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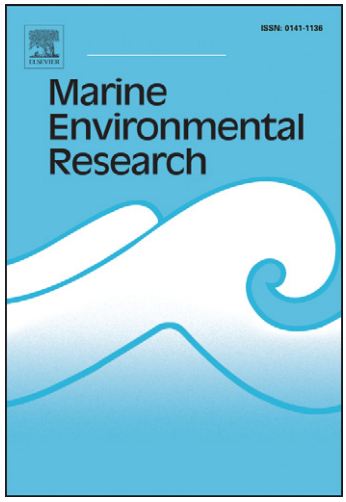

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Impacts of seawater desalination on the giant Australian cuttlefish *Sepia apama* in the upper Spencer Gulf, South Australia

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Abstract

With seawater desalination expanding rapidly, it is important that ecological studies are undertaken to determine the effects of brine discharge on the marine species in the area. The abundance of giant Australian cuttlefish (*Sepia apama*, Gray 1849) eggs and environmental data were recorded at nine sites near Point Lowly, Spencer Gulf, South Australia, an area where the largest desalination plant in the Southern hemisphere is proposed. In addition, the effects of different concentrations of desalination brine on the growth, survival and condition of cuttlefish embryos were investigated. The primary egg-laying sites for the cuttlefish were in the vicinity of Stony Point (sites 4 and 3) and the area with the least egg abundance was on the eastern and western areas around Point Lowly (sites 9 and 7) where no eggs were found. The survival of embryos decreased with an increase in salinity, with no embryos surviving to full term in salinities greater than 50 $^{0}/_{00}$. Mean weight and mantle length also decreased with increasing salinity. Besides elevated salinity, the brine also had increased concentrations of Ba, Ca, K, Sr and Mg relative to water near Point Lowly. Brine discharge from seawater desalination poses a potential threat to the unique spawning aggregation of the giant Australian cuttlefish, in the upper Spencer Gulf, South Australia.

Keywords: Sepia apama; Cuttlefish; Spencer Gulf; South Australia; Desalination; Brine

1. Introduction

With only 1% of freshwater available for agriculture, industrial and domestic purposes, freshwater resources are a precious commodity. Water scarcity is projected to increase across much of the globe with severe water shortages predicted to affect 2.7 billion people in over 80 countries in the next century (Barker et al., 2000). Water, like energy in the late 1970s, is likely to become the most critical natural resource issue to confront our environment and economy. The inherent need for freshwater has hence encouraged the rapid development of desalting technologies (URS, 2002).

Desalination refers to the wide range of technical methods designed to remove salts from waters of different qualities (Gleick et al., 2006). Seawater desalination is a process in which large volumes of feed water are drawn into a desalination plant from the ocean and salts are removed using, most commonly, reverse osmosis. The process as a whole is not without environmental and ecological implications. Impingement and entrainment of marine organisms via the intake pipes is a major environmental concern (York and Foster, 2005; Gleick et al., 2006). The most significant problem associated with seawater desalination however, is the disposal of the highly concentrated brine effluent produced by desalination plants as by-product which is often discharged into the sea (Arnal et al., 2005). Typical desalination brines contain approximately 50% more salt than the feed water (1.3-1.7 times the amount of salts) (Einav et al., 2002) and have a higher specific density (Gleick et al., 2006). Desalination brine can have salinities as high as ~70 $^{0}/_{00}$ to 80 $^{0}/_{00}$, although the operational technical limit is 70 $^{0}/_{00}$ (Arnal et al., 2005).

The impact of desalination brine on the marine environment takes place mainly at the point source, in the vicinity of the brine discharge pipe. Even though the brine contains natural marine ingredients, without prior mixing, its high specific weight causes it to sink to the sea floor forming a stratified system with the brine forming a bottom layer (Jibril and Ibrahim, 2001; Einav et al., 2002; Fernández-Torquemada et al., 2005). As the plume sinks, its effects potentially could extend over a range of hundreds of meters. Desalination discharge alters the amount of dissolved oxygen in the water if there is insufficient mixing, water temperature is increased due to the heat treatment within the process and turbidity can be increased at the outlet point (Gleick et al., 2006; Raventos et al., 2006). Desalination brine also may contain many contaminants and hazardous wastes (Gleick et al., 2006). These include anti-fouling agents, chlorine and acid which are unavoidably needed in large scale plants to treat the feed water and

pipelines. These constituents are not usually treated to remove toxicity before being discharged into the sea (Hashim and Hajjaj, 2005). Brine from seawater desalination can also contain high concentrations of elements which are typically found in seawater, including heavy metals such as lead, manganese, copper and zinc.

Although heavy metals and toxic chemicals can be detrimental to marine organisms, salinity is one of the most important physio-chemical factors to which they are exposed (D'Aniello et al., 1989). Marine organisms exist in osmotic balance with their environment and the osmotic stresses acting on different species depend upon individual adaptations and salinity tolerances within specific habitats. The repercussions of high salinity levels on marine ecosystems and organisms can take a variety of forms. Animals which are not adapted to such conditions often move away from the affected area (Young and Potter, 2002). Species richness and density can also decline where extreme salinities are prominent (Bayly, 1972; Vega-Cendejas and Hernández de Santillana, 2004). Increases in the concentration of salts may result in the dehydration of cells, and the inability to hypoosmotically regulate leading to a decrease of turgor pressure and mortality, especially in larvae, eggs and juveniles (Cintron, 1970; Aladin, 1991; Einav et al., 2002; Young and Potter, 2002).

