	Crustacean	EU868720.1	18S
Alpheus	*vanderbilti*	Crustacean	EU868730.1	18S
Alpheus	*gracilipes*	Crustacean	GQ131935.1	18S
Ammotretis	*rostratus*	Fish	HQ615528.1	18S
Aracana	*aurita*	Fish	KM588857	18S
Arripis	*truttaceus*	Fish	HQ615529.1	18S
Barbatia	*barbata*	Mollusc	AF207646.1	18S
Barbatia	*lacerata*	Mollusc	JN974509.1	18S
Barbatia	*lima*	Mollusc	JN974512.1	18S
Barbatia	*virescens*	Mollusc	JN974524.1	18S
Barbatia	*fusca*	Mollusc	JN974526.1	18S
Brachaluteres	*jacksonianus*	Fish	KM588858	18S
Calliostoma	*sakashitai*	Mollusc	AB365306.1	18S
Calliostoma	*javanicum*	Mollusc	EU530068.1	18S
Calliostoma	*consors*	Mollusc	EU530069.1	18S
Calliostoma	*zizyphinum*	Mollusc	EU530070.1	18S
Calliostoma	*antonii*	Mollusc	GQ160796.1	18S
Calliostoma	*iridium*	Mollusc	HE800785.1	18S
Calliostoma	*granulatum*	Mollusc	DQ093434.1	18S
Callorhinchus	*milii*	Fish	AY049813.1	18S
Centroberyx	*lineatus?*	Crustacean	KM588861	18S
Centroberyx	*affinis*	Crustacean	KM588888	18S
Chelmonops	*curiosus*	Crustacean	KM588850	18S
Clanculus	*cruciatus*	Mollusc	AF120514.1	18S
Clanculus	*atropurpureus*	Mollusc	EU530079.1	18S
Cleidothaerus	*albidus*	Mollusc	AY192690.1	18S
Cnidoglanis	*macrocephalus*	Fish	KM588845	18S
Contusus	species?	Fish	KM588869	18S
Contusus	species?	Fish	KM588886	18S
Cristiceps	*australis*	Fish	KM588837	18S
Cynoglossus	*semilaevis*	Fish	JN211973.1	18S
Cynoglossus	*broadhursti*	Fish	KM588833	18S
Dasyatis	*bennetti*	Fish	JN211927.1	18S
Diodon	*nicthemurus*	Fish	KM588878	18S
Diodora	*cayenensis*	Mollusc	L78884.1	18S
Diodora	*dysoni*	Mollusc	FJ977638.1	18S

Diodora	*listeri*	Mollusc	HM771452.1	18S
Diodora	*lineata*	Mollusc	HM771456.1	18S
Diodora	*ticaonica*	Mollusc	HM771457.1	18S
Diodora	*singaporensis*	Mollusc	HM771458.1	18S
Diodora	*graecae*	Mollusc	AY923877.1	18S
Dosinia	*discus*	Mollusc	L78863.1	18S
Emmelichthys	*nitidus?*	Fish	KM588830	18S
Engraulis	*australis*	Fish	KM588865	18S
Enoplosus	*armatus*	Fish	KM588881	18S
Eubalichthys	*mosaicus*	Fish	KM588844	18S
Eucrassatella	*donacina*	Mollusc	AJ581873.1	18S
Eucrassatella	*cumingi*	Mollusc	KC429350.1	18S
Foetorepus	*calauropomus*	Fish	KM588877	18S
Fusinus	*longicaudus*	Mollusc	HQ834039.1	18S
Genypterus	*tigerinus*	Fish	KM588874	18S
Glycymeris	*pedunculata*	Mollusc	AJ389631.1	18S
Glycymeris	sp.	Mollusc	X91978.1	18S
Glycymeris	*insubrica*	Mollusc	AF207647.1	18S
Glycymeris	*glycymeris*	Mollusc	KC429328.1	18S
Glyptauchen	*panduratus*	Fish	KM588832	18S
Gonorynchus	*greyi*	Fish	KM588846	18S
Gymnapistes	*marmoratus*	Fish	KM588843	18S
Heteroclinus	species?	Fish	KM588876	18S
Hyperlophus	*vittatus*	Fish	HQ615538.1	18S
Hyporhamphus	*regularis*	Fish	FJ710901.1	18S
Hypselognathus	*rostratus*	Fish	KM588868	18S
Ibacus	*chacei*	Crustacean	JN701609.1	18S
Ibacus	*peronii*	Crustacean	JN701611.1	18S
Ischnochiton	*yerburyi*	Mollusc	JQ339720.1	18S
Ischnochiton	*comptus*	Mollusc	AY377639.1	18S
Ischnochiton	*rissoi*	Mollusc	AY377640.1	18S
Ischnochiton	*australis*	Mollusc	AY377641.1	18S
Ischnochiton	*elongatus*	Mollusc	AY377642.1	18S
Kathetostoma	*laeve*	Fish	KM588866	18S
Lepas	*testudinata*	Crustacean	EU082406.1	18S
Lepas	*anatifera*	Crustacean	FJ906772.1	18S
Lepas	*anserifera*	Crustacean	FJ906775.1	18S
Lepas	*australis*	Crustacean	FJ906777.1	18S
Lepidotrigla	*papilio*	Fish	KM588847	18S
Lepidotrigla	*spinosa*	Fish	KM588871	18S
Leptomithrax	*sternocostulatus*	Crustacean	FJ812346.1	18S
Leviprora	*inops*	Fish	KM588853	18S
Lima	*lima*	Mollusc	AF120533.1	18S
Malleus	*albus*	Mollusc	HQ329350.1	18S
Malleus	*candeanus*	Mollusc	HQ329351.1	18S

