

Regional Sustainability Planning in the Upper Spencer Gulf

Investigating potential impacts of shipping on giant Australian cuttlefish

Final Report – April 2014

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Abbreviations

dB	Decibels
DEWNR	Department of Environment, Water and Natural Resources
DMITRE	Department for Manufacturing, Innovation, Trades, Resources and Energy
DPTI	Department of Planning, Transport and Infrastructure
EPA	Environment Protection Authority
GL	Gigalitre
LPG	Liquefied petroleum gas
Mtpa	Million tonnes per annum
NTU	Nephelometric turbidity units
PIRSA	Primary Industries and Resources South Australia
RESIC	Resources and Energy Sector Infrastructure Council
SARDI	South Australian Research and Development Institute
SATC	South Australian Tourism Commission
USG	Upper Spencer Gulf

Executive Summary

Spencer Gulf is the most significant economic growth area in South Australia. A lack of deep-water port facilities to meet export capacity has led to a number of companies proposing new port developments. An increase in shipping to the region is expected associated with mining and other infrastructure developments. Upper Spencer Gulf (USG) is world renowned for its breeding aggregation of giant Australian cuttlefish (*Sepia apama*). However, there is a lack of information on whether *S. apama* may be impacted by shipping noise and associated turbidity.

Several available datasets suggest significant declines in abundance and biomass of *S. apama* in USG, especially near the breeding aggregation site. Although a range of potential threats have been identified, there are no obvious explanations to account for their decline. Few studies have specifically investigated effects of shipping on cephalopods, although the literature suggests that shipping noise can cause physical, physiological and behavioural changes in some organisms.

The aims of this project were to: (1) undertake a desktop review of *S. apama* related research including population trends, threats, shipping impacts and management, (2) investigate the potential impacts of shipping on *S. apama* through field/laboratory experimentation and analysis, (3) incorporate outcomes from experimental research into an integrated model assessing population viability, and (4) discuss associated conservation and management implications for *S. apama* in USG.

For the experimental component of the project, *S. apama* adults and eggs were collected from False Bay, Whyalla, South Australia and transported to SARDI Aquatic Sciences Centre at West Beach. Eggs were kept in flow-through polystyrene holding tanks and exposed to shipping noise and turbidity on either a twice weekly or fortnightly basis (i.e. relevant to shipping frequency), with appropriate control treatments. Hatching success of eggs and embryonic development (length and weight of hatchlings) were examined for each treatment. Adults were held in individual flow-through conical tanks then placed in a resting respirometer and, after a suitable acclimation period (4 to 6 h), were exposed to reef and shipping noise in random order for approx. 2 h. Oxygen consumption in the chamber was measured to provide an indication of potential stress.

Shipping noise and turbidity had no significant effect on hatching success though hatchlings exposed to noise were 3-6% larger than the control group. Increased shipping frequency led to slightly increased hatching success and increased size of hatchlings, although this may relate to increased handling and movement of eggs in these treatments. Turbidity had no significant effect on hatching success or embryonic development. Given the lack of effect of these factors on hatching success it was not possible to incorporate these variables into a demographic model. Shipping noise also had no effect on the metabolic rate of adult *S. apama*. This research only investigated the effect of one component of sound, sound pressure. The experiments investigated lethal effects on egg development and sub-lethal effects on embryonic growth and adult metabolism, however shipping noise may impact longer term survival and influence behaviour of individuals. In addition, other factors associated with shipping that were not investigated may impact *S. apama*.

It is recommended that future research focuses on understanding the hearing range of *S. apama* and whether the particle displacement component of sound affects *S. apama*. Behavioural changes associated with shipping noise should also be investigated.

1. Introduction

The ocean is naturally a noisy environment caused by factors such as rain, wind, and waves, as well as noises from marine mammals, snapping shrimp and some fish. Continued increases in human activity associated with coastal development and oceanic transport has contributed to anthropogenic noise in the marine environment (Slabbekoorn et al. 2010; André et al. 2011). Such sources of noise pollution include construction and operational noises such as pile-driving, dredging, drilling, and offshore wind farms; shipping noises from freight, passenger crossing, fishing and recreational vessels; as well as other sounds such as sonar, seismic surveys and explosions (Götz et al. 2009; Slabbekoorn et al. 2010). Noise from shipping is pervasive throughout the marine environment, especially at low (<300 Hz) frequencies, with exposure to distant shipping elevating ambient noise levels by 15-20 dB. Shipping therefore has the potential to impact marine species through increased noise exposure (Merchant et al. 2012; McKenna et al. 2013). The species of interest for this report is the giant Australian cuttlefish, *Sepia apama*, the largest cuttlefish in the world.

Depending on the intensity or sound pressure level at the source, the pitch or frequency, the distance between the source and the receiver, and the cumulative effect when a source is long-lasting or repeated in time, sound can potentially affect marine organisms in various ways (Götz et al. 2009). Little is known about how excessive noise impacts marine life, but it is expected to cause physical, physiological and behavioural effects (André et al. 2011). Few studies have specifically examined effects of anthropogenic noise on cephalopods therefore this report also considers effects on other invertebrates and fish.

Sound can be thought of in terms of both particle motion and pressure fluctuation. For fish, while the ear (otolith), and in some species the swim bladder, serves to detect sound, the lateral line detects low frequency sound and water motion relative to the body (Slabbekoorn et al. 2010). Little is known about sound detection in cephalopods, although the statocysts¹ and the epidermal head and arm lines² are the

¹ The statoliths occur within the statocyst and function similarly to fish otoliths.

² The epidermal head and arm lines are hydrodynamic receptor systems similar to fish lateral lines.

likely organs for sound detection (Komak et al. 2005; Mooney et al. 2010). The affects of sound on cephalopods remains unclear, although the recent literature suggests that they are sensitive to low frequency sounds, as physical damage to the statocysts and suspected strandings associated with noise have occurred (Packard et al. 1990; André et al. 2011).

Shipping not only contributes noise to the environment, but may also lead to increased turbidity especially where the distance between the water line of the ship and bottom of its hull is close to the bottom of the ocean, as in coastal waters. Here, the rotating ship propeller creates a turbulent stream of fast moving water flow referred to as propeller wash that can move and erode bottom bed sediments. During shipping, sediments can be temporarily suspended by shallow water manoeuvres and propeller wash. This can increase localised turbidity and have a range of impacts on a variety of aquatic organisms. For example, light penetration and visibility can be reduced (Savino et al. 1994), gills can be clogged, organisms smothered and contaminated sediments resuspended. Shipping traffic can increase turbidity from calm conditions of two nephelometric turbidity units (NTU) to over 19 NTU during boat passage, although turbidity can be greater than 100 NTU following storm events (Quesenberry et al. 2007). While higher turbidity had no influence on the development of Lake herring (*Coregonus artedii*) eggs (Savino et al. 1994), the effects on cephalopod eggs are unknown. They are vulnerable as they are generally attached to a substrate, leaving them exposed to changing local conditions and unable to move to avoid increased turbidity and potential smothering effects from sedimentation. We are not aware of any studies that have investigated the effects of turbidity on cephalopods, although it may affect activities such as feeding and mating, which rely on visual cues.

1.1 Upper Spencer Gulf

Spencer Gulf is the most significant economic growth area in South Australia and an important region for the state's mining pipeline and associated infrastructure. A lack of deep-water port facilities to meet export capacity close to proposed developments has led to at least five companies proposing new port developments in Spencer Gulf and the Resources and Energy Sector Infrastructure Council

(RESIC) indicating that at least three new ports are required (RESIC 2011). If port developments proceed, for example at Port Bonython, then increased shipping in Spencer Gulf is likely. At Port Bonython the plan is to build and operate a common user bulk export facility, which is capable of handling Cape-size vessels. A three km long jetty that reaches into deep water is proposed along with enclosed conveyers and a ship loader. This proposed jetty would run parallel to the existing 2.4 km long Port Bonython jetty, which is used for export of petroleum products such as LPG and crude oil from Cooper Basin. The maximum-sized tanker that can currently be loaded has a capacity of 110 000 tonnes, and there are around 30 ships loaded per year. If the new port development at Port Bonython proceeds then an extra 122 ships per annum are envisaged. Thus, significant expansion of shipping is likely in the region.

Upper Spencer Gulf is renowned for having the only known breeding aggregation of *S. apama* in the world. Whilst the giant Australian cuttlefish, *Sepia apama*, is distributed and breeds in waters across the southern coastline of Australia, it forms an extraordinarily large breeding aggregation along a small stretch of rocky reef in USG (Steer et al. 2013). The existing Port Bonython facility and the proposed development of a deep-water port are in close proximity to the main aggregation area. Since the late 1990s, this *S. apama* aggregation has consisted of tens of thousands of individuals and is internationally recognized as an iconic natural phenomenon that has attracted considerable world-wide media and scientific attention. Recently, it has shown significant decreases in both abundance and biomass (Steer et al. 2013) raising some concern for the species and prompting the SA Government to set up a Cuttlefish Working Group. The working group involves representatives from Primary Industries and Regions South Australia (PIRSA), South Australian Research and Development Institute (SARDI), Department of Environment, Water and Natural Resources (DEWNR), Environment Protection Authority (EPA), Department of Planning, Transport and Infrastructure (DPTI), South Australian Tourism Commission (SATC), Department for Manufacturing, Innovation, Trade, Resources and Energy (DMITRE), the SA Conservation Council and Whyalla City Council.

The Australian Government recognises USG as a priority area and as such funded this research under the Sustainable Regional Development program. USG was selected as an eligible region based on the high rate of growth linked to potential mining developments and the associated infrastructure and services. The government works collaboratively with state and regional stakeholders to promote and inform environmental, social and economic sustainability within the region. There is a focus on matters of national environmental significance, protected under national environmental law, and other priority environmental values such as *S. apama*.

Although increased shipping is expected in USG and nearby where *S. apama* aggregate to breed, there has been no research which has specifically investigated the potential impact of turbidity and noise from shipping on *S. apama*. The objectives of the current project are to: (1) undertake a desktop review of any *S. apama* research including population trends, threats, shipping impacts and management (see Appendix A); (2) investigate the potential impacts of shipping on *S. apama* through field/laboratory experimentation and analysis; (3) incorporate outcomes from experimental research (if possible) into an integrated model assessing population viability; and (4) discuss associated conservation and management implications for *S. apama* in USG including future research needs.