Increases in salinity can produce smaller embryos. For example, a distinct relationship between salinity, egg size and embryonic development was found in the estuarine crab *Chasmagnathus granulata* (see Giménez and Anger, 2001). The smaller the hatchling, the greater the physical constraints imposed on the functional morphology of organs responsible for swimming and food capture (Boyle and Boletzky, 1996), which in turn, lessens the individuals' chances of survival. Importantly, salinity directly affects embryonic development in cephalopods (D'Aniello et al., 1989; Sen, 2005). Previous research on the effects of salinity within cephalopods has focused on *Loligo* spp., and few studies have investigated effects of salinity on *Sepia* spp. What has been found however is that salinity ranges for embryonic development and hatching success are species specific and higher salinities (28 $^{0}/_{00}$-38 $^{0}/_{00}$) appear to be optimal (Palmegiano and Dapote, 1983; Paulij et al., 1990; Cinti et al., 2004; Sen, 2005). Growth rates of cephalopods are also affected by salinity, where lower salinities increase statolith size (Villanueva et al., 2007), but also cause deformations of embryos (Paulij et al., 1990). At present there is no published information on the effects of high salinities (>42 $^{0}/_{00}$) especially those typical of desalination brine (~70 $^{0}/_{00}$ to 80 $^{0}/_{00}$) on the growth and survival of the cephalopod embryo or juvenile stage.

Determining tolerance levels and subsequent health of giant Australian cuttlefish embryos to the potential environmental pressures administered from desalination will aid in managing the population to ensure its long term survival. A proposal exists to build the largest seawater desalination plant in the Southern hemisphere at Port Bonython in the upper Spencer Gulf, South Australia. Effluent consisting of highly concentrated brine will be discharged in the vicinity of the breeding ground of *S. apama*, thereby having the potential to impact the population. *S. apama* form a unique annual spawning aggregation, not exhibited by any other cuttlefish species in the world, during winter in the upper Spencer Gulf (Hall and Hanlon, 2002; Hall and Fowler, 2003). The Gulf is considered an inverse estuary with high natural salinities (~40 $^{0}/_{00}$ – 43 $^{0}/_{00}$ near Point Lowly) (Nunes and Lennon, 1986). The breeding ground for *S. apama* lies within ~ 2-8 m of relatively shallow water with large areas of benthic rocky substratum. *S. apama* require a hard surface upon which their eggs can be laid and the rocky reef areas at Point Lowly through to Black Point provide this unique habitat.

The overall objective of this study was to determine the potential impacts of seawater desalination on the egg stage of *S. apama* in the upper Spencer Gulf. Therefore, this project aims to determine: (1) the distribution and abundance of clutches of eggs of *S. apama* throughout the breeding aggregation, (2) environmental parameters and water quality in the vicinity of Port Bonython, pre-desalination, and (3) the effects of increased salinity on the embryonation period, survival and condition of cuttlefish hatchlings via a laboratory experiment.

2. Methods

2.1 Study site and study species

Data collection and field sampling were made at nine sites in the coastal waters between Black Point and Point Lowly in the upper Spencer Gulf (Fig. 1, Table 1). This area is where the dense spawning aggregation of *S. apama* occurs every winter from May to August. The key breeding ground occurs along approximately 8 km of coastline (with a subtidal reef area of 0.64 km^2) from Point Lowly west towards Black Point (Fig. 1). The coastline consists of a platform of plate-like fragments of dense quartzite bedrock (Gostin et al., 1984), which extends out beyond the intertidal zone and

gradually becomes low relief subtidal rocky reef out to 70-130 m off shore (~8 m depth) (Hall and Fowler, 2003). Vast areas of this rocky substratum provide ideal egg attachment surfaces where females lay clusters of individual lemon-shaped eggs on the underside of sub-tidal crevices, rocks and overhangs (Cronin and Seymour, 2000; Hall and Fowler, 2003). The embryonic development time of 3-5 months varies according to the time at which the egg was laid. Eggs laid in May for example, will develop over four months and hatch in October and eggs laid later in the season, in August, experience warmer water temperatures and hatch in November (Hall and Fowler, 2003).

This unique habitat lies within the oceanographic region of Spencer Gulf, South Australia. This particular gulf system is a semi-enclosed body of water, often termed an inverse estuary, approximately 300 km long with a maximum width of 130 km and a typical depth of 40 m at the southern opening (Fig.1a). In the channels of the northern reaches it is around 15-20 m whilst most coastal zones range between 2 and 8 m depth near Black Point, Point Lowly and south of Whyalla. The area receives little rainfall, has minimal runoff, little input of groundwater and high evaporation. The head of the gulf therefore exhibits hypersaline conditions where salinity can reach 48 $^{0}/_{00}$ in late summer (Nunes Vas et al., 1990). Oceanic salinity values are found at the entrance to the gulf.

2.2 Abundance of clutches

Egg abundance was determined during July 2007 and 2008 at each of the 9 sites by underwater visual strip transects undertaken on SCUBA. Underwater visual survey techniques are an effective, non-destructive method for estimating the abundances of marine organisms (Edgar et al., 2004), but may underestimate the abundance of eggs which are generally laid on the underside of rocks and well-hidden. We therefore only present data on number of clutches of eggs, and would expect any bias in counts (e.g. under counting) to be consistent across sites. Six replicate transects of 20 m length were sampled at each site. Two to three divers counted the number of clutches of eggs while searching to 1 m either side of the transect line; the area covered per site was 240 m^2. Clutches were defined as a group of two or more eggs.

2.3 Water quality

Water quality was analysed and environmental parameters determined in July and August 2007 during the peak egg developmental period of the giant Australian cuttlefish. Samples for analysis of nutrients and trace elements were taken from the 9 sites within the known breeding ground where numbers of clutches of eggs were estimated (Fig. 1b; Table 1).

Surface water samples for analysis of nutrients and water chemistry were taken (approximately 15 cm below surface) via 20 ml plastic sterilised syringes (n=6) at each of the 9 sites. Samples for nutrient analysis (n=3) were then filtered through 0.45 µm glass fibre filters into 15 ml sample containers and stored frozen prior to analysis. Nutrient samples were then analysed for concentrations of dissolved ammonia ($NH_{3/4}^{+}$), oxidised nitrogen (NO_x), and orthophosphate (OP) on a Lachet FIA (Flow Injection Analysis) Automated Ion Analyser.