Malleus	*regula*	Mollusc	HQ329353.1	18S
Malleus	*malleus*	Mollusc	AB594370.1	18S
Malleus	*irregularis*	Mollusc	AB594375.1	18S
Maxillicosta	*scabriceps*	Fish	KM588875	18S
Meuschenia	*scaber*	Fish	KM588883	18S
Mimachlamys	*varia*	Mollusc	L49051.1	18S
Mimachlamys	*nobilis*	Mollusc	JN974533.1	18S
Mustelus	*antarcticus*	Fish	KM588834	18S
Neoodax	*balteatus*	Fish	KM588880	18S
Neoplatycephalus	*richardsoni*	Fish	KM588884	18S
Neosebastes	*bougainvillii*	Fish	KM588831	18S
Nyctiphanes	*simplex*	Crustacean	DQ900732.1	18S
Octopus	*berrima*	Mollusc	KM588829	18S
Odax	*acroptilus*	Fish	KM588870	18S
Omegophora	*armilla*	Fish	KM588873	18S
Oplegnathus	*woodwardi*	Fish	KM588863	18S
Orectolobus	*ornatus*	Fish	AY049843.1	18S
Ostrea	*edulis*	Mollusc	EU660787.1	18S
Ostrea	*chilensis*	Mollusc	EU660789.1	18S
Pagrus	*auratus*	Fish	KM588841	18S
Paguristes	*cadenati*	Crustacean	JN800614.1	18S
Paguristes	*sericeus*	Crustacean	KF182486.1	18S
Paguristes	*puncticeps*	Crustacean	KF182487.1	18S
Paguristes	*grayi*	Crustacean	KF182488.1	18S
Paguristes	*triangulatus*	Crustacean	KF182489.1	18S
Pandalus	*montagui*	Crustacean	EU868792.1	18S
Parapercis	*haackei*	Fish	KM588835	18S
Parascyllium	*ferrogineum*	Fish	KM588882	18S
Parequula	*melbournensis*	Fish	KM588852	18S
Pecten	*maximus*	Mollusc	EU660803.1	18S
Pecten	*jacobaeus*	Mollusc	EU660806.1	18S
Pelates	*octolineatus*	Fish	KM588848	18S
Pempheris	*ornata?*	Fish	KM588864	18S
Pilumnus	*floridanus*	Crustacean	HM638020.1	18S
Pinna	*muricata*	Mollusc	AJ389636.1	18S
Pinna	*carnea*	Mollusc	KC429337.1	18S
Portunus	*sanguinolentus*	Crustacean	EU284152.1	18S
Portunus	*pelagicus*	Crustacean	FJ812347.1	18S
Portunus	*trituberculatus*	Crustacean	KF266707.1	18S
Pristiophorus	*cirratus*	Fish	AY049849.1	18S
Processa	*guyanae*	Crustacean	EU868802.1	18S
Processa	*japonica*	Crustacean	GQ131926.1	18S
Pseudorhombus	*jenynsii*	Fish	KM588842	18S
Sassia	*remensa*	Mollusc	JX241140.1	18S
Scomber	*japonicus*	Fish	JN211936.1	18S

Scomber	*japonicus*	Fish	JN211941.1	18S
Sepia	*elegans*	Mollusc	AF120507.1	18S
Sepia	*officinalis*	Mollusc	AJ606935.1	18S
Sepia	*officinalis*	Mollusc	AY557471.1	18S
Sepioteuthis	*lessoniana*	Mollusc	AY557480.1	18S
Seriolella	*brama*	Fish	KM588872	18S
Sillaginodes	*punctata*	Fish	KM588838	18S
Sillago	*asiatica*	Fish	JN211852.1	18S
Sillago	*asiatica*	Fish	JN211853.1	18S
Sorosichthys	*ananassa*	Fish	KM588887	18S
Sphyraena	*novaehollandiae*	Fish	KM588836	18S
Sphyraena	*obtusata*	Fish	KM588862	18S
Squalus	*acanthias*	Fish	M91179.1	18S
Squatina	*californica*	Fish	AY049858.1	18S
Stigmatopora	*argus*	Fish	KM588867	18S
Sutorectus	*tentaculatus*	Fish	KM588851	18S
Thamnaconus	*modestoides*	Fish	JN211768.1	18S
Thamnaconus	*degeni*	Fish	KM588860	18S
Thyrsites	*atun*	Fish	KM588889	18S
Meuschenia	*freycineti*	Fish	KM588885	18S
Torquigener	*pleurogramma*	Fish	KM588840	18S
Trachurus	*novaezelandiae*	Fish	KM588839	18S
Trachurus	*declivis*	Fish	KM588854	18S
Trichomya	*hirsuta*	Mollusc	EU289830.1	18S
Tugali	*gigas*	Mollusc	AF335561.1	18S
Tugali	*parmophoidea*	Mollusc	HM771444.1	18S
Vincentia	*conspersa*	Fish	KM588855	18S
Vincentia	*badia*	Fish	KM588856	18S
Vincentia	*conspersa*	Fish	KM588879	18S
Zebrias	*zebra*	Fish	EF126044.1	18S

Table S4. PCR and sequencing primers for metagenomic analysis with *16S rRNA* gene.