2. Methods

2.1 Collection of *S. apama* eggs and adults

Sepia apama adults and eggs were collected from False Bay, Whyalla, South Australia (Figure 1). Specimens were transported to South Australian Research and Development Institute (SARDI) Aquatic Sciences Centre, West Beach, in aerated tanks. Upon arrival, adults were transferred into individual flow-through conical tanks and eggs were placed into flow-through polystyrene holding tanks (hereafter referred to as holding tanks). Eggs were sewed to polystyrene squares (10 eggs per square) using nylon line to suspend them away from the bottom of the holding tanks and to simulate their orientation and spatial dynamics in nature. Eggs were randomly assigned to holding tanks such that there were 10 eggs per tank. All eggs

were approximately the same size and stage of development and had been recently laid.



Figure 1. Point Lowly region near Whyalla, USG showing False Bay where adults and eggs were collected for experiments.

2.2 Shipping sound

Shipping sounds of 100-20 000 Hz for egg experiments were based on five field recordings of two idling vessels — a pilot boat in the Whyalla marina (16 m long) and a dry bulk ship at inner harbour Port Adelaide (161 m long) — overlaid with natural background rocky reef noise. Shipping sounds of 100-20 000 Hz were obtained for adult experiments from field recordings of three ships of 149-157 m length that arrived or departed from inner harbour Port Adelaide. Multiple reef recordings of 100-20 000 Hz were collected from four locations between Point Lowly and Black Point along the Point Lowly coastline where the eggs were collected (Figure 1). These recordings were used for both egg and adult experiments.

The sounds were collected using an underwater hydrophone (HTI-96-MIN) connected to a Sony (PCM M10) recorder. Recordings were then imported and edited using Audacity® 2.0.3. Tracks were first edited to remove unwanted noise (e.g. dolphin clicks, hydrophone cable dragging) and then spliced together using random 20 s intervals from each track. Underwater speakers (University Sound® Series UW30) were connected to a 12 W universal amplifier (Kemo® Electronic, M032N) and power supplied using 12 V batteries (Powertech 12V 350mA). Sound was played through a MP3 player (Philips GoGEAR RAGA MP3 player) at similar frequency and volume to recorded sounds. For the egg development experiment, sound tracks of the two idling vessels and reef noise were created to play for 12 h, with the MP3 player programmed to repeat at the end of the track. For the adult experiment, sound tracks were created to play reef noise and shipping noise each in 30 min blocks for 2 h.

2.3 Egg development

A three factor experimental design was used to examine the effects of shipping frequency (twice weekly or fortnightly) and noise (shipping sound vs no shipping sound), as well as turbidity (turbidity vs no turbidity) on hatching success, and weight and length of hatchlings (Figure 2). There were three replicate holding tanks per treatment. Fortnightly exposure was chosen to represent current shipping levels to Port Bonython (Santos jetty in Figure 1), whereas twice weekly was chosen to represent a future increased shipping level.

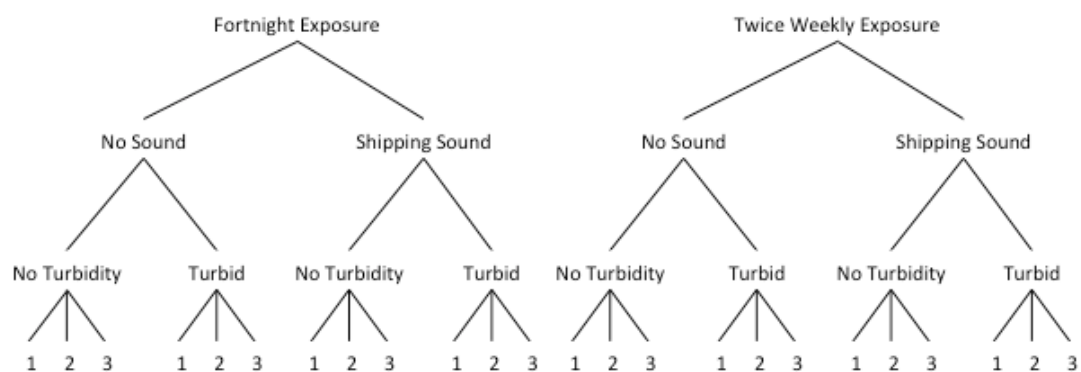


Figure 2. Experimental design used to examine effects of shipping frequency (fortnight exposure vs twice weekly exposure), noise (no sound vs shipping sound)

and turbidity (no turbidity vs turbid) on hatching success and size of *S. apama* hatchlings. Control treatments were either no sound, no turbidity or no sound and no turbidity combined. There were three replicate holding tanks per treatment (labelled 1, 2, and 3 above) with 10 eggs per holding tank.

Sepia apama eggs were moved from holding tanks to experimental treatment tanks for exposure to treatments as required (see Figure 2). This setup comprised two large conical bottom tanks which acted as sumps (320 L); each of these was fitted with a double ring aeration supply and a submersible pump, which was used to pump the water up to the experimental tanks (Figure 3). Two experimental tanks, similar to the polystyrene holding tanks, were placed on top of each conical bottom tank, supported with marine grade ply wood (Figure 3). Each experimental tank contained an UW30 underwater speaker (Electro-Voice® University Sound® Series) and a 29 cm air stone. To create turbidity, approximately 500 g of bentonite clay was added to one of the recirculating sumps to give a turbidity of 70 NTUs. This clay was kept in suspension by the aeration systems and pumps. The turbidity level was based on previous turbidity experiments that had found effects on organisms (Benfield and Minello 1996; Reid et al. 1999; Quesenberry et al. 2007). The turbidity level is well above the background levels of turbidity found in USG; extensive monitoring by the EPA in 2012 has shown that the average turbidity throughout USG is in the order of 0.16-0.435 NTU (Gaylard, email, 19 February 2014).

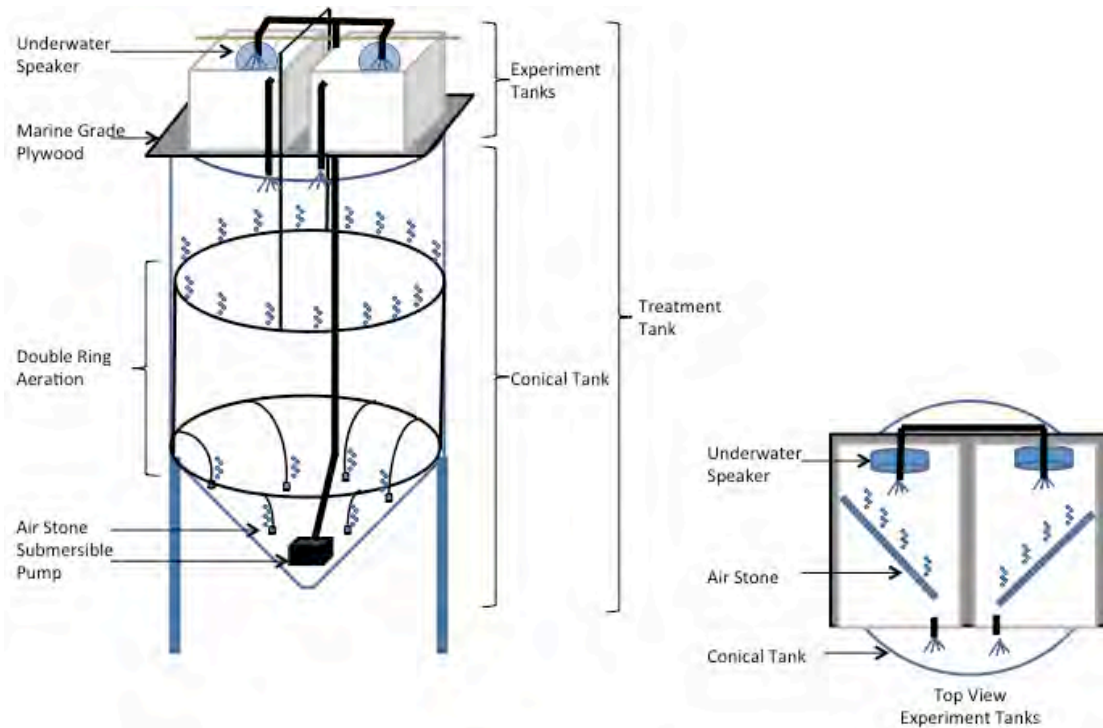


Figure 3. Set-up of egg experiment showing two experimental tanks and the conical bottom tank. Eggs were moved from holding tanks to the experimental tanks for exposure to experimental treatments according to the design in Figure 2.

Eggs were randomly placed into 36 L polystyrene holding tanks, with flow-through water and aeration, and allowed to develop through to hatching (Figure 4). The temperature ranged from 13.5°C at the beginning of the experiment to 15.5°C at the end of the experiment. The salinity was between 37 and 38 ppt. The salinity and temperatures, as well as the change in temperature, reflected field conditions in USG. Eggs were moved to experimental tanks for a 24 h period. To simulate shipping noise events the eggs were exposed to the noise either fortnightly or twice weekly (shipping frequency) for a period of 19-21 h (Figure 2). Ships currently idle at Port Bonython for approximately 30 h, however, due to the battery capacity of the MP3 player the eggs could not be exposed to sound treatments for as long. They were therefore exposed to shipping noise for 19 to 21 h. Eggs were monitored throughout the experiment for signs of development and as *S. apama* hatched they were immediately removed from the tanks, weighed, euthanized and stored in 70 per cent ethanol. Dorsal mantle length was measured using digital calipers

(ProSciTech SUC3-150) and each individual was weighed on a microbalance (Mettler Toledo PB3002 DeltaRange R).



Figure 4. Set-up of flow-through polystyrene holding tanks used to monitor development of *S. apama* eggs.

2.4 Physiological response of adults

Stress in organisms can often be estimated based on metabolic rate, with stressed individuals breathing harder and faster and consuming more oxygen than relaxed individuals. The impact of noise on metabolism (i.e. oxygen consumption) was measured for each adult *S. apama* (n=8) using a 10.92 L resting respirometer (hereafter referred to as respirometer) with a working section of 170 x 500 mm (diameter x length) (Figure 5). To reduce ambient noise, the entire respirometry system was placed in a quiet room separate from the main building at SARDI Aquatic Sciences Centre and the respirometer was placed inside a sound insulated bath using a 20 mm thick layer of polystyrene foam. Pump noise was minimized by

leading all tubing from the respirometer into a second separate polystyrene insulated bath containing the re-circulating pump and flushing pump. Both respirometer baths were provided with filtered, UV sterilized and fully oxygenated flow-through seawater at $14.8 \pm 0.1^\circ\text{C}$ (mean \pm S.E.) and 38.5 ppt salinity which ensured stable temperatures during measurements.



Figure 5. Set-up of respirometer used to measure oxygen consumption of adult *S. apama* in response to shipping noise.