2.4 Water chemistry

Samples for water chemistry (n=3) were also filtered through 0.45 µm glass fibre filters but placed into acid washed 30 ml sample containers containing 500 µL of nitric acid (HNO_3) [70%] and refrigerated for trace element analysis. These samples were analysed by the National Measurement Institute (NMI) for trace elements (Calcium (Ca), Magnesium (Mg), Potassium (K), Strontium (Sr), Barium (Ba), Iron (Fe), Zinc (Zn), Manganese (Mn) and Copper (Cu)). A Perkin Elmer 6000 DRC (Dynamic Reaction Cell) Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) was used to detect the concentrations within each sample. High resolution ICP-MS was used to determine Zn concentrations to remove interference of molecular ions originating from NaCl, S, Mg, K, and Ca. Lutetium and indium were used as internal standards to correct for ICP-MS drift. Cu and Mn were omitted from further analyses because the readings were below detection limits.

2.5 Environmental parameters

A YSI 6600 Multi-parameter Water Quality Meter (CTD sonde) was used to obtain data on depth, temperature, salinity, pH and dissolved oxygen (DO) at each site. The sonde was slowly lowered from the side of a boat and took recordings from the surface to the bottom for 90 seconds with 3-7 second intervals between each reading.

On the first sampling occasion readings were taken for all sites and replicate drops were conducted. On the second sampling occasion only one set of recordings were taken and some sites were not sampled due to rough sea conditions.

2.6 Embryo growth experiment

2.6.1 Egg collection

Sepia apama eggs were collected from Stony Point (site 4; 32°59"5'S, 137°43"1'E) in the upper Spencer Gulf, South Australia (Fig. 1b), during July 2007. Newly laid eggs that were soft, bright white and opaque in appearance were collected (Hall and Fowler, 2003). Eggs were gently prised off the underside of rocks by hand and placed into mesh catch bags. Eggs (n=120) were then transferred to a plastic bucket underwater to prevent dehydration and transported to the University of Adelaide in an insulated 20 L foam box whilst being aerated.

2.6.2 Experimental set up

Eggs were maintained in a controlled temperature room at the University of Adelaide and temperature was adjusted to simulate the conditions in the upper Spencer Gulf based on temperature estimates recorded from Ward Spit (approximately 10 km from Point Lowly) in 2006 (Saunders, unpublished data). Temperature was therefore slowly increased during the experimental period (July-November) from 13.8°C to 18°C (±1°C). One standard fluorescent tube was used to illuminate the room and was adjusted for brightness. Light was regulated on a 12 h light: 12 h dark photoperiod. The room was monitored daily to ensure temperature and lights were functioning correctly.

2.6.3 Seawater and brine collection

Seawater was collected from the South Australian Research and Development Institute (SARDI), Aquatic Sciences Centre, West Beach, South Australia. The seawater originated from 1 km off shore at a depth of 10 m off the metropolitan coast of Adelaide in Gulf St Vincent. The water was passed through a settlement tank and primary sand filter before storage at the facility. The water was collected from SARDI on a weekly basis and stored at constant temperature (~18°C) in a 2000 L tank in the aquarium room at the University of Adelaide.

Desalination brine was collected on two occasions from the Penneshaw desalination plant, Kangaroo Island, South Australia. The plant has been operating on a small scale since 1999 producing 100 ML per year of freshwater. The plant uses Reverse Osmosis (RO) technology to separate the salts from the seawater. Due to the small volume of water which is desalinated, no chemicals are used, minimising the impact of this brine on the marine environment. The starting salinity of the brine when collected was 52 $^{0}/_{00,}$ but this increased to 55 $^{0}/_{00}$ over the course of the experiment due to evaporation. Brine was transported to the laboratory in a 1,000 L tank and once at the laboratory, was stored in the aquarium room in a 1,000 L tank at constant temperature.

2.6.4 Experimental design

Eggs were carefully suspended onto 100 x 50 mm pieces of polystyrene floats using a needle and fishing line. Eggs (*n*=12) were suspended underneath the foam and in close proximity to each other to simulate their orientation and spatial dynamic in nature. Eggs were transferred into a 40 L tank and left to acclimate for 5 days. Salinity was increased at a rate of 2 $^{0}/_{00}$ each day until the required salinity treatment was reached. Eggs were acclimatised for 2 days prior to being moved into the specific treatment tanks of various salinities. The experiment involved five treatments (control of 39 $^{0}/_{00}$, 40 $^{0}/_{00,}$ 45 $^{0}/_{00,}$ 50 $^{0}/_{00}$ and brine of 52 $^{0}/_{00}$ – 55 $^{0}/_{00}$) with two replicate tanks per treatment. Tanks were aerated continually using a HAILEA air pump (Model V-60, super silent power) attached to plastic hoses and air stones.

2.6.5 Tank water quality

Within the controlled temperature room a flow through system with a biological filter was not feasible. Water changes within treatments were therefore done manually. pH was maintained at 7.8-8.2. To maintain water quality for the requirements of cuttlefish (NH_4 < 0.5 mg/L, NO_2 < 0.2 mg/L and NO_3 < 50 mg/L) (Hanley et al., 1998; Minton, 2004) the levels of these three parameters were tested using an Aquarium Pharmaceuticals (API) liquid test kit. Nutrient tests were conducted mostly during the two consecutive days after a water change. However during the initial two weeks of the experiment nutrient analyses were conducted prior to a water change. Water changes (50-75 %) were conducted every 3-5 days to maintain water quality and levels of trace elements which are needed for cephalopod development (Hanley et al., 1998). Water was changed within specific treatments when significant levels of nutrients within these

tanks were detected. Water changes involved floating eggs out of tanks to avoid disturbance during the water change, removing built up detritus and refilling tanks with treatment water which was a mixture of seawater and desalination brine of the required salinity.