16S	
Forward PCR primers	
16Sar.SA501	AATGATACGGCGACCACCGAGATCTACACATCGTACGTCATCCATGCTGCGCCTGTTTATCAAAAACAT
16Sar.SA502	AATGATACGGCGACCACCGAGATCTACACACTATCTGTCATCCATGCTGCGCCTGTTTATCAAAAACAT
16Sar.SA503	AATGATACGGCGACCACCGAGATCTACACTAGCGAGTTCATCCATGCTGCGCCTGTTTATCAAAAACAT
16Sar.SA504	AATGATACGGCGACCACCGAGATCTACACCTGCGTGTTCATCCATGCTGCGCCTGTTTATCAAAAACAT
16Sar.SA505	AATGATACGGCGACCACCGAGATCTACACTCATCGAGTCATCCATGCTGCGCCTGTTTATCAAAAACAT
16Sar.SA506	AATGATACGGCGACCACCGAGATCTACACCGTGAGTGTCATCCATGCTGCGCCTGTTTATCAAAAACAT
16Sar.SA507	AATGATACGGCGACCACCGAGATCTACACGGATATCTTCATCCATGCTGCGCCTGTTTATCAAAAACAT
16Sar.SA508	AATGATACGGCGACCACCGAGATCTACACGACACCGTTCATCCATGCTGCGCCTGTTTATCAAAAACAT
Reverse PCR primers	
16SMarR.SA701	CAAGCAGAAGACGGCATACGAGATAACTCTCGATCCTGCAATGACTYHAYAGGGTCTTCTCGTC
16SMarR.SA702	CAAGCAGAAGACGGCATACGAGATACTATGTCATCCTGCAATGACTYHAYAGGGTCTTCTCGTC
16SMarR.SA703	CAAGCAGAAGACGGCATACGAGATAGTAGCGTATCCTGCAATGACTYHAYAGGGTCTTCTCGTC
16SMarR.SA704	CAAGCAGAAGACGGCATACGAGATCAGTGAGTATCCTGCAATGACTYHAYAGGGTCTTCTCGTC
16SMarR.SA705	CAAGCAGAAGACGGCATACGAGATCGTACTCAATCCTGCAATGACTYHAYAGGGTCTTCTCGTC
16SMarR.SA706	CAAGCAGAAGACGGCATACGAGATCTACGCAGATCCTGCAATGACTYHAYAGGGTCTTCTCGTC
16SMarR.SA707	CAAGCAGAAGACGGCATACGAGATGGAGACTAATCCTGCAATGACTYHAYAGGGTCTTCTCGTC
16SMarR.SA708	CAAGCAGAAGACGGCATACGAGATGTCGCTCGATCCTGCAATGACTYHAYAGGGTCTTCTCGTC
16SMarR.SA709	CAAGCAGAAGACGGCATACGAGATGTCGTAGTATCCTGCAATGACTYHAYAGGGTCTTCTCGTC
16SMarR.SA710	CAAGCAGAAGACGGCATACGAGATTAGCAGACATCCTGCAATGACTYHAYAGGGTCTTCTCGTC
16SMarR.SA711	CAAGCAGAAGACGGCATACGAGATTCATAGACATCCTGCAATGACTYHAYAGGGTCTTCTCGTC
16SMarR.SA712	CAAGCAGAAGACGGCATACGAGATTCGCTATAATCCTGCAATGACTYHAYAGGGTCTTCTCGTC
Forward Read primer	
16Sar.R	TCATCCATGCTGCGCCTGTTTATCAAAAACAT
Reverse Read primer	
16SMar.R	ATCCTGCAATGACTYHAYAGGGTCTTCTCGTC

Reverse Index-Read primer	
16SMar.IR	GACGAGAAGACCCTRTDRAGTCATTGCAGGAT

Table S5. PCR and sequencing primers for metagenomic analysis with *18S rRNA* gene.

18S	
Forward PCR primers	
18SMarF.SA501	AATGATACGGCGACCACCGAGATCTACACCTACTATAGGATGATATTTGTGGCCGTTCTTAGTTGGTGGAG
18SMarF.SA502	AATGATACGGCGACCACCGAGATCTACACCGTTACTAGGATGATATTTGTGGCCGTTCTTAGTTGGTGGAG
18SMarF.SA503	AATGATACGGCGACCACCGAGATCTACACAGAGTCACGGATGATATTTGTGGCCGTTCTTAGTTGGTGGAG
18SMarF.SA504	AATGATACGGCGACCACCGAGATCTACACTACGAGACGGATGATATTTGTGGCCGTTCTTAGTTGGTGGAG
18SMarF.SA505	AATGATACGGCGACCACCGAGATCTACACACGTCTCGGGATGATATTTGTGGCCGTTCTTAGTTGGTGGAG
18SMarF.SA506	AATGATACGGCGACCACCGAGATCTACACTCGACGAGGGATGATATTTGTGGCCGTTCTTAGTTGGTGGAG
18SMarF.SA507	AATGATACGGCGACCACCGAGATCTACACGATCGTGTGGATGATATTTGTGGCCGTTCTTAGTTGGTGGAG
18SMarF.SA508	AATGATACGGCGACCACCGAGATCTACACGTCAGATAGGATGATATTTGTGGCCGTTCTTAGTTGGTGGAG
Reverse PCR primers	
18SMarR.SA701	CAAGCAGAAGACGGCATACGAGATAAGTCGAGTACGTGCCATATGGGCATCACAGACCTGTTAT
18SMarR.SA702	CAAGCAGAAGACGGCATACGAGATATACTTCGTACGTGCCATATGGGCATCACAGACCTGTTAT
18SMarR.SA703	CAAGCAGAAGACGGCATACGAGATAGCTGCTATACGTGCCATATGGGCATCACAGACCTGTTAT
18SMarR.SA704	CAAGCAGAAGACGGCATACGAGATCATAGAGATACGTGCCATATGGGCATCACAGACCTGTTAT
18SMarR.SA705	CAAGCAGAAGACGGCATACGAGATCGTAGATCTACGTGCCATATGGGCATCACAGACCTGTTAT
18SMarR.SA706	CAAGCAGAAGACGGCATACGAGATCTCGTTACTACGTGCCATATGGGCATCACAGACCTGTTAT
18SMarR.SA707	CAAGCAGAAGACGGCATACGAGATGCGCACGTTACGTGCCATATGGGCATCACAGACCTGTTAT
18SMarR.SA708	CAAGCAGAAGACGGCATACGAGATGGTACTATTACGTGCCATATGGGCATCACAGACCTGTTAT
18SMarR.SA709	CAAGCAGAAGACGGCATACGAGATGTATACGCTACGTGCCATATGGGCATCACAGACCTGTTAT
18SMarR.SA710	CAAGCAGAAGACGGCATACGAGATTACGAGCATACGTGCCATATGGGCATCACAGACCTGTTAT

18SMarR.SA711	CAAGCAGAAGACGGCATACGAGATTCAGCGTTTACGTGCCATATGGGCATCACAGACCTGTTAT
18SMarR.SA712	CAAGCAGAAGACGGCATACGAGATTCGCTACGTACGTGCCATATGGGCATCACAGACCTGTTAT
Forward Read primer	
18SMarF.R	GGATGATATTTGTGGCCGTTCTTAGTTGGTGGAG
Reverse Read primer	
18SMarR.R	TACGTGCCATATGGGCATCACAGACCTGTTAT
Reverse Index-Read primer	
18SMarR.IR	ATAACAGGTCTGTGATGCCCATATGGCACGTA

Appendix B. Giant Australian cuttlefish symposia

The following pages contain the agenda, key messages from each talk and list of attendees.