To verify that noise levels in the respirometer system were below natural ambient levels on rocky reefs where *S. apama* reside, a hydrophone was used to record the sound pressure levels inside the respirometer. This preliminary trial revealed that pump noise in the system was below the sound pressure levels of natural reef and boat noises across frequencies of 0-20 Hz, and also below the suspected hearing range of *S. apama* (Packard et al. 1990). The playback of shipping noises and natural reefs in the respirometer were then calibrated to closely mimic sound pressure levels recorded in the field.

Prior to each trial, a *S. apama* was moved from the individual flow-through conical tank and placed in the respirometer and left to acclimatize for a minimum of 4-6 h

until oxygen consumption of the test subject reached a steady state level. The trial was then started and the oxygen consumption of the test subject was measured during playback of a randomly chosen sound for a period of 30 min. The test subject was then left to relax without any noise disturbance for a further 30 min before the next randomly chosen sound was played for 30 min. Each *S. apama* was exposed to reef noise and shipping sounds in random order.

Throughout the trials, 180 s flush and 420 s oxygen measurement periods were applied following the intermittent flow respirometry methodology of Steffensen et al. (1984) and Steffensen (1989). The flushing period ensured the oxygen concentration within the respirometer never decreased below 80 per cent of air saturation and removed any CO₂ build-up. During the experiments, oxygen levels within the swimming respirometer were measured and monitored using a fibre optic oxygen meter (Hach). MO_2 (mgO₂kg⁻¹h⁻¹) was calculated with LabChart v. 6.1.3 (ADInstruments, Dunedin, New Zealand) software as the slope of the linear regression of oxygen concentration decline over time for each determination cycle (e.g. Bushnell et al. 1994; Schurmann and Steffensen 1997). At the end of each trial, the test subject was removed and background respiration (e.g. from bacteria) was measured as oxygen depletion in the empty respirometer. Whenever background respiration was near 5 per cent of the oxygen consumption of the test subject, the respirometer was treated with a bleach solution and thoroughly flushed prior to the next *S. apama* being tested.

3. Results

3.1 Egg development

Sepia apama developed and hatched from all experimental treatments, although there was some variability among holding tanks of each experimental treatment (Figure 6). Hatching success for all treatments ranged from 68 to 100 percent with the highest hatching success for eggs exposed to shipping noise and no turbidity on a twice weekly basis (Figure 6). Shipping noise and turbidity had no significant effect on hatching success of *S. apama*. *Sepia apama* eggs from twice weekly

treatments had a slightly higher hatching success rate than those from fortnightly treatments (Figure 6).

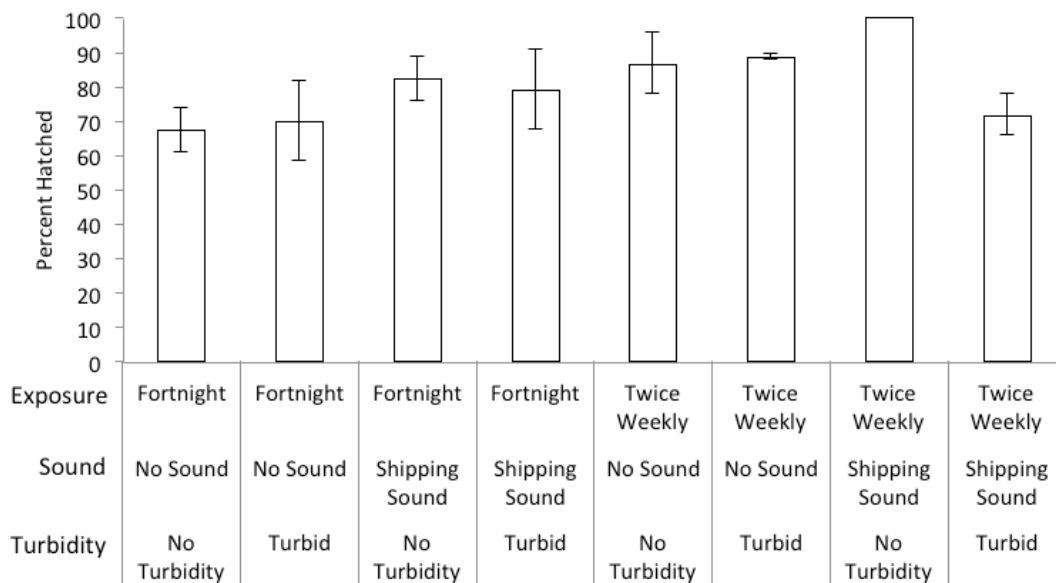


Figure 6. Percent of successfully hatched *S. apama* from tanks exposed to fortnightly or twice weekly shipping noise or turbidity. Control treatments were either no shipping noise, no turbidity or no shipping noise and no turbidity combined. Shown are mean percent hatched (\pm standard error) from three replicate holding tanks for each of the eight treatments.

Measurements of hatchlings found significant individual treatment effects of shipping noise and shipping frequency on *S. apama* hatchling size. *Sepia apama* eggs that were exposed to shipping noise were slightly larger than those not exposed to shipping noise (Figure 7a, b). Similarly those exposed to treatment conditions on a twice weekly basis were slightly larger than those exposed on a fortnightly basis (Figure 7e, f). Although significant differences between treatments were found, they were relatively small (3-7%) and probably not biologically important (Figure 7). Turbidity had no significant influence on the size of hatchlings (Figure 7c, d).

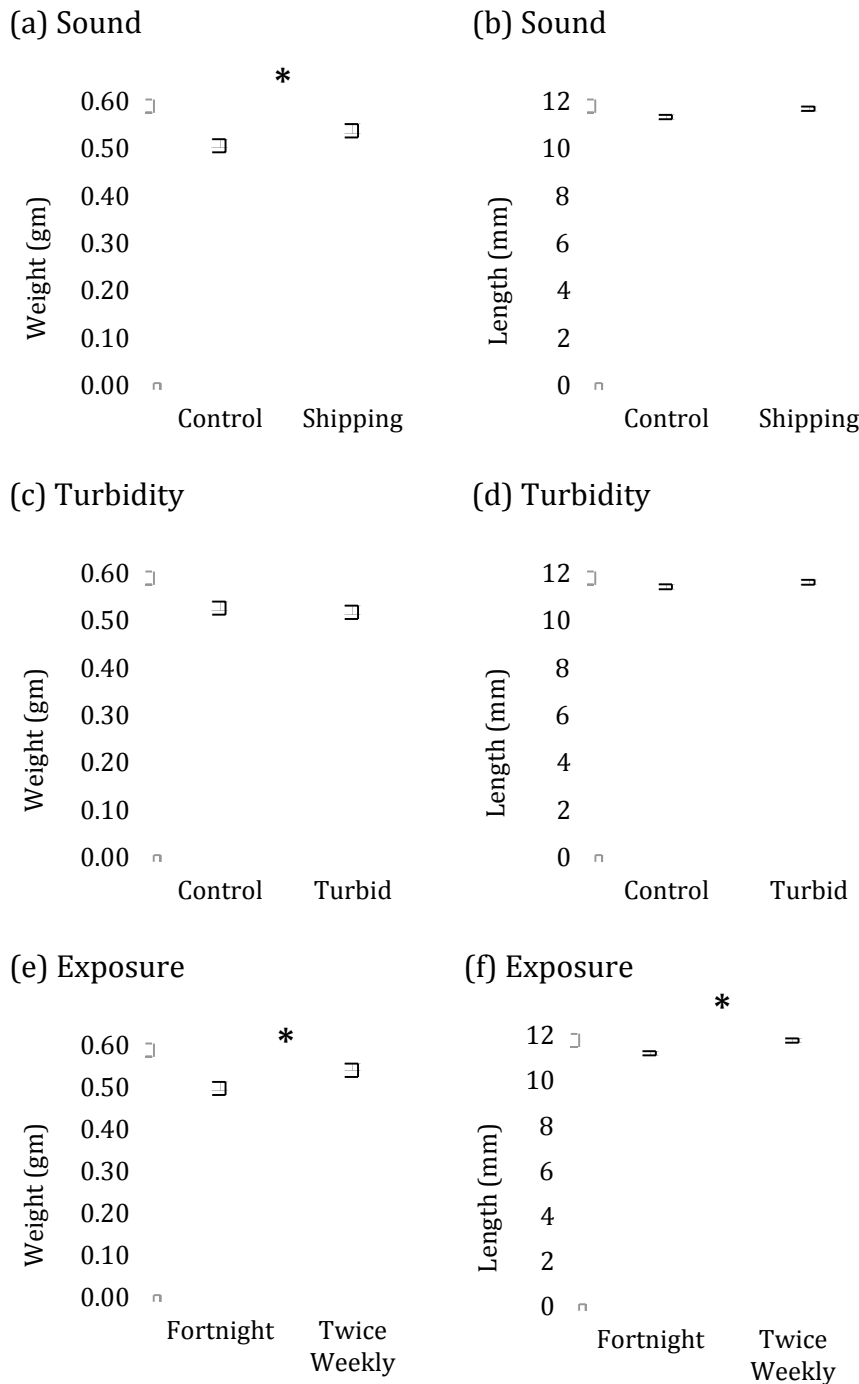


Figure 7. Weight and length of *S. apama* hatchlings (mean \pm standard error) for treatments of sound (a, b), turbidity (c, d) and exposure (e, f). * Represents significant differences between treatment parameters based on ANOVA.

Given there was no significant effect of shipping noise or turbidity on development of *S. apama* eggs, it was not possible to incorporate these variables into the demographic model. Therefore, simulation studies investigating the potential

impacts of shipping on *S. apama* viability under future shipping scenarios were not undertaken.

3.2 Physiological response of adults

Metabolic rate of adult *S. apama* exposed to shipping noise was not significantly different from either resting metabolic rate prior to exposure to sound or when exposed to reef noise (Figure 8).

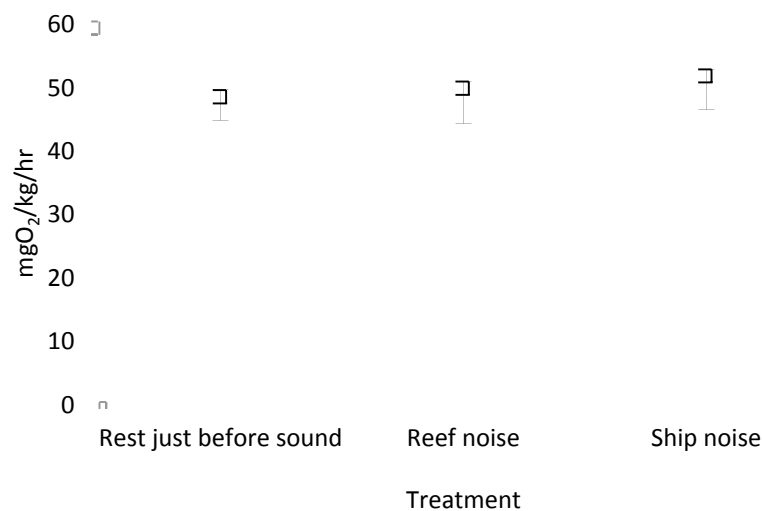


Figure 8. Resting metabolic rate of adult *S. apama* (mean \pm standard error, $n=8$) prior to exposure to reef or shipping noise (rest just before sound) and following exposure to reef noise or ship noise.