Concentrations of trace elements within experimental tanks were sampled on two occasions during the experimental period (August and September). Samples for water chemistry were analysed in a similar manner to the field samples.

2.6.6 Length and weight of hatchlings

Eggs were monitored daily until time of hatching. Date of hatching was recorded for each individual and minimum length of time to hatching determined based on eggs being laid on the day of collection. Percent survival was determined based on the number of individuals per tank per treatment which survived to hatching. Hatchlings were removed immediately from tanks once hatched and placed into an ice slurry. Length was measured using Mitutoyo digital blade type callipers (± 0.05 mm) and wet weight, using an electronic balance (± 0.01 g).

2.6.7 Field samples

Ten hatchlings were collected from Stony Point just prior to hatching in October to determine condition of wild cuttlefish. Length and weight measurements of hatchlings were determined in the laboratory using electronic callipers and an electronic balance.

2.7 Statistical analyses

Field data (number of clutches, nutrients, and trace elements) were analysed using two-factor (site, time) ANOVAs. All factors were treated as random. Homogeneity of variances was tested using Cochran's C test. If significant, data were transformed using Ln (X+1), but if this transformation did not lead to homogeneity of variances, data analyses were made on non-transformed data. Where significant differences in ANOVAs were found Student-Newman Keuls (SNK) post-hoc tests were used to determine which sites or times differed.

Laboratory data (hatchling length and weight, and trace elements) were analysed using a two-factor ANOVA (treatment and tanks nested within treatment), however no significant variation among tanks was found therefore data were pooled and analysed by treatment. The relationship between survival of eggs and salinity was determined using a logistic regression where the log likelihood was minimised.

3. Results

3.1 Abundance of clutches

The number of clutches of eggs showed a significant difference among sites, but similar patterns were seen for both years (Fig. 2; $F_{8, 8} = 12.9$, $P < 0.001$). Several sites had no eggs (e.g. sites 7 and 9 in year 1 and sites 2, 6, 7, 8 and 9 in year 2). Sites 1, 3 and 4 had significantly greater numbers of clutches than all other sites. Where clutches were present, between 1 and 21 clutches were found per site.

3.2 Field water quality and chemistry

Oxidised nitrogen (NO_x) concentrations were significantly greater in August compared to July (Fig. 3a; Table 2). Orthophosphate (OP) and ammonia ($NH_{3/4}^+$) showed an interaction between sites and time of sampling because for one time (July) there were no significant differences among sites whereas for August, differences among sites were found (Fig. 3b and c; Table 2). In August, site 6 and site 8 had significantly higher concentrations of OP and $NH_{3/4}^+$ than the other sites, and than July values (Fig. 3; Table 2).

Zinc showed no significant difference among sites or times (Fig. 4g, Table 3). Concentrations of Ba and Fe were significantly greater during August than July, but did not vary among sites (Fig. 4a and d, Table 3). For the remaining four elements (Sr, Ca, K, Mg) a significant interaction between site and time was found largely because for July there were no significant differences among sites, whereas for August some sites differed (Fig. 4b, c, e and f). Concentrations of Sr, Ca, K and Mg at site 8 were significantly lower than all other sites with the exception of site 6 (Sr, Ca, K, Mg) and 9 (Ca, K, Mg) (Fig. 4).

342

343 *3.3 Field environmental parameters*

344

345 Measurements of the environmental parameters showed little variation by depth
346 therefore mean ± standard error for each site and time was calculated throughout the
347 entire water column (Table 4). Water temperature did not vary among sites on each of
348 the sampling occasions (July <0.35° C mean difference among sites; August maximum
349 difference 1.07° C). Mean water temperature in August (14.62 ± 0.05° C) was 2.21° C
350 greater than in July (12.40 ± 0.04° C). Salinity was also constant across all sites during
351 both months. The mean salinity across all sites within the breeding ground was 38.77 $^{0}/_{00}$
352 (± 0.05). The highest average salinity was recorded at site 9, 39.42 $^{0}/_{00}$ during July
353 (Table 4). Dissolved oxygen varied among sites (Table 4). Sites 4 and 9 had the highest
354 levels of DO on average than any of the other sites. The minimum mean value of
355 dissolved oxygen for any site in either of the two sampling periods was 6.06 mg/L (site
356 1 in August) suggesting that the water was well oxygenated. pH readings were constant
357 throughout each month, but were marginally higher across all sites during August
358 compared to July. The average pH in July was 8.37 (± 0.02) compared to August which
359 was 8.48 (± 0.02). The maximum depth of the sites varied from ~2 m to just under 6 m,
360 and showed some variation between the two sampling times, which was largely due to
361 how close to the shore the boat could get during the rough weather in August.

362

363 *3.4. Embryo growth experiment*

364

365 *3.4.1 Water quality and chemistry*

366 The water quality, salinity and temperature in the experimental tanks are
367 summarised in Table 5. The level of NH_4 and NO_3 were within optimum concentrations
368 (NH_4 < 0.5 mg/L and NO_3 < 50 mg/L) throughout the experimental period within all
369 treatments. Levels of NO_2 were elevated (>0.2 mg/L) in higher salinity treatment tanks.
370 Salinity did not fluctuate greatly within treatments, however within the brine treatment
371 there was an average gradual salinity increase of 3.42 $^{0}/_{00,}$ over the experimental period.
372 pH levels were maintained at 7.8 within all treatments, however pH was elevated in the
373 brine treatment (range 7.8-8.2 for 55 treatment). Water temperature increased within all
374 treatments by 4.50° C (± 0.50° C) and closely matched the measured temperatures

recorded in upper Spencer Gulf in 2006. Thus, experimental treatments were exposed to a similar temperature regime to nature.