Appendix C. List of researchers and project staff

- Professor Bronwyn Gillanders, University of Adelaide
- Professor Stephen Donnellan, SA Museum
- Dr Thomas Prowse, University of Adelaide
- Dr Damien Fordham, University of Adelaide
- Dr Mike Steer, SARDI Aquatic Sciences
- Dr Skye Woodcock, University of Adelaide
- Dr Steven Myers, University of Adelaide
- Dr Chris Izzo, University of Adelaide
- Dr Jacob Johansen, University of Adelaide
- Dr Keith Rowling, Primary Industries and Regions South Australia

GIANT AUSTRALIAN CUTTLEFISH SYMPOSIUM

When: Friday 20th November 2015 (13:00 – 17:00)
Where: SARDI (Aquatic Sciences) – Lecture Theatre
2 Hamra Ave, West Beach, 5024

BACKGROUND

A number of research projects have been undertaken to investigate concerns about a significant decline in Giant Australian Cuttlefish spawning population at Point Lowly. While the exact cause of the decline remains difficult to identify, the research to date has been valuable in eliminating possible causes and progressing our knowledge of this species. This symposium will synthesize the results of these studies.

13:00 – 13:10 **INTRODUCTION**

13:10 – 13:30 **WHAT'S GOING ON WITH THE CUTTLEFISH POPULATION?** (*Mike Steer -SARDI*)

- General history of the spawning population
- Biology & ecology
- Knowledge gaps.

13:30 – 13:45 **IS POLLUTION A POTENTIAL DRIVER?** (*Sam Gaylard – EPA)*

- Ambient water quality.
- Heavy metals.
- Noise pollution.

13:45 – 14:00 **WHAT IS THE EXTENT OF FISHING ON THE AGGREGATION?** (*Craig Noell – SARDI)*

- Commercial/Recreational sector.
- By-catch.
- Mitigation strategies.

14:00 – 14:15 **WHAT ABOUT THE ENVIRONMENTAL EFFECTS?** (*Bronwyn Gillanders – UoA)*

- Effects of temperature/salinity.
- Field evidence.
- Global trends.

14:15 – 14:30 **WHAT IS THE SCALE OF THE ISSUE?** (*Steve Donnellan – SA Museum)*

- Taxonomic resolution.
- Extent of the population (movement & migration).

14:30 – 14:45 *AFTERNOON TEA*

14:45 – 15:00 **HOW VIABLE IS THE SPAWNING POPULATION?** (*Thomas Prowse – UoA)*

- Population modeling, scenario testing.

15:00 – 15:15 **WHAT ARE WE DOING ABOUT IT?** (*Keith Rowling - PIRSA)*

- Management implications.
- Community engagement.

15:15 – 15:30 **FUTURE SURVEY OPTIONS** (*Kara Edmonds – BHP Billiton*)

- ROV trials.
- Collaborative approach.
- Data continuity.

15:30 – 15:45 **WRAP-UP** (*Mike Steer – SARDI)*

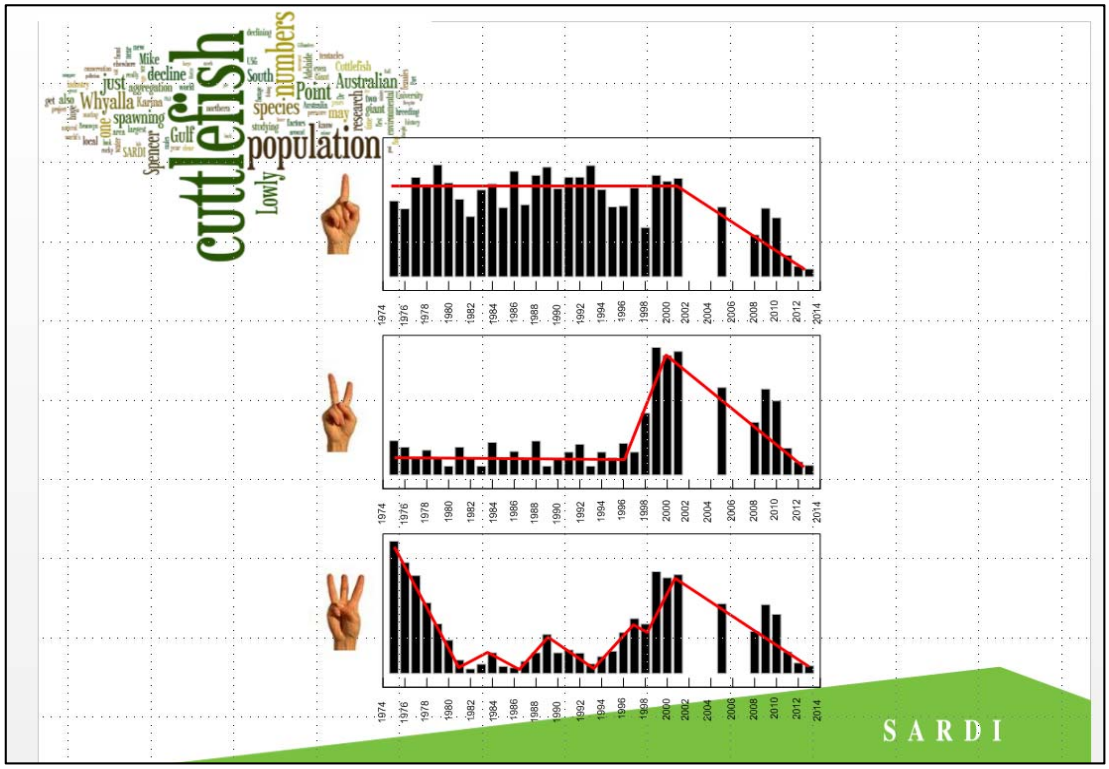
- Weight-of-evidence summary.
- Future research direction.