4. Discussion

Overall the sensory ecology of cephalopods remains poorly known despite the general increase in anthropogenic-induced underwater noise. This research investigated responses to shipping noise for two life history stages, developing embryos and adults, using two approaches. *Sepia apama* egg development and adult respirometry were not affected by the sound pressure levels associated with shipping noise or by turbidity in the current experiment. These results contrast with several other experimental studies of the effects of noise (shipping, pile driving, seismic air guns) on invertebrates and fish, although this may reflect the lack of

reporting of non-significant results (but see Bolle et al. 2012). It may also reflect the fact that *S. apama* may be more sensitive to particle displacement, which was not examined (see below). The vast majority of literature is on fish and marine mammals, yet comparisons between fish and cephalopods should be viewed cautiously as cephalopods lack a swim bladder. Previous research has suggested that the presence and type of swim bladder was correlated with injury at high sound levels and that species lacking a swim bladder showed no visible injuries (Halvorsen et al. 2012).

Besides a lack of lethal effects on hatching success, there was also no indication of sub-lethal effects on growth, i.e. length and weight of hatchlings or metabolism of adults (oxygen consumption), in the experiments. In fact hatchling weight was actually marginally higher by 6 per cent with exposure to shipping noise. Whether cephalopod hatchlings suffer some effect from shipping noise was not tested. They may, for example, consume less food when exposed to shipping noise such that growth effects are observed. A permanently high sound level led to reduced growth and reproduction rates in shrimp (*Crangon crangon*) because they consumed less food (Lagardere 1982). Similar studies have not been undertaken on cephalopods.

While this study on egg development only focused on lethal effects and sub-lethal effects on embryonic growth, it is important to realise that exposure of eggs or adults to shipping noise may cause effects that did not lead to death within the experimental period, but may impact subsequent long-term survival. Similarly, exposure to noise may also affect behaviour of *S. apama* and hence lead to predation and starvation risks. Several studies have shown, for example, fish exposed to anthropogenic noise have reduced digging behaviour (required for maintenance of shelters), decreased defence against predators, altered social interactions (Bruitjes and Radford 2013) and altered foraging behaviour (Payne et al. in press). Anthropogenic noise also disrupted settlement stage fish from distinguishing natural reef sound (Holles et al. 2013). The hearing ability of fish has also been impaired by noise exposure, but such changes may not necessarily be permanent (Caiger et al. 2012).

Several studies have also investigated physiological effects of shipping noise on invertebrates and fish, but not on cephalopods. While no effect of shipping noise on oxygen consumption of adult *S. apama* was found, shore crabs exposed to ship noise consumed more oxygen compared to those exposed to ambient noise (Wale et al. 2013). Higher oxygen consumption, which reflects metabolic rate, may lead to reduced growth and increased stress, thereby reducing survival (Wale et al. 2013). A metabolic response was also found in shrimp (*Crangon crangon*) exposed to higher sound pressure where the ammonium excretion rate and oxygen consumption rate were higher (Regnault and Lagardere 1983). Differences between *S. apama* and crustaceans may reflect the two groups responding to different components of sound.

Turbidity also had no significant effect on *S. apama* embryonic development and hatching success. We are not aware of other studies that have investigated such an effect on cephalopod egg development. The orientation of *S. apama* eggs and water currents may mean that they are less susceptible to sediment accumulation and turbidity of water than benthic invertebrates such as corals (see Erftemeijer et al. 2012). Once hatched, *S. apama* may use visual cues for prey and predator detection, navigation and for reproductive behaviours. Recent experiments on cuttlefish (*Sepia prashadi* and *S. pharaonis*) demonstrated that cuttlefish respond to predators even in turbid waters (Cartron et al. 2013a; Cartron et al. 2013b).

Significantly higher hatching success was found for *S. apama* eggs exposed to shipping noise and turbidity treatments on a twice weekly basis. These treatments also produced hatchlings that were larger and heavier than the control treatments. These effects may reflect increased handling of the twice weekly treatments compared to the fortnightly treatments. Although all treatments were examined for hatchlings, the increased water movement associated with moving treatments into experimental tanks may have led to greater aeration of eggs. Unfortunately, due to space constraints it was not possible to move all treatments to experimental tanks.

Few studies have specifically examined effects of anthropogenic noise on cephalopods (but see Fewtrell and McCauley 2012). Squid (*Sepioteuthis australis*) exposed to air gun noise were found to eject ink at the first gun signal and then

move backwards with jetting motions when exposed to repeated sounds (Fewtrell and McCauley 2012). Vessels conducting seismic surveys using compressed air guns (which produce high intensity low frequency sound waves, 20-500 Hz) were thought responsible for stranding of giant squid (*Architeuthis*) in three locations in the northern hemisphere (Guerra et al. 2011). Two studies that examined the influence of sound (50-400 Hz sinusoidal wave sweep) on four cephalopod species (cuttlefish *Sepia officinalis*, octopus *Octopus vulgaris* and squids *Loligo vulgaris* and *Illex coindetti*) found that all individuals exposed to sound presented lesions with damage to parts of the statocyst and the appearance of these lesions became more pronounced in specimens observed 96 h after sound exposure. In both studies control treated cephalopods showed no lesions in the statocysts (André et al. 2011; Solé et al. 2013).

Sound consists of particle motion and sound pressure. Cephalopods appear to be sensitive to low-frequency particle motion rather than high-frequency motion and may not detect sound pressure (Packard et al. 1990; Kaifu et al. 2011). Only sound pressure and not particle displacement was tested, which may explain the lack of significant results. Cephalopods are sensitive to low frequency sound between 20 and 200 Hz (Packard et al. 1990), although the absolute sensitivity and spectral range of hearing is likely to vary among different cephalopod species (Slabbekoorn et al. 2010).

5. Conclusion

This study sought to investigate the potential impacts of shipping on *S. apama* through laboratory experiments. The literature suggested that the effects of sound on cephalopods was unclear, although they may be sensitive to low frequency sounds, as both physical damage to statocysts and strandings have occurred in the past. With increased shipping likely in Upper Spencer Gulf, associated with new port developments, there was potential for increases in shipping noise, shipping frequency and associated turbidity. This region is also renowned for its breeding aggregation of *S. apama*. The effects of shipping noise, shipping frequency and turbidity on hatching success and hatchling size was examined. In addition, the

effects of shipping noise on oxygen metabolism of adults, which is an indicator of potential stress, was examined.

The laboratory investigations of the effects of turbidity and the sound pressure component of shipping noise suggest that these two factors do not impact *S. apama* in regards to the parameters measured. The particle displacement component of sound was not investigated; other factors not investigated included whether there are differences in distribution between quiet and noisy environments, whether there are behavioural effects that may subsequently impact survival or whether shipping sounds mask biologically relevant sounds. Several recent reviews point to the need for a broader examination of the effects of anthropogenic noise (see future research needs).

6. Implications of the study for giant Australian cuttlefish conservation

Although this study found no significant effect from shipping noise or turbidity on recently hatched individuals which were exposed to treatment conditions for the three months of their development, only the sound pressure component of noise was examined. Similarly, short-term exposure of adults to shipping noise suggested that they were not stressed. However, in the field *S. apama* may avoid noise and move elsewhere, but this would be difficult to ascertain. Additional research (see future research needs below) is required before definitive conclusions regarding impacts of shipping can be made.

The experiments were only able to expose developing eggs to shipping noise for less than 24 h due to logistical constraints. This is less than the approximate time (30 h) that a ship is idling at the current Port Bonython jetty. With future projections of increased shipping in the region *S. apama* may be exposed to shipping noise on a more regular basis. Currently, a ship visits Port Bonython approximately every fortnight, but if the proposed new port development goes ahead then a ship may visit every three days.

The experiments investigated lethal effects on egg development and sub-lethal effects on embryonic growth and metabolism. Shipping noise may also impact longer term survival and influence behaviour thereby affecting the spatial distribution of organisms and their ability to respond to predators and prey, as well as reproductive activities (Francis and Barber 2013). If behavioural changes are found it is also important to determine whether these changes have fitness consequences. Currently there is also a lack of information on community-level effects associated with shipping noise and turbidity and whether there are flow-on effects to *S. apama*.

Sepia apama eggs exposed to two of the three treatments, namely shipping noise and increased shipping frequency, showed significant effects on size of hatchlings. The differences were relatively small (3-7%) with hatchlings exposed to shipping noise and twice weekly treatments being slightly larger. Larger individuals may be able to swim and feed better than smaller individuals, but this would require further testing to properly ascertain fitness consequences. It should also be noted that these differences may reflect increased handling and aeration of treatment eggs compared to control eggs. No effect of turbidity was found.

7. Future research needs

Experiments focused on lethal effects on egg development and sub-lethal effects associated with growth and metabolism. They also focused on short-term exposure which may not reflect responses to noise of longer duration. Noise can cause physical damage (e.g. lesions in the statocyst) and influence behaviour, which was not investigated. Anthropogenic noise is increasing in the marine environment and is not just comprised of commercial shipping noise. Other anthropogenic noise sources, such as recreational boating and noise associated with jetty maintenance and construction, also need to be considered. Below, areas involving noise that require future research are highlighted.

Future needs for noise related research:

- Determining the sensory capabilities of *S. apama* is vitally important, because there will be no direct effect if the sound frequency is outside their hearing range. An understanding of the hearing range of *S. apama* would also allow future experiments to target the appropriate range.
- No effects of sound pressure on egg development and adult metabolism were found, but whether shipping noise effects hatchlings is unknown.
- Several studies suggest that the greatest effects are likely to occur in relation to behaviour. Understanding whether noise distracts *S. apama* needs to be fully explored.
- Most noise-related impacts involve behavioural responses that affect temporal patterns, spatial distributions or movements, foraging efficiency and predatory behaviour, mate attraction and territorial defence (Francis and Barber 2013). Site abandonment is well known for birds, but largely unexplored for other organisms. A search in USG for alternative spawning sites failed to find *S. apama* in other areas (Steer, 13 February 2014) and there was no relation between *S. apama* abundance and shipping numbers (Steer et al. 2013). The potential for avoidance behaviour that may prevent *S. apama* from inhabiting the reef area in close proximity to shipping requires further investigation, although understanding the effects of noise on spatial distribution may be difficult to ascertain.
- Whether cephalopods exposed to shipping noise show similar responses to predators as those not exposed to noise could be investigated. Similarly, how noise may affect reproductive mating behaviour is unknown.
- If behavioural responses are found, the next step would be to determine whether behavioural changes have fitness consequences such as reduced reproductive success, and reduced foraging ability.
- Studies integrating the effects of noise exposure beyond the level of the individual are also required (Francis and Barber 2013). Community-level consequences resulting from altered species interactions and population responses of multiple species are lacking.