An increase in elemental concentration with an increase in salinity occurred for Ba, Ca, K, Sr and Mg (Fig. 5 a, b, c, e and f), but this increase was only significant for 4 of the 5 elements (not significant for Ba). There was no significant difference between treatments for Fe and Zn (Fig. 5 d and g).

3.4.2 Hatching success

Hatching success was similar between the 39 $^{0}/_{00}$ (control, no brine) and 40 $^{0}/_{00}$ treatments, but then decreased for 45 $^{0}/_{00}$. In the 50 $^{0}/_{00}$ and 55 $^{0}/_{00}$ treatments there was total mortality of eggs ($F_{4, 5}$ = 340.18, P<0.0001; Fig. 6a). A logistic regression was fitted to the data [percent survival = $\exp(B_0 + B_1 \times \text{salinity})/(1 + \exp(B_0 + B_1 \times \text{salinity})$ where $B_0 = 30.256 \pm 5.979$, and $B_1 = -0.666 \pm 0.132$]. Thus, there was ~ 7 % decrease in survival for every 1 $^{0}/_{00}$ increase in salinity. It was also noted that one embryo in the 45 $^{0}/_{00}$ was malformed. For those treatments where individuals survived through to hatching, the minimum average time to hatching was 99 days. There was no difference between the length of time to hatching among the three treatments ($F_{2, 59}$ = 0.3323, P = 0.738; Fig. 6b).

3.4.3 Length and weight of hatchlings

There was a significant difference between the mantle lengths and weights of the hatchlings (Mantle length: $F_{3, 68}$ = 9.514, P < 0.001; Fig 7a; Weight: $F_{3, 68}$ = 9.501, P < 0.001; Fig 7b). Field-collected specimens were significantly larger and heavier than any of the treatment individuals. Of the laboratory treatments, individuals from 45 $^{0}/_{00}$ were significantly smaller in length and weight than those from 39 $^{0}/_{00}$ and 40 $^{0}/_{00}$.

4. Discussion

The primary egg-laying area for *S. apama* lies between Point Lowly and Black Point in the upper Spencer Gulf (Hall and Hanlon, 2002). Within this region certain areas had a greater number of clutches. Differences in cephalopod egg abundance between sites within a particular region are common. A previous study found that the number of *S. apama* eggs varied among areas within a single site of the breeding aggregation, although the difference was not statistically significant (Hall and Fowler, 2003). Spatial variability of egg abundance on a small spatial scale (within a 1 km) has also been found for squid species (Moltschaniwskyj and Pecl, 2003). The differences between sites may be attributed to the fine-scale variability of substrate within the breeding aggregation. The study area in the upper Spencer Gulf is made up of a hard substrate which constitutes a conspicuous and finite area. The area with the highest number of clutches maintained a clear slaty bed rock which was the most suitable for egg-laying (Gostin et al., 1984; Hall and Fowler, 2003). Knowledge of primary egg-laying sites can contribute to a more informed decision as to where an intake and discharge pipe for seawater desalination should be placed.

The benthic eggs are exposed to water surrounding them during the austral winter. Values for nutrients and environmental variables near Point Lowly were generally considered moderate to good according to the ANZECC guidelines (ANZECC, 2000). Several sites (6 and 9) did exceed trigger values for nutrients (ammonia and orthophosphate) at one sampling time. Caution will therefore be required to ensure that the brine discharge does not lead to elevated nutrient concentrations. Although it is difficult to find guidelines for many of the trace elements for Australian waters, trace elements are likely to be increased in brine, which if not dispersed may lead to elevated levels in the vicinity of giant Australian cuttlefish eggs. Salinity is already elevated in upper Spencer Gulf due to the lack of freshwater input and inverse estuary nature of the gulf (Nunes Vas et al. 1990). Salinity was lower on average during this sampling period (38.78 ‰) compared to mean salinities of 40 ‰ recorded in March, 1984, 41 ‰ in August, 1975 and 42.6 ‰ in July, 1976 (Johnson, 1981; Nunes and Lennon, 1986). The brine is expected to have double the concentration of salts (70-80 ‰), higher temperature and turbidity than ambient seawater, and lower dissolved oxygen levels. In addition, concentrations of trace

436 elements may be increased by ~50% (Vanhems 1992, cited in Einav et al. 2002).
437 Continual discharge of brine from a desalination plant could potentially cause changes
438 in nutrients, trace elements and environmental parameters which may negatively impact
439 the environment.
440
441 Increased concentrations of desalination brine had an inhibitory effect on
442 hatching success and the growth and development of *S. apama* embryos. Embryos from
443 treatments whose salinities were closest to those found in nature had the most successful
444 hatch rate. The salinities in the field during the peak egg developmental period range
445 from ~38 ‰ to 42 ‰ (Johnson, 1981). Significantly fewer embryos survived to full
446 term in salinities of 45 ‰ and complete mortality occurred in treatments greater than
447 this concentration. Salinity ranges for embryonic development in cephalopods are
448 species specific, and previous research has shown that between 34 ‰ and 42 ‰ is
449 optimal (D'Aniello et al., 1989; Paulij et al., 1990; Cinti et al., 2004; Sen, 2004). The
450 current study has indicated that salinity which increases above 40 ‰ will lead to a
451 decrease in survivorship of *S. apama* embryos and that with every 1 ‰ increase in
452 salinity above 40 ‰ survival of embryos will decrease by ~7 %.
453
454 Although physiological uptake of oxygen and nutrients by cuttlefish embryos
455 occurs through the egg capsule by diffusion and the egg acts as a protective structure
456 (Cronin and Seymour, 2000), osmotic stress has been inferred as a possible cause for
457 malformations in developing cephalopod embryos (Paulij et al., 1990). A malformation
458 of a single embryo in the 45 ‰ treatment was observed. The individual survived almost
459 to the hatching phase, however by the completion of the experiment, had died and its
460 morphology had become unidentifiable. In the absence of any circulatory mechanism to
461 aid oxygen transport to the tissue, oxygen must pass by diffusion from the external
462 environment through the egg capsule to the embryo (Cronin and Seymour, 2000).
463 Increased salinity causes a diffusion limitation to the respiration of the embryos. The
464 solubility of gases, such as oxygen, is decreased in hypersaline water because the salts
465 reduce the solubility of gases (Sherwood et al., 1991; Porter et al., 1999). Osmotic stress
466 probably demanded a lot of energy which could not be used for development (Paulij et
467 al., 1990). The increased mortality with the increased salinity of desalination brine may
468 have also encouraged the inhibitory effects of microscopic bacteria or pathogenic fungi.
469 The fine layer of algal growth which covered the outer layer of the eggs was increased