15:45 *DRINKS & NIBBLES*

KEY MESSAGES

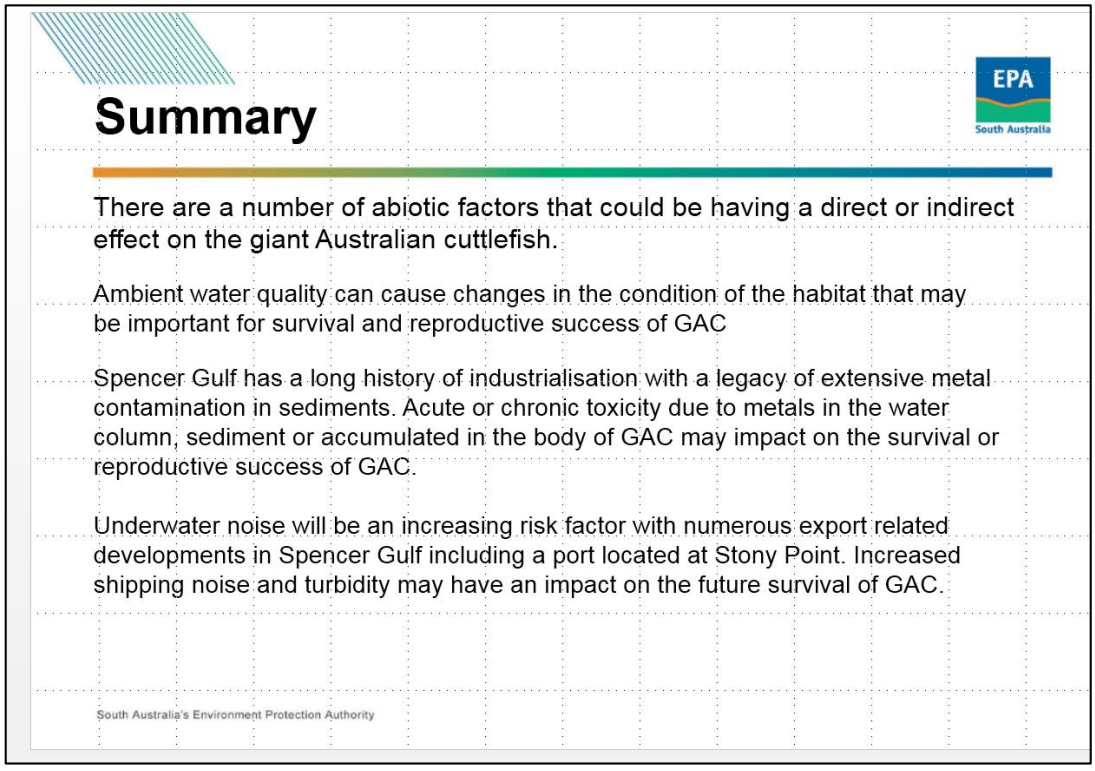
WHAT'S GOING ON WITH THE CUTTLEFISH POPULATION? (*Mike Steer -SARDI*)

- Provided the general history of the Point Lowly Cuttlefish spawning aggregation, including the development of the fishery, management strategies, population surveys and recent decline in population abundance and biomass.
- Integrated our understanding of the biology and ecology of Giant Australian Cuttlefish building upon the research of Hall and Fowler (2003) **FRDC 98/151**. *The fisheries biology of the cuttlefish* Sepia apama *Gray, in South Australia.*
- Discussed the potential 'causes' of the decline as detailed in Steer et al. (2013) **FRDC 2011/054** *Monitoring the relative abundance and biomass of South Australia's iconic Giant Australian Cuttlefish breeding population.*
- Presented three alternative hypotheses that may explain the trends in cuttlefish population dynamics.
- This set up the successive presentations.



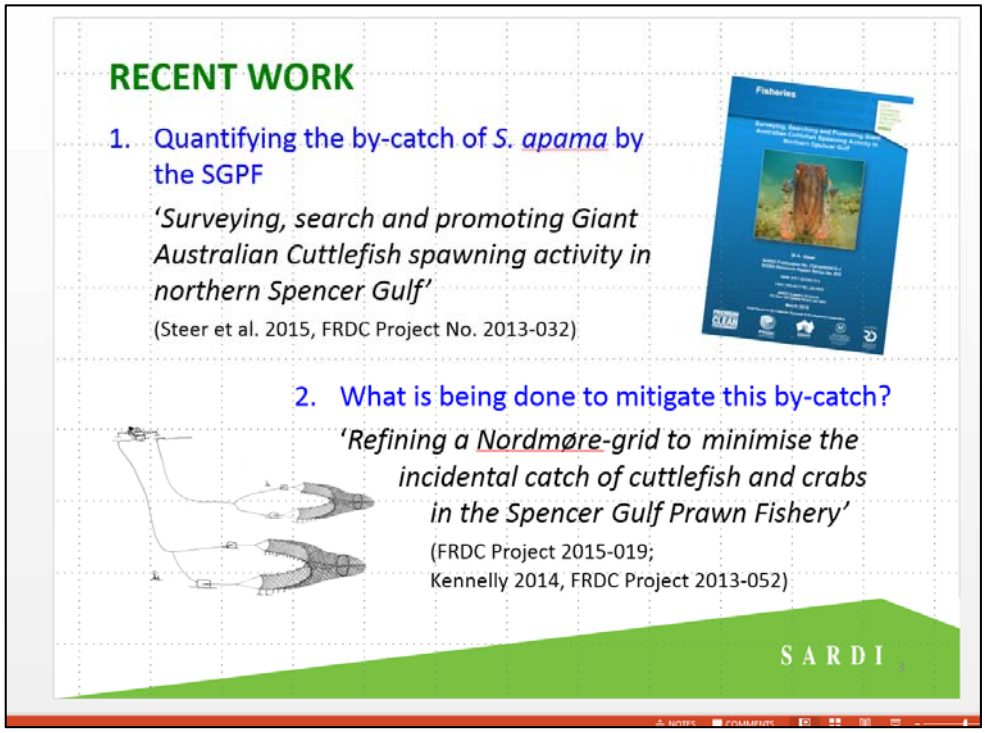
IS POLLUTION A POTENTIAL DRIVER? (*Sam Gaylard – EPA)*

- Explored the potential effects of ambient water quality, bioaccumulation of heavy metals and underwater noise on the spawning aggregation. Drawing information from Steer (2015) **FRDC 2013/032** *PIRSA: Surveying, searching and promoting cuttlefish spawning activity in northern Spencer Gulf,* and Woodcock et al (2014) *Regional sustainability planning in the upper Spencer Gulf investigating potential impacts of shipping on Giant Australian Cuttlefish. Final Report to Department of the Environment.*
- Observed difference in habitat and water chemistry where an expected result of the broadscale regional rather than localised influences with no observed effect on the spawning population.
- Giant Australian Cuttlefish collected from the spawning grounds had higher concentrations of heavy metals compared to cuttlefish collected from southern spencer Gulf. Metal loads were well within their known tolerances.
- Underwater noise had no adverse effect on embryo development, hatching success or adult respiration, however, more research is required.