A whole of Gulf view is required that considers the full range of activities (e.g. fishing, aquaculture, shipping, power, marine parks etc) occurring in the region and how they interact. Spencer Gulf is currently on the verge of major expansion in industrial activity, with associated increases in other activities (e.g. port development, desalination, shipping), yet the gulf is a relatively pristine system. The anticipated expansion in activity has the potential to lead to many resource-use conflicts, not just between industry and the environment, but also between different industries. It is necessary to consider and map individual and cumulative impacts, possible trade-offs required and develop models encompassing ecosystem-based marine spatial management as advocated in the Spencer Gulf Ecosystem Development Initiative. This initiative represents an opportunity for South Australia to use Spencer Gulf as a model for integrated marine management and for this to occur well before significant development in the Gulf.

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Appendix A. Desktop review

1. Summary

Upper Spencer Gulf (USG) in South Australia has been recognised as a priority area for regional sustainability planning given its significant potential for industrial development. Giant Australian cuttlefish, *Sepia apama*, is an iconic species in USG that forms the only known breeding aggregation of cuttlefish in the world. This document provides a brief background to the species, and investigates trends in abundance and biomass from two complementary approaches: one focused in USG and the other focused along the breeding aggregation area at Point Lowly. Both data sets suggest significant declines in abundance and biomass in USG especially near the breeding aggregation site. At the breeding aggregation, abundance has dropped by 90% since 1999 and biomass by 95% raising some concern for the population. Given significant declines in abundance and biomass of *S. apama* potential factors contributing to the decline and threats into the future are briefly examined. Based on examination of correlations between a range of factors and *S. apama* abundance and biomass there were no clear influences of abiotic, biotic or other potential threats, with the possible exception of rainfall. Finally, potential impacts of shipping on cephalopods are examined. This review suggests that there is potential for shipping noise to cause physical, physiological and behavioural changes, although few studies have specifically investigated effects of shipping on cephalopods.

2. Introduction

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 rock reef in 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 *S. apama* 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) area near Point Lowly in USG. Abundances as high as 105 cuttlefish per 100 m² 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 three years later, became a year-round closure. In March 2013 the entire northern Spencer Gulf (from a line between Wallaroo and Arno Bay) was closed to the taking of cuttlefish for an initial period of 12 months and subject to annual review. The core area of the breeding aggregation has therefore received conservation protection for some time. Until recently, *S. apama* still formed part of commercial and recreational fisheries.

USG in South Australia has been recognised as a priority area under the Department of Sustainability, Environment, Water, Population and Communities' Sustainable Regional Development program, which forms part of the strategy for regional sustainability planning. The USG region has been recognised as a significant growth area with opportunities for expansion of mining, and a large number of new mineral/mineral processing ventures possible. Associated with such development will be increased shipping and associated infrastructure (port development, desalination plants).

3. Information on population trends (abundance and biomass)

While *S. apama* are distributed throughout southern Australia, the only location where abundance and biomass have been routinely estimated is in USG. Here, abundance (and biomass) of *S. apama* has been estimated using two approaches:

- (1) *S. apama* has been sampled as part of the annual juvenile snapper trawls which have been undertaken in April of each year (2000-2010 inclusive),
- (2) Estimates of abundance of *S. apama* along the Point Lowly breeding aggregation area have been undertaken in ten of the 15 years inclusive of 1998 and 2012 primarily during the peak spawning time.

Both these estimates of abundance provide an indication of temporal change of the population in Spencer Gulf, but cover different areas of USG and use different methods (see section 3.1 and 3.2 for details). They are therefore not directly comparable.

3.1. Estimates of abundance from juvenile snapper trawls

In April of each year (2000-2010 inclusive) scientific trawl surveys have been undertaken in USG targeting newly recruited juvenile snapper. These surveys also catch *S. apama* and can be used to estimate their abundance in USG. The April surveys occur just prior to *S. apama* arriving on the breeding aggregation site at Point Lowly and therefore may represent individuals moving onto the aggregation site from locations throughout the USG. Over 100 trawls were undertaken in the USG between 2000 and 2005 and approximately 70 trawls over the period 2006-2010 (Figure 1, 2). Each trawl sample traversed approximately 0.5 nautical miles of seabed and used a small, purpose-built otter-trawl (Fowler and Jennings 2003). The percentage of *S. apama* catch for each trawl ranged from 6% to 100%, with at least 50% of the catch being counted for more than 85% of the trawls. Total *S. apama* biomass was only recorded for each trawl for 2002-2010.

Two previous publications have presented some (2000 and 2001, Hall and Fowler 2003) or all (Gillanders and Payne In Press) of the *S. apama* trawl survey data as abundance distribution maps (Figure 1), but there have been no quantitative

analyses of the data. The data are currently being analysed as part of a Fisheries Research Development Corporation (FRDC) project (e.g. Prowse et al. submitted). The distribution abundance maps show that *S. apama* is patchily distributed, with a number of trawl samples having no *S. apama*, and that the distribution varied through time (Figure 1).

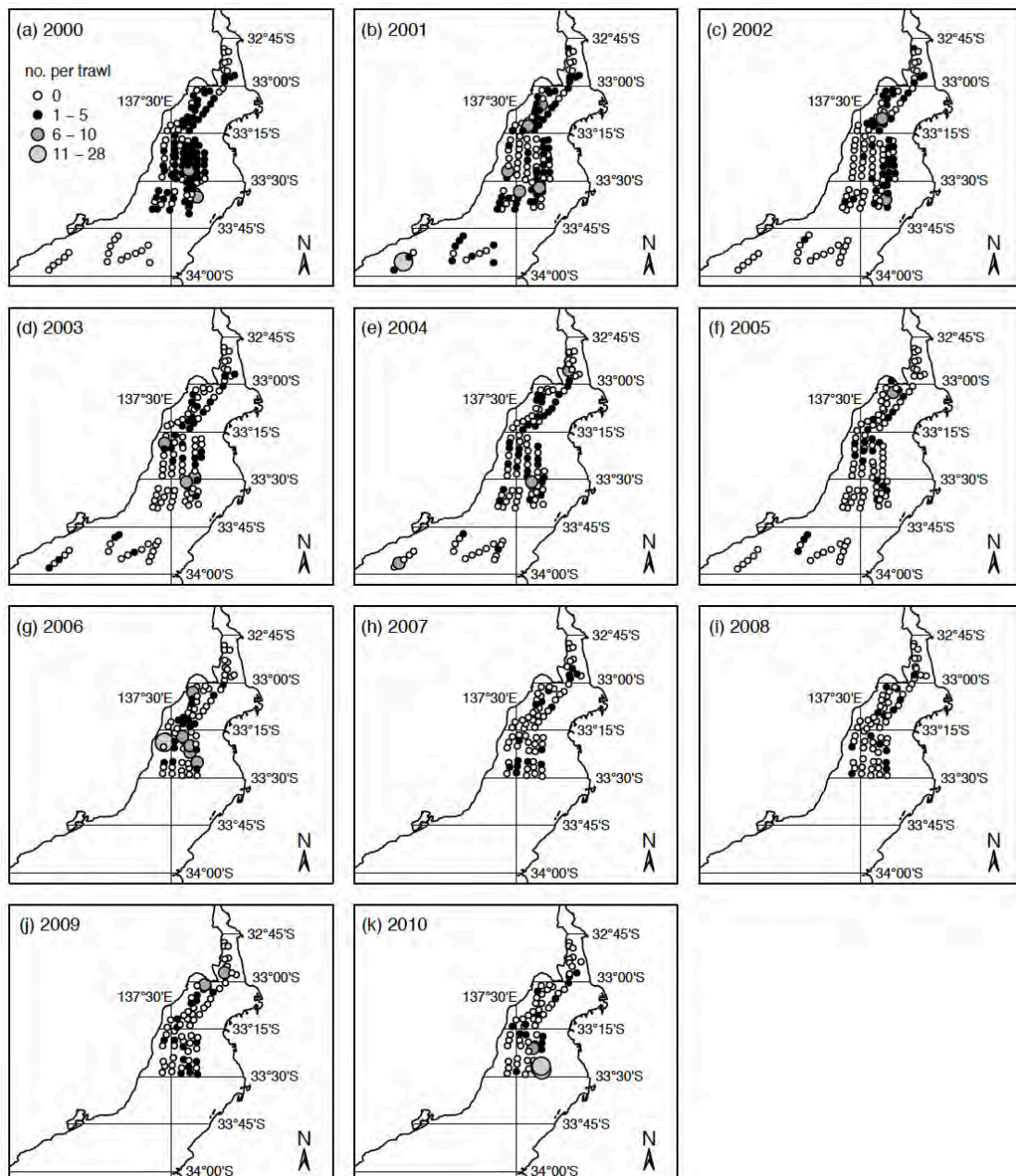


Figure 1. Distribution and abundance of *S. apama* in USG during April of each year. Open circles indicate trawl samples in which no *S. apama* were obtained and the

size of closed circles represents the abundance of *S. apama* for each trawl sample. From: Gillanders and Payne (In Press).

As part of a current FRDC project on *S. apama*, Bayesian techniques are being used to analyse *S. apama* abundance from these USG trawl surveys. Bayesian techniques were used to investigate abundance of *S. apama* in northern and southern harvest zones of USG with the northern harvest zone including the breeding aggregation area (Figure 2). Bayesian models showed that *S. apama* abundance in USG is spatially structured and decreased over the period 2000-2010 for the two harvest zones, although the decline was less clear for the southern harvest zone (Figure 3). This decline throughout USG suggests that declining winter abundance on the Point Lowly breeding grounds (see section 3.2) is not due to the failure of *S. apama* to aggregate, nor to the aggregation occurring elsewhere, but is part of a systemic decrease in the size of the USG population (Prowse et al. submitted).