within treatments containing more brine. Pathogenic infections resulted in mortality of oysters when they were exposed to desalination brine (Mandelli and McIlhenny, 1971).

The inhibition of normal metabolic activity also caused the embryos in higher salinities to be smaller than hatchlings in the ambient salinity treatment. Previous research has shown that abiotic factors such as salinity have the potential to decrease the nutritional condition of developing larvae, as indicated by their length and weight (Folkvord et al., 1996). The decrease in mantle size as an effect of salinity has been noted previously and described as a malformation (Paulij et al. 1990). Also, correlations with size of hatchlings and hatching success have been determined for cephalopod embryos grown in low salinities (Palmegiano and Dapote, 1983; Fagundez and Robaina, 1992; Cinti et al., 2004; Sen, 2004; Sen, 2005; Villanueva et al., 2007); however this is the first study which indicates a decrease in weight and mantle length in salinities greater than 42 ‰. The yolk reserves of individual eggs are the only energy source for development of the embryo; the smaller the hatchling, the greater the physical constraints imposed on the functional morphology of organs responsible for swimming and food capture, therefore once hatched survivability may also be decreased (Boyle and Boletzky, 1996).

Development time of cuttlefish embryos was what was expected of eggs laid in July. Embryonic development varies between 3 and 5 months depending when the eggs were laid as development of *S. apama* eggs is mostly influenced by water temperature (Hall and Fowler, 2003). The mean developmental period of 99 days also supports the findings for *S. apama* eggs grown *in situ* in another study where the developmental time was 100 days in a controlled temperature environment ranging from 16° C to 18° C (Hall and Fowler, 2003).

Desalination brine concentrates not only salts, but metals and trace elements during the process of extracting fresh water (Talavera and Quesada Ruiz, 2001). Although *S. apama* eggs may already be exposed to heavy metals and trace element concentrations, due to the breeding aggregations proximity to major industry, increased concentrations of trace elements may have been a cause of mortality in the experiment as concentrations of some trace elements were far greater than those found within waters near the breeding aggregation. Metals retard embryos from hatching at concentrations equal to or lower than those causing mortality and the effects of metals on embryos are often increased as a function of exposure duration (Macdonald et al., 1988). *Loligo vulgaris* embryos reared in different concentrations of trace elements only

504 developed normally in a concentration range of 360.7-601.2 mg/L of calcium, 351.9-
505 586.5 mg/L for magnesium and 1166.6-1652.7 mg/L for potassium. Above or below
506 these ranges mortality occurred and surviving hatchlings experienced reduced mobility
507 (D'Aniello et al., 1989). In the current study, the calcium levels were within this
508 survivability range, however magnesium concentrations were above this threshold for
509 the 50 ‰ and 55 ‰ tanks. The concentrations of potassium were also above natural
510 levels in the experimental tanks of 45 ‰, 50 ‰, and 55 ‰. Hatchlings were less active
511 within the 45 ‰ treatment tanks which may be attributed to the high levels of
512 magnesium in the water (D'Aniello et al., 1989). Once hatched the cuttlefish in this
513 treatment were sluggish and very few inked to escape capture (Dupavillon, personal
514 observation). By comparison, cuttlefish in the control (39 ‰) and 40 ‰ treatments
515 swam actively and inked multiple times in defence against capture.
516 With the potential increased concentrations of trace elements and metals
517 reaching the ocean through the discharge of desalination brine, it is vital to determine its
518 effects upon all life stages of *S. apama*. Research on the bioaccumulation of zinc into
519 early life stages of cuttlefish indicates that metals are taken up by cuttlefish eggs
520 (Bustamante et al., 2002). They appear to remain concentrated within the capsule
521 membrane of the egg, which thus acts as an efficient shield protecting the embryo
522 against exposure. Once hatched however the juvenile hatchlings assimilate heavy metals
523 into their tissues quite readily (Bustamante et al., 2002). Immediately after hatching,
524 rapid increases of Cu, Fe and Zn concentrations in cuttlefish tissues have been found.
525 This suggests that hatchlings are highly dependent on essential metals to fulfil their
526 metabolic demands. It therefore follows that salts and metals are rapidly taken up once
527 the hatchling is in contact with seawater (Miramand et al., 2006). Exposure to effluents
528 of desalination plants may lead to accumulation of trace elements (Hanna and Muir,
529 1990).
530 A large-scale desalination plant which discharges concentrated brine effluent
531 into the vicinity of *S. apama's* breeding aggregation could possibly be detrimental to the
532 future survival of the population. These findings are important to the design and
533 development of a desalination plant in this area and can possibly be used to infer
534 impacts upon other benthic organisms. Pelagic organisms, such as teleosts are able to
535 move away from intolerable conditions such as discharged desalination brine. Benthic
536 organisms, for which certain life stages are mobility impaired, must have pre-
537 adaptations to withstand such environmental fluctuations. Risks of increased salinity

and increased concentrations of nutrients and trace metals on the eggs of cuttlefish are primarily associated with the properties of hypersaline water and the ecology of the eggs themselves. *S. apama* eggs are laid in shallow water, and remain in the benthic environment during their long developmental period; movement away from adverse conditions is therefore not feasible. Hypersaline water is denser than normal seawater and therefore sinks and accumulates on the bottom. In a laboratory setting the embryos of *S. apama* do not survive the effects of desalination brine. A reduced number of hatchlings would be expected at a very small increase in salinity, and therefore it is essential that any outlet pipe is in a region away from the *S. apama* breeding aggregation and that the discharged flow returns to background salinity levels relatively quickly.