WHAT IS THE EXTENT OF FISHING ON THE AGGREGATION? (*Craig Noell – SARDI)*

- Quantified cuttlefish bycatch in Northern Spencer Gulf as presented in Steer (2015) **FRDC 2013/032** *PIRSA: Surveying, searching and promoting cuttlefish spawning activity in northern Spencer Gulf.*
- Presented preliminary results of two by-catch reduction device research projects. Kennelly (2014) **FRDC 2013/052** *Bycatch Reduction Devices (BRDs) to reduce the incidental catch of cuttlefish in the Spencer Gulf Prawn Trawl Fishery* and Noell et al. **FRDC 2015/019** *Refining a Nordmore-grid to minimise the incidental catch of cuttlefish and crabs in the Spencer Gulf Prawn Fishery.*
- Giant Australian Cuttlefish by-catch is negligible, and preliminary results indicated that BRD trials look promising.
- The Spencer Gulf and West Coast Prawn Fisherman's Association is pro-active to ensure that the iconic Giant Australian Cuttlefish population is not compromised by fishing activity. They also have a strong focus on maintaining their MSC accreditation.

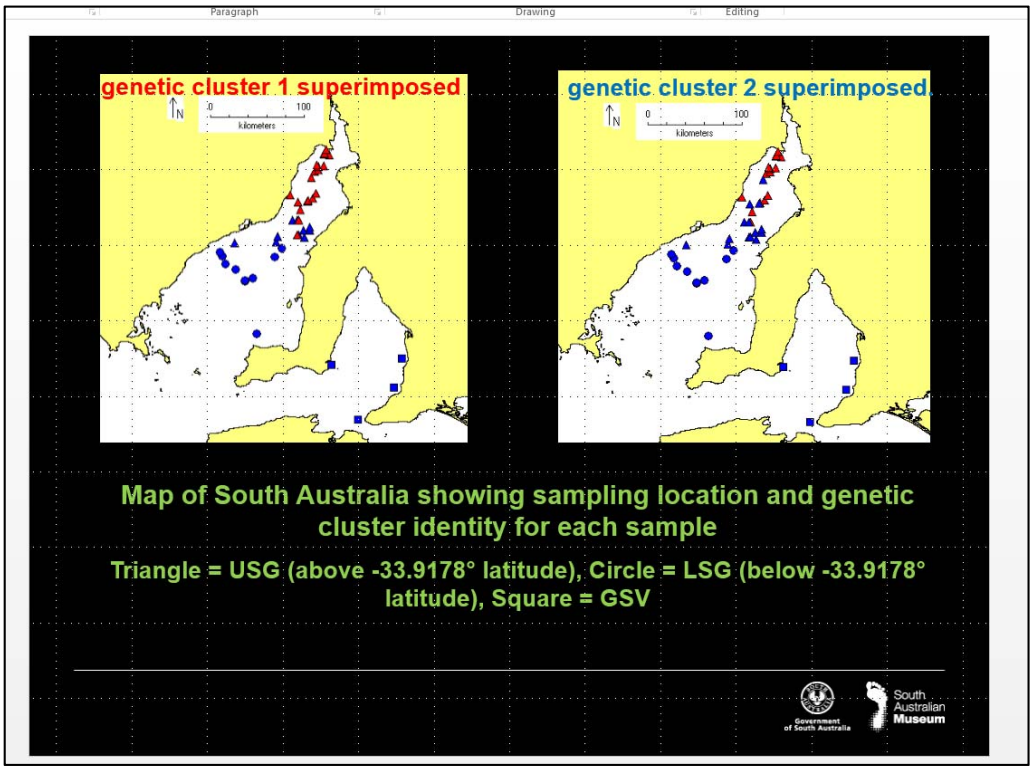


WHAT ABOUT THE ENVIRONMENTAL EFFECTS? (*Bronwyn Gillanders – UoA)*

- Field investigation of temperature effects as presented in Steer (2015) **FRDC 2013/032** *PIRSA: Surveying, searching and promoting cuttlefish spawning activity in northern Spencer Gulf.*
- Laboratory investigation of temperature/salinity/oxygen on embryos and adults as presented in Gillanders (in prep) **FRDC 2013/010** *Giant Australian Cuttlefish in South Australian waters.*
- Also explored global trends in cephalopod abundance – through a workshop funded by the Environment Institute hosted by the University of Adelaide in January 2015 (with international cephalopod experts – A. Arkhipkin, G. Pierce, J. Semmens, Zoe Doubleday, T. Prowse, B. Gillanders and M. Steer).
- Key message – Temperature has a governing effect on cephalopod population dynamics provided that food is not limited, temperatures do not go beyond the species thermal range and salinity remains ambient.