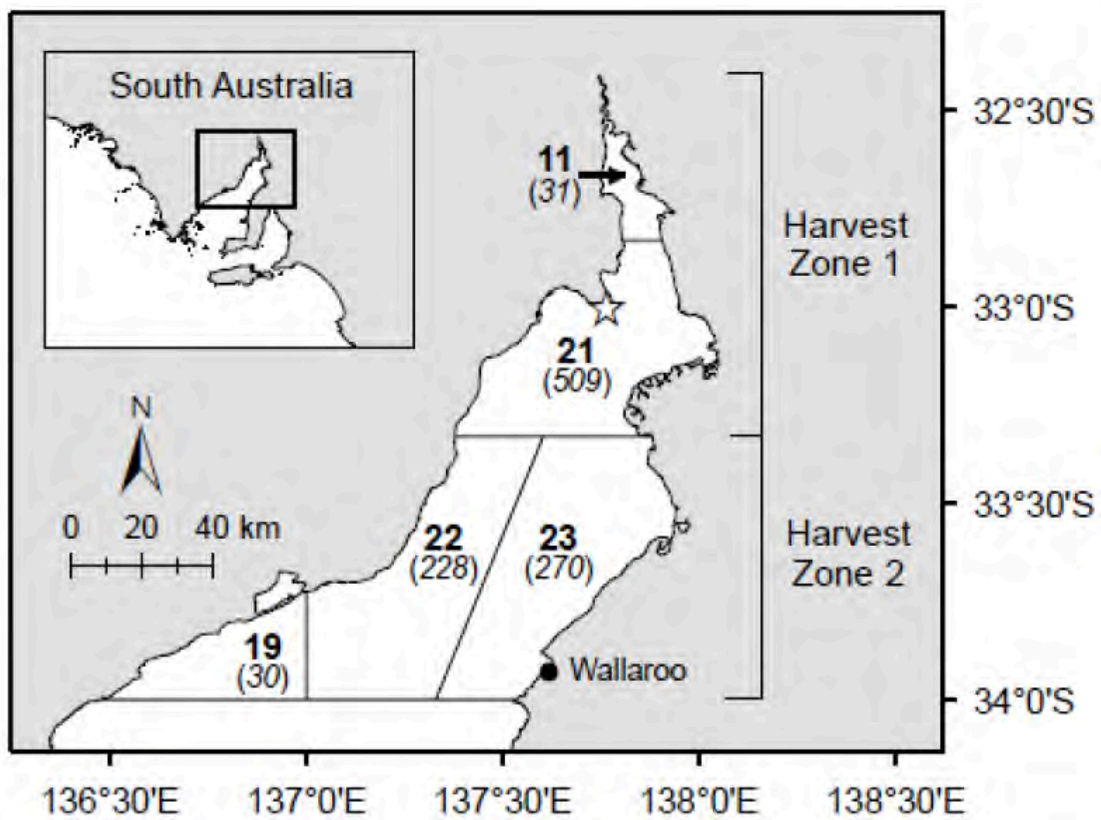


Figure 2. The USG region, showing five Marine Fishing Areas (MFA; bold numbers) designated for the South Australian Marine Scalefish Fishery. For each MFA, italicised

numbers in brackets represent the total number of trawls performed over 2000-2010 for SARDI's juvenile snapper surveys. MFA 21 contains the *S. apama* aggregation site at Point Lowly, which is marked with a star. Also shown are the two harvest zones referred to in the text and used for Bayesian statistical analyses: Harvest Zone 1 in the north (MFAs 11 & 21) and Harvest Zone 2 in the south (MFAs 19, 22 & 23). From: Prowse et al. (submitted).

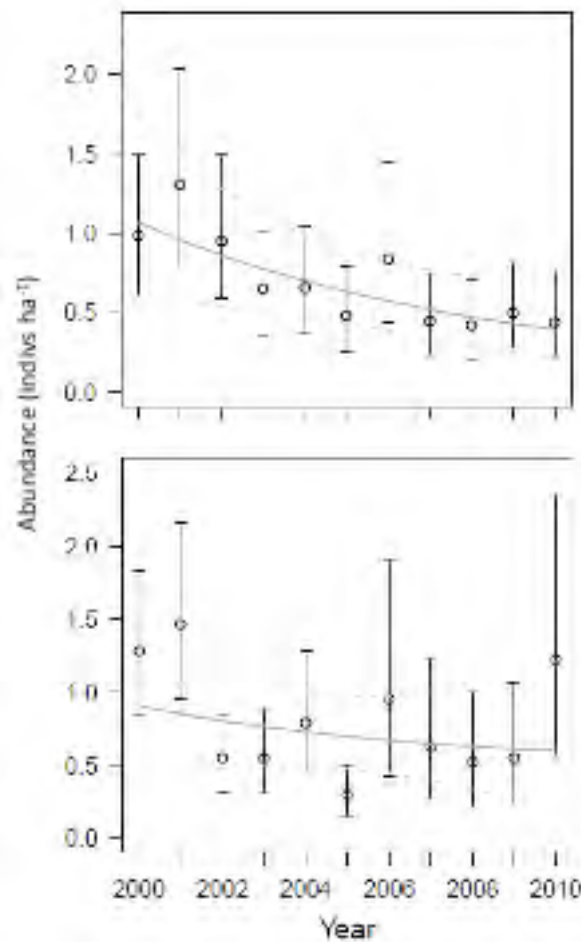


Figure 3. Estimates of *S. apama* abundance for the northern (top graph) and southern (bottom graph) harvest zones defined for USG. Northern harvest zone comprises marine fishing areas (MFA) 11 and 21, whereas southern harvest zones encompass MFA 19, 22 and 23 (see Figure 2). Shown are random year effects (points) and fitted trends (lines), together with 95% Bayesian credible intervals, from a spatial abundance model. From: Prowse et al. (submitted).

3.2. Estimates of abundance along the Point Lowly breeding aggregation area

Abundance and biomass of spawning *S. apama* on the Point Lowly breeding aggregation area (see Figure 2) have been estimated by counting the number and visually estimating the size (mantle length) and sex of all *S. apama* along four or eight 50 x 2 m belt transects at a number of sites (Figure 4). These surveys made by SCUBA divers provide an average estimate of *S. apama* abundance per 100 m² and an estimate of average weight (biomass) per 100 m². Ten of the thirteen sites surveyed are used for overall estimates of abundance and biomass (exceptions: Backy Point which has not been surveyed every year, and One Steel Wall and Santos Jetty, where access has been restricted in some years due to shipping traffic) (Steer et al. 2013). Most of the sites used for overall estimates of abundance and biomass have surveys undertaken at shallow and deep depths (exceptions: False Bay, Point Lowly East and Fitzgerald Bay), in which case there are 8 replicate belt transects per site (Steer et al. 2013).

The surveys were initially undertaken as part of an FRDC funded project that also comprised part of Hall's PhD thesis (1998-2001 inclusive) (Fowler and Jennings 2003). This research project was initiated following targeting of *S. apama* on the breeding aggregation by fishers in 1997 and early 1998. No surveys were undertaken from 2002-2004 inclusive, and then a survey was undertaken in 2005 following concerns about declining abundances (Steer and Hall 2005). No surveys were again undertaken in 2006 and 2007. BHP Billiton then conducted surveys from 2008-2011 as part of its Olympic Dam environmental impact statement project (BHP Billiton 2009b; Hall 2012) and has an ongoing commitment to surveys. SARDI Aquatic Sciences undertook surveys in 2012 (and 2013 – data not yet released) and also refined the methodology for future surveys (Steer et al. 2013). All these surveys have used similar methodology with counts and estimates of biomass made by SCUBA divers at a number of sites using the belt transect methodology described above. The number of surveys per year has varied, although the aim has always been to ensure that at least one survey is conducted during the peak winter period when *S. apama* aggregate to breed.

Abundance of *S. apama* was low in 1998, however estimates in this year were made after substantial fishing on the breeding aggregation (Figure 5) (Hall and Fowler 2003). Mid-way through 1998 the breeding aggregation area was closed to fishing. In 1999 abundance was estimated at 182 642 (\pm 34 422) individuals, and remained similar through to 2001, after which time numbers have generally decreased (Hall and Fowler 2003; Steer and Hall 2005; BHP Billiton 2009a; BHP Billiton 2011). Dramatic decreases in abundance occurred in 2011 and 2012 (BHP Billiton 2011; Steer et al. 2013). The estimate of 18 530 animals in 2012 represents a 90% reduction in population size from the peak in 1999. A similar trend was found for biomass where a 95% reduction over a 13 year time period was observed (Figure 6). Abundance and biomass estimates for 2013 have not yet been released (Steer, SARDI Aquatic Sciences, pers. comm.).



Figure 4. Point Lowly region showing location of sites used to survey *S. apama*. From: Steer et al. (2013). The star in Figure 2 shows the location of Point Lowly.

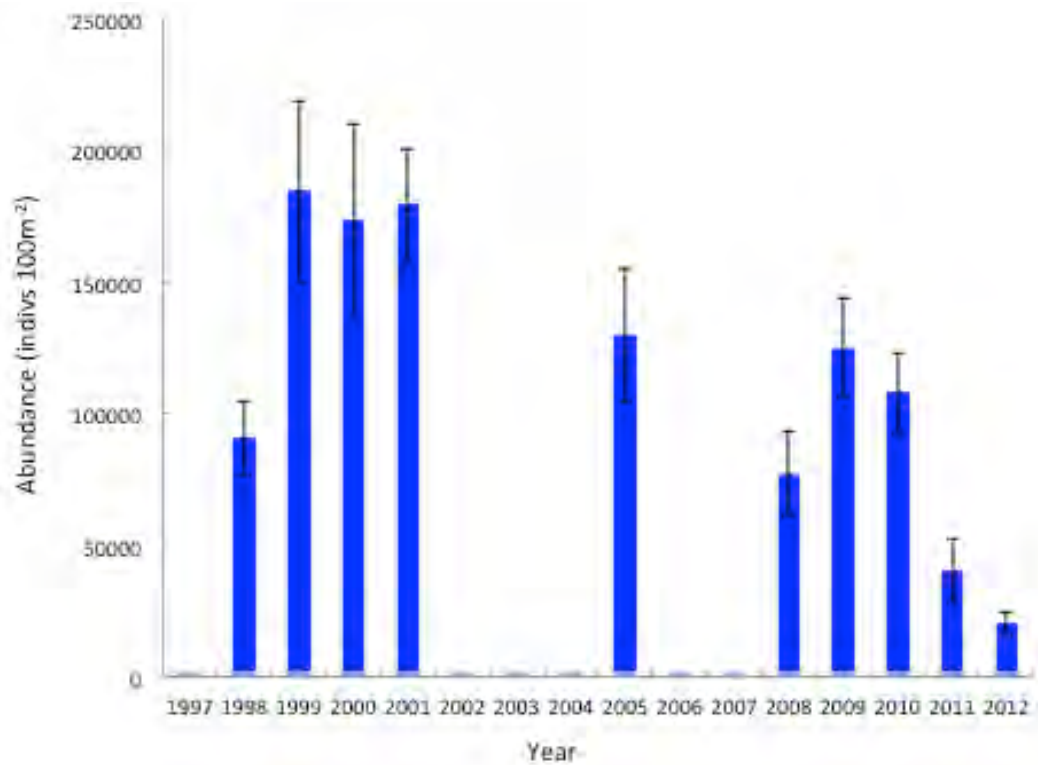


Figure 5. Estimates of *S. apama* abundance (mean and standard error) along the Point Lowly breeding aggregation area. Note, no estimates were made in 1997, 2002-2004 and 2006-2007, and estimates in 1998 were made after substantial fishing on the breeding aggregation. The breeding aggregation area has been closed to fishing since mid 1998. From: Steer et al. (2013).