The potential placement of the feed water and discharge pipe for the desalination plant needs to be carefully considered. Feed water, containing elevated concentrations of salt, such as those found in the upper Spencer Gulf, and high levels of nutrients and heavy metals should be avoided. These constituents are doubled in the discharge brine and at such high concentrations are detrimental to a wide variety of marine organisms (Epifanio and Srna, 1975; Talavera and Quesada Ruiz, 2001). The region of high egg abundance should be avoided also as a feedwater and discharge point, as not only will the brine have an effect on the developing embryos, but the infrastructure of the pipes may disturb this unique egg-laying habitat. Impingement and entrapment of the eggs and adult cuttlefish in these areas may also be possible (Gleick et al., 2006). These areas supply the population with the most offspring and therefore should be properly protected and conserved.

5. Conclusion

This study has focused on the benthic egg stage of the giant Australian cuttlefish in terms of the effects of brine. Brine typically has increased levels of turbidity, temperature and salinity and decreased levels of dissolved oxygen. Turbidity in particular may also affect the adult stages since their mating system relies on visual cues (Hall and Hanlon, 2002). Therefore, future studies need to focus on examining the potential impacts of desalination brine on adults as well. The strength of one generation is highly dependant on the strength of the previous generation since cuttlefish only live for 12 to 18 months (Hall and Fowler, 2003). Therefore, any detrimental affects from discharge brine may be catastrophic for the population as a whole. The finding that the

embryos of *S. apama* can not survive increased levels of salinity and certain trace elements is useful for the planning of desalination and for gaining insights into the physiology of the species itself. Locally, knowledge of the key egg-laying sites within the breeding aggregation will enable more cautious decision making from companies proposing to proceed with large-scale industry of any kind within the unique spawning grounds. Water quality, water chemistry and environmental parameters which have been established in the breeding aggregation also form a baseline data set.

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Table 1

Locations of study sites between Black Point and Point Lowly, upper Spencer Gulf, South Australia, showing latitude and longitude (decimal degrees). See Fig. 1*b* for figure of sites.

Site	South	East
1	32.991	137.720
2	32.993	137.727
3	32.995	137.739
4	32.996	137.752
5	32.996	137.758
6	32.994	137.773
7	32.000	137.782
8	32.100	137.787
9	32.994	137.785

776 Table 2
777 Two-factor ANOVA results for the concentrations of dissolved nutrients (oxidised nitrogen, ammonia and
778 orthophosphate) in seawater samples
779
780

		Oxidised Nitrogen (NO_x)	Ammonia ($NH_{3/4}^+$)	Orthophosphate (OP)
Source of variation	*d.f.*	MS	MS	MS
Site	8	0.1264	0.0027	0.2660
Time	1	1.9134 ***	0.0043	0.0050
Site x time	8	0.0443	0.0031 ***	0.0270 ***
Residual	36	0.0710	0.0004	0.0034

781 Note: MS indicates mean squares; NO_x data were *ln* (x) transformed; Cochran's *C* test was non-
782 significant for NO_x and $NH_{3/4}^+$, but significant for OP ($P< 0.01$); * $P<0.05$, ** $P<0.01$, *** $P<0.001$.
783

Table 3
Two-factor ANOVA results for the concentrations of trace elements in seawater samples within the breeding aggregation in the upper Spencer Gulf

		Barium (Ba)	Calcium (Ca)	Iron (Fe)	Magnesium (Mg)	Potassium (K)	Strontium (Sr)	Zinc (Zn)
Source of variation	*d.f.*	MS	MS	MS	MS	MS	MS	MS
Site	8	0.566	1721.296	5.973	18637.963	4404.167	675046.296	49.866
Time	1	2.579*	5400.000	107.245*	63379.630	16016.667	2322962.960	187.787
Site x time	8	0.350	2233.333*	14.342	22746.296*	5462.500*	827546.296*	51.956
Residual	36	0.170	907.407	12.832	9253.704	1970.370	304259.259	35.751

Note: MS indicates mean squares; Cochran's *C*- test ($P < 0.05$) was significant for all elements. * $P<0.05$; ** $P<0.01$, *** $P<0.001$.

Table. 4
Mean values (± standard error, s.e.) of environmental parameters of seawater (temperature, salinity, dissolved oxygen, pH) and depth as measured in July and August 2007, within the breeding aggregation near Point Lowly, upper Spencer Gulf. Values for depth are the maximum depth at which measurements were recorded for each site. Note: unless otherwise indicated, s.e. = 0 because in July and August the same values were recorded across all depths. Note: (-) = No data recorded.