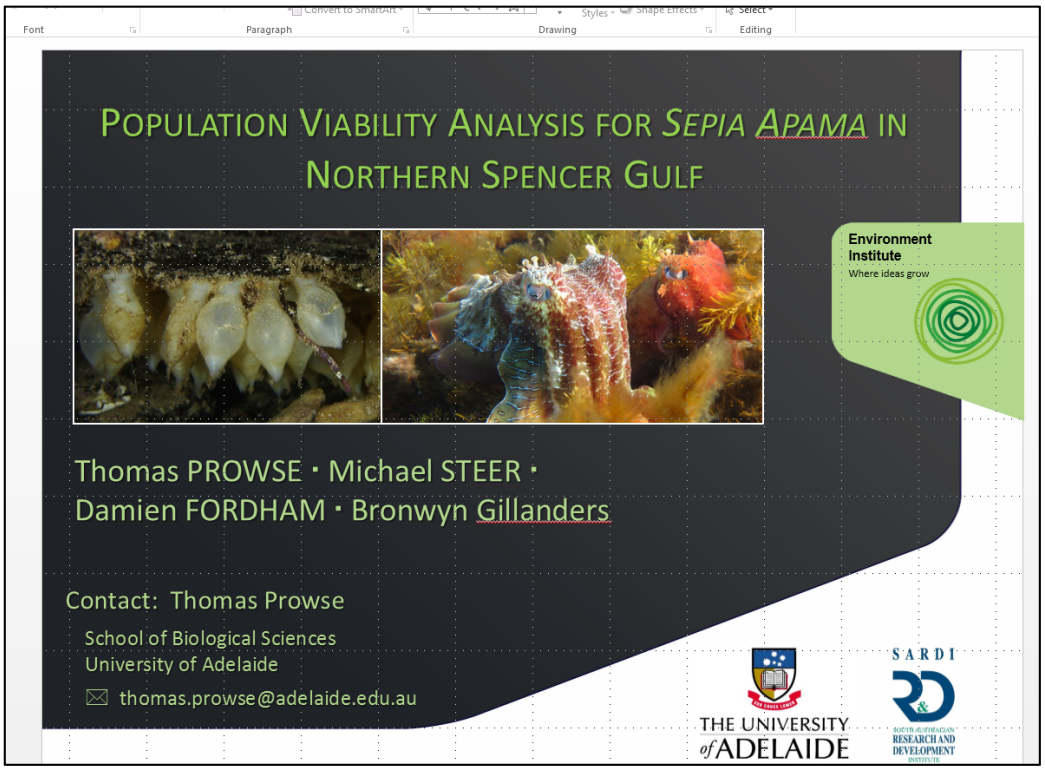
WHAT IS THE SCALE OF THE ISSUE? (*Steve Donnellan – SA Museum)*

- Defined the taxonomic extent of Giant Australian Cuttlefish as in Gillanders (in prep) **FRDC 2013/010** *Giant Australian Cuttlefish in South Australian waters.*
- Clear genetic distinction, northern population (North of Wallaroo) is isolated.



HOW VIABLE IS THE SPAWNING POPULATION? (*Thomas Prowse – UoA)*

- Modelled viability of the population as in Gillanders (in prep) **FRDC 2013/010** *Giant Australian Cuttlefish in South Australian waters* and using data collected from Steer (2015) **FRDC 2013/032** *PIRSA: Surveying, searching and promoting cuttlefish spawning activity in northern Spencer Gulf.*
- Main findings:
  - Substantial population-level variability is inevitable
  - Commercial fishing outside MFA 21 poses negligible risk
  - Bycatch from prawn trawling poses negligible risk
  - Intensive fishing on the aggregation sites, and future desalination discharge, are the greatest threats to population viability
  - Closure of cuttlefish harvesting in Marine Fishing Area 21 should substantially enhance population viability

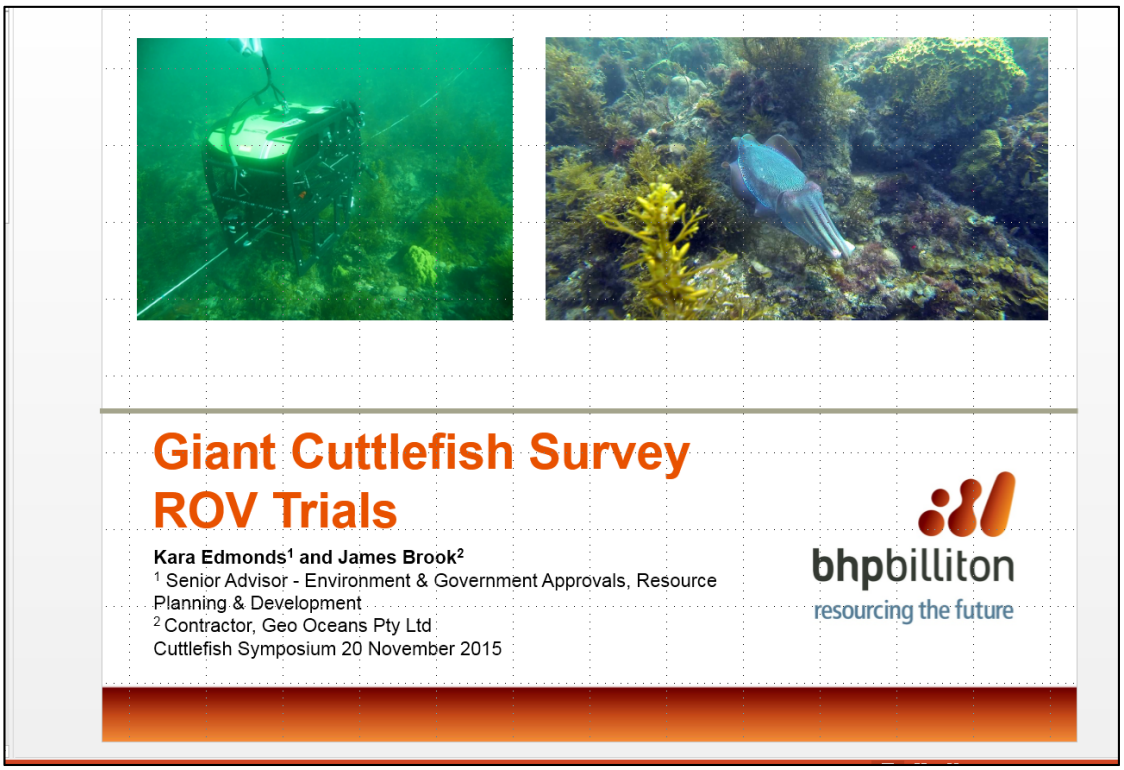


WHAT ARE WE DOING ABOUT IT? (*Keith Rowling - PIRSA)*

- Presented management history.
- Indicated that the False Bay closure is unlikely to be removed.
- Also indicated that the broadscale northern Spencer Gulf closure will continue through 2016.