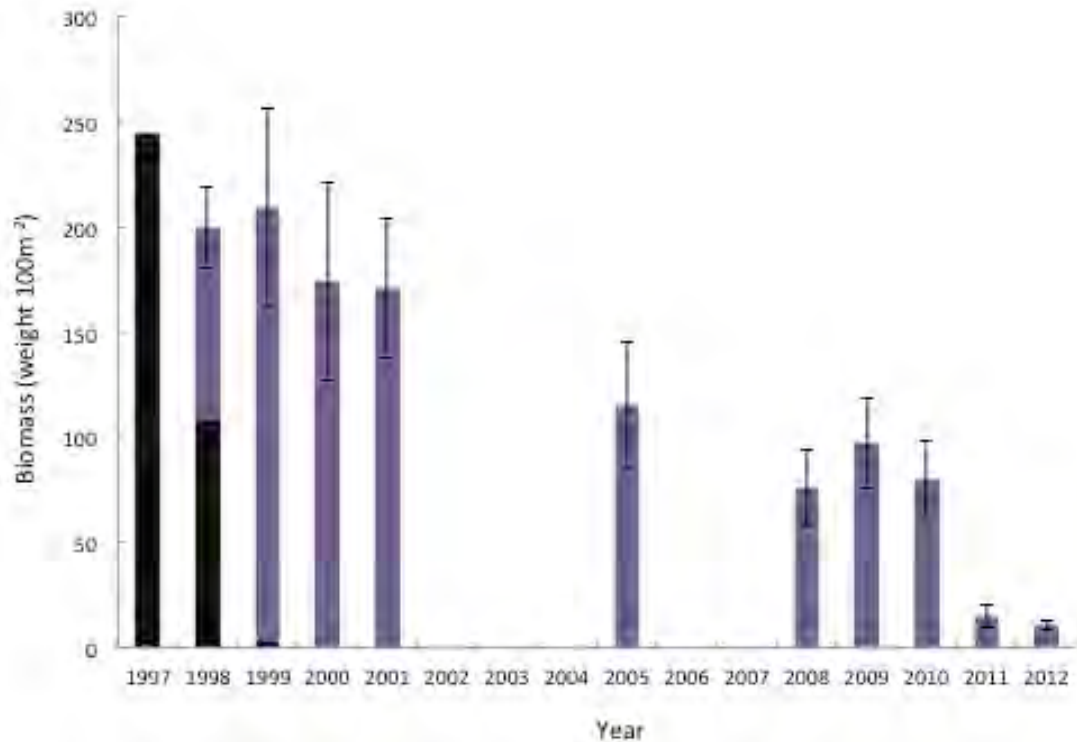


Figure 6. Estimates of *S. apama* biomass (mean and standard error) along the Point Lowly breeding aggregation area. Note, no estimates were made in 1997, 2002-2004 and 2006-2007. Solid black bars represent fishery catch from MFA 21 in 1997 and 1998 prior to the closure to fishing in the area in mid 1998. Catch from MFA 21 has been negligible since 1998. For location of MFAs see Figure 2. From: Steer et al. (2013)

4. Potential threats to giant Australian cuttlefish

Given both approaches for estimating abundance and biomass have shown declines through time, Steer et al. (2013) explored the potential cause of the *S. apama* decline providing a preliminary investigation of factors that may require further investigation. Further exploration of factors contributing to the decline of *S. apama* in USG forms part of a current FRDC project. Below we summarise potential factors (Table 1). Broadly, these factors include potential for a natural cycle in abundance, a range of abiotic and biotic factors, and a range of possible threats associated with development some of which may not currently be occurring. Where possible the variables were correlated with abundance and biomass of *S. apama* on the breeding aggregation (i.e. with data presented in Figure 5 and 6).

The decline in abundance of *S. apama* may represent a natural process (Table 1). Cephalopod populations are known to show considerable fluctuation in abundance including on 10-20 year cycles (see references in Steer et al. 2013). Anecdotal newspaper reports suggest dead and dying cuttlefish (species not specified) occurred on beaches in 1910 and 1947. These may represent the end of the spawning season for a species that naturally senesces, but it is unclear whether they came from potential aggregations areas. In addition, the exact site these dead and dying cuttlefish originated from is unknown. Commercial fishing data only show increases in abundance of *S. apama* taken in commercial catches in the late 1990s although fishing occurred earlier (Hall and Fowler 2003), and anecdotal fishers reports suggest that *S. apama* were not always in such high abundance (Steer et al. 2013). Studies along the Point Lowly Peninsula involving SCUBA divers during winter months in the 1980s suggest few cuttlefish were present in 1982, but large numbers were observed in 1986 (Steer et al. 2013). Thus, it is possible that the high abundances seen in the late 1990s represent a natural cycle, but it is not clear why numbers may fluctuate.

There were insufficient long-term data to adequately investigate the influence of many abiotic factors on *S. apama* abundance and biomass (Table 1). Of those factors investigated only rainfall showed a significant correlation with *S. apama* abundance. Increased rainfall in late summer and autumn led to a decrease in abundance of *S. apama* but whether this reflects changes in salinity, pollution via terrestrial runoff or a direct influence on water clarity is unknown (Steer et al. 2013). In addition, in many cases annual (and or monthly) abiotic variables were correlated with *S. apama* abundance, but there is potential for short-term spikes or pulses to affect *S. apama*. Although annual shipping traffic at Port Bonython showed no correlation with *S. apama* abundance it is unknown how noise impacts *S. apama* (see section 5).

The effects of a range of biotic factors including predators, prey and human influences (e.g. fishing) did not provide evidence of impacts on *S. apama* abundance

and biomass, although again annual data were often used (e.g. for all predators and prey) (Table 1). Calamary, *Sepioteuthis australis*, have increased through time suggesting that factors contributing to the decline of *S. apama* are not affecting all cephalopods (Steer et al. 2013). As *S. apama* declined, opportunities for *S. australis* to increase may have occurred. Competition between *S. australis* and *S. apama* for space and resources may now be occurring (Steer et al. 2013).

Industrial and other development in the region has potential to influence *S. apama* abundance previously and in the future (Table 1). Of other potential threats not addressed earlier coastal development including port development and associated increased shipping, as well as potential saline discharges from desalination plants may affect *S. apama*. These developments have potential to impact *S. apama* through noise impacts, as well as effects associated with changes in turbidity and salinity which may affect early life history stages (eggs) and adults. Although effects of saline discharge on embryo development of *S. apama* have been investigated (Dupavillon and Gillanders 2009), there has been no research on the potential impacts of shipping (noise and turbidity) on *S. apama* or the potential impact of saline discharge on adults.

Table 1. Potential factors contributing to a decline in *S. apama* as summarised from Steer et al. (2013) Additional threats listed by Halpern et al. (2007) have been included as have other proposed and potential activities in USG.

Factor	Evidence	Other comments
Natural process	No reports of <i>S. apama</i> in early 1980s, but present in 1986; historical newspaper reports suggest dead & dying cuttlefish in USG but not clear where they came from	Species of cuttlefish not listed in newspaper reports
Abiotic factors		

Climate change	Sea level, sea temperature (see below), ocean acidification, ozone/UV – potential long-term effects possible but unlikely to have been significant changes in last 10-15 years	Potential for longer term impacts; Prowse et al. (submitted) found decline in abundance in USG could not be attributed to climatic factors
Temperature	Average annual temperature showed no correlation with <i>S. apama</i> numbers. Lagged monthly temperature 0-12 months prior to peak spawning also did not affect abundance	Potential for short-term spikes or pulses in temperature to affect embryo survival
Turbidity/Sediment	Effect unknown	No long-term turbidity data available
Onshore wind (used as proxy for water clarity)	No correlations found with abundance for annual or monthly data	
Salinity	High salinity can impact embryo development	No long-term salinity data available
Rainfall (proxy for freshwater input)	Rainfall inversely correlated with abundance	Potential effect of salinity, terrestrial runoff or influence on water clarity
Hypoxia	No known hypoxic areas in USG	
Pollution	Nutrients, metal pollutants, other toxicants, hydrocarbons, noise (see	Variety of potential sources (Whyalla Steelworks, Port Pirie,

	details below)	Whyalla Waste Water Treatment Plant, sea cage aquaculture, SANTOS hydrocarbon processing plant)
Nutrient input	No correlation between nutrient load (annual) and <i>S. apama</i> abundance or biomass	Nutrients may have an indirect effect as influence growth of ephemeral algae
Metal pollutant input	Weak positive correlation between Pb, Mn & Zn levels and <i>S. apama</i> biomass (i.e. decline in metals correlated with decline in <i>S. apama</i>)	Spawning area unlikely to have been exposed to localised metals in vicinity of Port Pirie & Whyalla steelworks
Other toxicant input	Unknown	No information available
Hydrocarbon input	Range of invertebrates show no evidence of hydrocarbon residues	Hydrocarbon contamination from Santos unlikely to impact <i>S. apama</i>
Noise pollution	No correlation between current shipping levels (2.4 vessels per month) and abundance or biomass of <i>S. apama</i> ; unknown how shipping noise per se may impact <i>S. apama</i>	Uncertain whether offshore shipping traffic may interrupt potential movement and migration pathways for <i>S. apama</i> entering and exiting Point Lowly spawning grounds
Biotic influences		
Predators	Dolphins, New Zealand fur seals, snapper, Australian salmon,	Where possible, correlations made between <i>S. apama</i>

	yellowtail kingfish (see details below)	abundance and annual catch of predators; spikes in abundance of predators may however occur, but difficult to know whether these impact abundance of <i>S. apama</i>
Dolphins, <i>Delphinus delphis</i> and <i>Tursiops aduncus</i>	Dolphins known to feed on <i>S. apama</i>	No estimates of local dolphin abundance, but anecdotal reports of an increase in abundance of resident dolphins
NZ fur seals, <i>Arctocephalus forsteri</i>	Unlikely to influence <i>S. apama</i> abundance; further research underway investigating diet of <i>A. forsteri</i>	Relative abundance & foraging intensity of <i>A. forsteri</i> in USG unknown
Snapper, <i>Chrysophrys auratus</i>	No correlation between annual commercial catch of <i>C. auratus</i> and abundance or biomass of <i>S. apama</i>	
Australian salmon, <i>Arripis truttaceus</i>	No correlation between annual commercial catch of <i>A. truttaceus</i> and abundance or biomass of <i>S. apama</i>	
Yellowtail kingfish, <i>Seriola lalandi</i>	No correlation between <i>S. lalandi</i> escapes and <i>S. apama</i> abundance or biomass	Nearby <i>S. lalandi</i> aquaculture; No data on wild <i>S. lalandi</i> abundance

Prey	Western king prawns, blue crabs	Diet of <i>S. apama</i> currently under investigation
Western king prawns, <i>Penaeus (Melicertus)</i> <i>latisulcatus</i>	No clear relations between annual estimates of commercial catch of <i>P.</i> <i>latisulcatus</i> and abundance or biomass of <i>S. apama</i>	
Blue crabs, <i>Portunus</i> <i>armatus</i>	Inverse correlation between annual estimates of <i>P. armatus</i> CPUE and <i>S. apama</i> abundance and biomass	Suggests that <i>S. apama</i> predation mediates blue crab abundance
Species invasion	No known species invasion in USG	
Harmful algal bloom	No known HAB in USG	
Diseases & parasites	Full necropsies of <i>S.</i> <i>apama</i> in 2004 and again in 2010 found few parasites on <i>S. apama</i>	Personal communication from Whittington (SA Museum) & Catalano (University of Adelaide)
Habitat	Habitat not currently limited in terms of potential egg-laying sites	Blooms of <i>Hinckesia</i> <i>sordida</i> observed but unknown if affects egg laying and egg development
Fishing – Marine scalefish	Initial decline was a result of fishing; increasing areas of USG have been closed to removal of <i>S.</i> <i>apama</i> ; catch negligible	Taken primarily for bait
Fishing – Spencer Gulf	Taken as bycatch; no	Current by-catch

prawn fishery	relation between trawl effort and <i>S. apama</i> numbers	monitoring of <i>S. apama</i> is underway as part of regular stock assessment surveys
Fishing – illegal/unreported	Some illegal fishing reported but full extent of illegal and unreported catch unknown	
Fishing - recreational	USG now closed to recreational fishing; impact on <i>S. apama</i> by this sector largely unknown	
Tourism	Unknown effects of diving and snorkling but little change in behaviour of <i>S. apama</i> observed whilst diving; likely to be minimal	
Aquaculture	Nearby <i>S. lalandi</i> aquaculture (see above); effects unknown	
Other potential threats		
Coastal engineering (e.g. breakwalls)	May provide additional habitat but dependent on structure	
Coastal development (e.g. Ports & dredging)	Turbidity may impact adult breeding behaviour	<i>S. apama</i> require visual cues for reproduction
Benthic structures	May provide additional habitat depending on	

	orientation	
Ocean mining	Possible impacts through noise	Mining leases exist in SG waters
Desalination plant	See salinity; possibility for other pollutants & turbidity effects; unknown how saline discharge might affect migratory routes onto/off breeding aggregation	Previous experiments conducted on eggs; current experiments underway examining salinity and temperature effects and their interaction
Defence	Depends on activities in water	

5. Potential impacts of shipping on cephalopods

5.1. Sound in the marine environment

The underwater environment is filled with ambient (natural) sounds, however over the past century, anthropogenic (human induced) sounds in and near the water have increased, adding artificial sounds to the environment (Slabbekoorn et al. 2010; André et al. 2011). The sources of artificial sounds in the marine environment include constructions or operational noises (pile-driving, dredging, drilling, offshore wind farms), shipping (freight, passenger, fishing and recreational) as well as other sounds such as sonar, seismic surveys and explosions (Götz et al. 2009; Slabbekoorn et al. 2010). Sound travels five times faster and at higher amplitudes and can travel greater distances in water in comparison to air (Slabbekoorn et al. 2010). Depending on the intensity (sound pressure level) at the source, the pitch (frequency), the distance between the source and the receiver, and the cumulative effect when a source is long-lasting or repeated in time, sound can potentially affect marine organisms (Götz et al. 2009). Little is known on how excessive noise is

impacting marine life, but it is expected to cause physical, physiological and behavioural influences (André et al. 2011).

Sound can be thought of in terms of both particle motion and pressure fluctuation. For fish, the ear serves to detect sound, whereas the lateral line while detecting low frequency sound, is primarily considered to detect water motion relative to the body (Slabbekoorn et al. 2010). Marine animals regularly utilise acoustic signals for important biological activities such as intraspecific communication (reproductive behaviour), predator avoidance, habitat identification, foraging and orientation (Mooney et al. 2012).

5.2. Sound sensitivity in cephalopods

Little is known about sound perception in cephalopods, although the recent literature suggests that cephalopods are sensitive to low frequency sounds (Packard et al. 1990; André et al. 2011). How sound detection is received by cephalopods remains unclear. The statocyst in cephalopods (which functions similar to fish otoliths) and the epidermal head and arm lines (hydrodynamic receptor systems similar to fish and amphibian lateral lines) are the likely organs for sound detection (Komak et al. 2005; Mooney et al. 2010). Cephalopod bodies have a similar density to water and lack pressure to particle motion transducers such as swim bladders coupled to their statocyst (Hu et al. 2009; Mooney et al. 2010). A statocyst detector system, which may primarily be for measuring acceleration and orientation, is innately equipped to also detect the particle motion component of a sound field for an animal with an impedance similar to the surrounding medium (Mooney et al. 2010). The threshold values measured as particle acceleration for three species (cuttlefish, *Sepia officinalis*, octopus, *Octopus vulgaris* and squid, *Loligo vulgaris*) declined towards the lower frequencies in the range between 1 Hz and 100 Hz; within this experiment animals were responding to particle motion rather than to sound pressure (Packard et al. 1990). Juvenile cuttlefish (*Sepia officinalis*) displayed behavioural responses to a range of frequencies (10-600 Hz) associated with local water movement; these responses were greatest around frequencies of 20, 45, 75, 180 and 300 Hz (Komak et al. 2005). Squid (*Loligo pealeii*) were similarly

found to respond to the acceleration and particle motion components of a sound field, not pressure (Mooney et al. 2010). The directional capabilities of the squid statocyst may allow squid to establish sound direction, while the lateral line may further allow detection of relative water motion around the squid's body (Mooney et al. 2010).

5.3. How noise affects cephalopods and their response to sound in general

An increase in the low frequency ambient and anthropogenic noise where cephalopods are sensitive may lead to a range of behavioural and physiological effects, as well as increased masking (Mooney et al. 2010). Vessels conducting seismic surveys using compressed air guns are believed to be responsible for stranding of giant squid (*Architeuthis*) in three locations, the northern coast of Spain where five individuals stranded within a month in 2001, four along the Asturian coast in a single week in 2003 and two in 2004 in Newfoundland waters within 17 days (Guerra et al. 2011). Air guns used in seismic surveys produce high intensity low frequency sound waves (20-500 Hz) (Guerra et al. 2011; Fewtrell and McCauley 2012). Two studies on the influence of sound (50-400 Hz sinusoidal wave sweep) on four cephalopod species (cuttlefish, *Sepia officinalis*, octopus, *Octopus vulgaris* and squids, *Loligo vulgaris* and *Illex coindetti*) found that all individuals exposed to sound presented lesions with damage observed on the macula static princeps, crista sensory epithelia and to hair cells, and the appearance of these lesions became more pronounced in specimens observed 96 hours after sound (André et al. 2011; Solé et al. 2013). In both studies control treated cephalopods showed no lesions in the statocysts (André et al. 2011; Solé et al. 2013). A study looking at the changes in behaviour when exposed to air gun noise found similar behavioural alterations in fish and squid, whereby squid (*Sepioteuthis australis*) were found to eject ink at the first gun signal and then move backwards with jetting motions when exposed to repeated sounds (Fewtrell and McCauley 2012). Komak et al. (2005) suggest that the readiness to respond to water movement of certain frequencies with a particular behaviour may be age dependent. One month old cuttlefish (*Sepia officinalis*) appeared to be more responsive in regards to movement and only one month cuttlefish displayed burrowing behaviour in comparison to three month old cuttlefish (Komak et al. 2005). Sound detection in cephalopods

could be driven by predator avoidance, navigation and detecting the presence of prey.

5.4. Response of cephalopods to shipping noise in particular

Noise from shipping is pervasive throughout the marine environment, especially at low (<300 Hz) frequencies, and a key concern is the effects of chronic noise exposure on marine species (Merchant et al. 2012). At these low frequencies, ambient noise levels are elevated by 15-20 dB when exposed to distant shipping (McKenna et al. 2013). The influence of shipping sounds on fish has contributed to increases in cortisol levels, hearing impairment, masked hearing thresholds and behavioural responses (e.g. avoidance, swimming speed and direction and schooling behaviour) (Götz et al. 2009). Shore crabs exposed to ship-noise consumed more oxygen compared to those exposed to ambient noise; a higher metabolic rate is suggested to lead to decreased growth and increased stress lowering survival (Wale et al. 2013). While studies on cephalopod exposure to low frequency anthropogenic noise from shipping have not been investigated, it is believed prolonged exposure to shipping noise may contribute to masking of the biologically relevant sounds, chronic stress, long-term behavioural responses, shifts in attention or induced acoustic traumas to the receptor system (Mooney et al. 2010; Merchant et al. 2012).

6. Conclusion

There was clear evidence for declines in abundance of *S. apama* not only on the breeding aggregation area at Point Lowly, South Australia but also throughout USG. The trend for decline of *S. apama* in USG is however clearer in the northern harvest zone. There is no clear evidence as to what may be causing the decline in abundance although rainfall was correlated with *S. apama* abundance such that an increase in rainfall in late summer and autumn may lead to a decrease in abundance of *S. apama* on the breeding aggregation area. USG is an area of economic growth and there is potential for new port developments near the breeding aggregation area and associated increases in shipping in the region. The influence of shipping (noise and turbidity) on cephalopods in general is not well known and there have been no studies on *S. apama*, yet shipping noise has potential to cause physical,

physiological and behavioural changes. This research project will investigate the potential impact of shipping noise and turbidity on development of *S. apama* embryos and the potential impact of shipping noise on physiology of *S. apama* adults. Results will inform regional sustainability planning for USG.

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