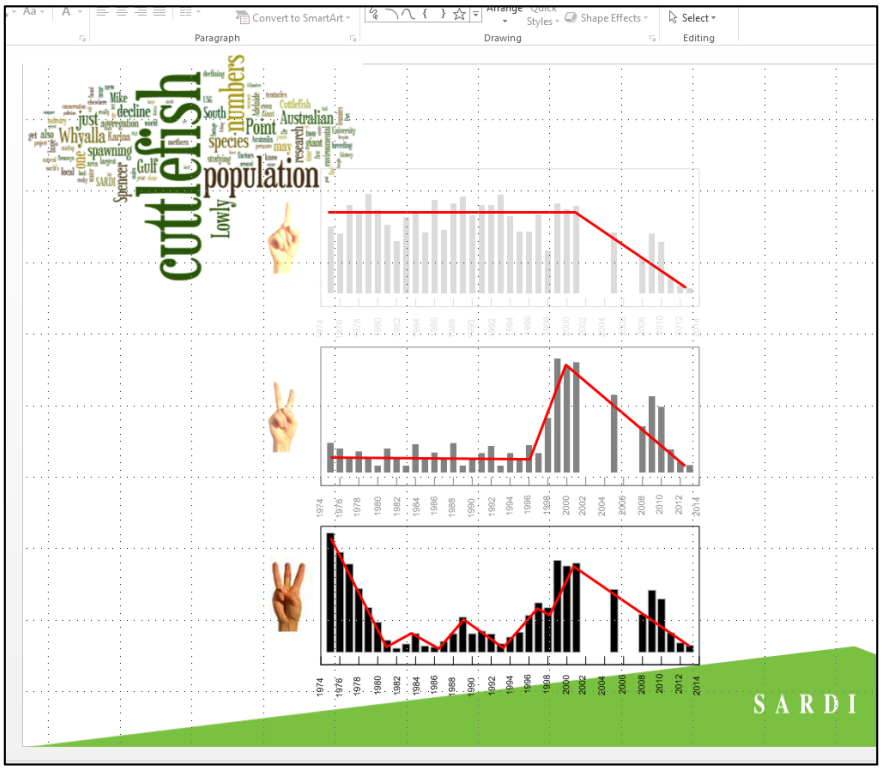
FUTURE SURVEY OPTIONS (*Kara Edmonds – BHP Billiton*)

- BHP trialed an ROV survey as an alternative to dive surveys.
- ROV-based surveys can obtain estimates of the breeding population abundance that are comparable with diver-based surveys.
- Post-field review of the video transects may still be required.
- A stereo camera system can more accurately measure size, and hence improve biomass estimates.
- The stereo camera system can provide added value for quality assuring calculations of the camera field of view.
- It would be prudent to continue periodic diver surveys, even if less frequently, to confirm that:
- the ROV-based population estimates continue to be similar to those based on 'diver unobstructed' data;
- a consistent difference from the estimates based on 'diver all' data is maintained.



WRAP-UP (*Mike Steer – SARDI)*

- Synthesizing all information to date from the various FRDC projects - Natural fluctuation appears to be the most parsimonious explanation of the recent cuttlefish decline.
- Remaining knowledge gap – trophodynamics, however, we have the capability to explore this in the near future with developed models through **FRDC 2011/205** Gillanders et al, *Spencer Gulf Research Initiative: development of an ecosystem model for fisheries and aquaculture*.



ATTENDEES

GIANT CUTTLEFISH SYMPOSIUM FRIDAY 20 NOVEMBER 2015			
ASHBY	CRISPIAN	FRDC	
BARRETT	SKYE	SARDI	
BASTIAN	JOHN	SGEDI BOARD	
BEGG	GAVIN	SARDI	
BROCKLEHURST	KATE	NATURAL RESOURCES EYRE PENINSULA	
BROOK	JAMES	BHP/CCSA	
BRYARS	SIMON	DEWNR	
CARROLL	JONATHON	SARDI	
CLARK	SIMON	SGEDI BOARD	
CLARKE	STEVEN	SARDI	
COONEY	CATHY	EPA CGCWG	
DEVENEY	MARTY	SARDI	
DONNELLAN	STEVE	SA MUSEUM	
DOROUDI	MEHDI	PIRSA	
EARL	JASON	SARDI	
EDMONDS	KARA	BHP	
FEENSTRA	JOHN	SARDI	
FLEER	DAVID	SARDI	
FOUREUR	BRIAN	SARDI	
GAUT	ALEX	CONSERVATION SA	
GAYLARD	SAM	EPA CGCWG	
GILLANDERS	BRONWYN	UNI OF ADE/CGCWG	
GLUIS	MARK	SARDI	
GRAMMER	GRETCHEN	SARDI	
GREAR	BRENTON	DEWNR CGCWG	
HARRISON	SHELLEY	MPSWG	
HILL	GREG	BHP BILLITON	
HOOPER	GRAHAM	SARDI	
JONES	ALICE	UNI OF ADELAIDE	
JUNGE	CLAUDIA	UNI OF ADELAIDE	
LAKE	DAVID	SATC CGCWG	
LI	XIAOXU	SARDI	
LINTON	VICKI	DEWNR	
LLOYD	MATT	SARDI	
LOO	MAYLENE	SARDI	
MALTHOUSE	PAUL	SARDI	
MATTHEWS	DAMIAN	SARDI	
MAYFIELD	STEPHEN	SARDI	
MCGARVEY	RICK	SARDI	
MITCHELL	JIM	FLINDERS UNI	
NOELL	CRAIG	SARDI	
PAGE	BRAD	DEWNR	
PROWSE	THOMAS	UNI OF ADE	
REINHOLD	SARAH-LENA	SARDI	
ROWLING	KEITH	PIRSA	
SHEPHERD	SCORESBY		
STEER	MICHAEL	SARDI	

GIANT CUTTLEFISH SYMPOSIUM FRIDAY 20 NOVEMBER 2015			
STONE	DAVID	SARDI	
SWAN	LOUISE	SANTOS	
TONKIN	IAN	SARDI	
TSOULAKAS	JO	PIRSA/CGCWG	
TYLER	JIM	UNI OF ADE	
WAYCOTT	MICHELLE	DEWNR	
WILTSHIRE	DAVID		
Huveneers	Charlie	Flinders Uni	