Site	Temperature (C°)		Salinity ($^0/_{00}$)		Dissolved O_2 (mg/L)		pH		Depth (m)	
	July	August	July	August	July	August	July	August	July	August
1	12.49 (± 0.01)	14.75	38.80 (± 0.01)	38.58	7.11 (± 0.13)	6.06 (±0.12)	8.39	8.48	2.58	5.24
2	12.47 (± 0.01)	14.84	38.84 (± 0.01)	38.59	7.58 (± 0.06)	6.19 (± 0.06)	8.43	8.48	3.81	3.01
3	12.32 (± 0.01)	-	38.81 (± 0.01)	-	7.86 (± 0.16)	-	8.40	-	3.18	-
4	12.45	14.73	38.67	38.63	9.50 (± 0.09)	8.52 (± 0.11)	8.38	8.49	4.72	2.78
5	12.63 (± 0.01)	14.48	38.64	-	8.78 (± 0.23)	7.40 (± 0.11)	8.39	8.47	2.54	2.24
6	12.28	15.00	38.85 (± 0.01)	38.58	6.72 (± 0.11)	8.97 (± 0.03)	8.37	8.55	1.96	3.79
7	12.32	-	38.81 (± 0.02)	-	6.61 (± 0.17)	-	8.37	-	2.42	-
8	12.28	-	38.94	-	8.19 (± 0.07)	-	8.36	-	5.69	-
9	12.40	13.93	39.42	38.86	9.45 (± 0.09)	9.41 (± 0.04)	8.21 (± 0.01)	8.42	5.90	4.08

Table 5

Salinity, temperature, pH and seawater quality (mean ± s.e.) for experimental treatment tanks, including range of values (in brackets), mean ± s.e., and sample size (*n*).

Treatment	Salinity ($^0/_{00}$), (mean)	Temperature (°C)	pH	NH_4 (mgL^{-1})	NO_2 (mgL^{-1})	NO_3 (mgL^{-1})
39	39.60 (± 0.03)		7.8 ± 0	0.06 ± 0.03	0.18 ± 0.02	2.17 ± 0.59
	(39.04 – 39.98)	(13.34 –18.11)		(0 – 0.50)	(0 – 0.25)	(0 – 10.00)
	n = 50	*n* = 50	*n* = 6	*n* = 26	*n* = 43	*n* = 23
40	40.44 (± 0.03)		7.8 ± 0	0.05 ± 0.02	0.18 ± 0.01	2.70 ± 0.48
	(40.10 – 41.05)	(13.29 -18.03)		(0 – 0.35)	(0.10 – 0.25)	(0 – 5.00)
	n = 49	*n* = 49	*n* = 6	*n* = 24	*n* = 43	*n* = 25
45	45.28 (± 0.02)		7.8 ± 0	0.07 ± 0.02	0.32 ± 0.02	5.93 ± 0.54
	(45.06 – 45.62)	(13.24 –18.07)		(0 – 0.35)	(0.10 – 0.50)	(0 – 10.00)
	n = 51	*n* = 51	*n* = 4	*n* = 24	*n* = 45	*n* = 27
50	50.20 (± 0.04)		7.8 ± 0	0.03 ± 0.02	0.4 ± 0.03	8.64 ± 1.19
	(49.62 – 50.43)	(13.09 –18.51)		(0 – 0.35)	(0.15 – 1.00)	(0 – 20.00)
	n = 54	*n* = 54	*n*= 6	*n* = 24	*n* = 42	*n* = 22
55	54.55 (± 0.15)	(13.42 –17.93)	8.0 ± 0.1	0	0.49 ± 0.04	9.58 ± 1.08
	(52.79 – 56.21)	*n* = 48	(7.8 – 8.2)	0	(0.15 – 1.00)	(0 – 20.00)
	n = 48		*n* = 8	*n* = 26	*n* = 42	*n* = 24

Table 6

Single-factor ANOVA results for the concentrations of trace elements in samples taken from experimental treatments.

		Barium (Ba)	Calcium (Ca)	Iron (Fe)	Magnesium (Mg)	Potassium (K)	Strontium (Sr)	Zinc (Zn)
Source of variation	*d.f.*	MS	MS	MS	MS	MS	MS	MS
Treatment	4	2.931 *ns*	0.071***	1.582 *ns*	0.073***	41220.000***	6376750.000***	25.450*ns*
Residual	15	1.247	0.001	1.239	0.001	1051.667	201666.667	50.850

Note: MS indicates mean squares; * $P<0.05$; ** $P<0.01$, *** $P<0.001$, *ns* = Not significant.

Figure captions

Fig.1. (a) Map of the South Australian Gulf system showing the shape and orientation of Spencer Gulf and (b) the location of the key breeding ground for the aggregation of the giant Australian cuttlefish in the northern Spencer Gulf. Sites 1-9 extend from Black Point through to Point Lowly. Figure 1a taken from Hall and Fowler (2003) and Figure 1b from Google Earth.

Fig. 2. Mean (± s.e.) number of clutches of cuttlefish eggs at nine sites within the breeding aggregation during 2007 and 2008.

Fig. 3. Concentrations of (a) oxidised nitrogen (NO_x), (b) orthophosphate (OP) and (c) ammonia ($NH_{3/4}^{+}$) in seawater samples from nine sites within the breeding aggregation during July and August 2007. Shown are mean values (± s.e.)

Fig. 4. Trace element concentrations (Ba, Ca, K, Fe, Sr, Mg and Zn) (a-g) in seawater from nine sites within the breeding aggregation during July and August 2007. Shown are mean values (± s.e.). Note: units vary among graphs.

Fig. 5. Trace element concentrations (Ba, Ca, K, Fe, Sr, Mg, and Zn) (a-g) in experimental treatment tanks. Tanks 1 and 2 represent the replicate tanks within each treatment. Shown are mean values (± s.e.). Note: units vary among graphs.

Fig. 6. Mean (± s.e.) (a) percent survival (%) and (b) number of days to hatching of cuttlefish embryos which were reared in different concentrations of desalination brine. Note: $39^{0}/_{00}$ treatment was a control and contained no brine.

Fig. 7. Mean (± s.e.) (a) mantle length (mm) and (b) weight of cuttlefish collected from Stony Point (site 4) in November 2007 (field) and of hatchlings from experimental tanks (39‰, 40‰ and 45‰) at the time of hatching.

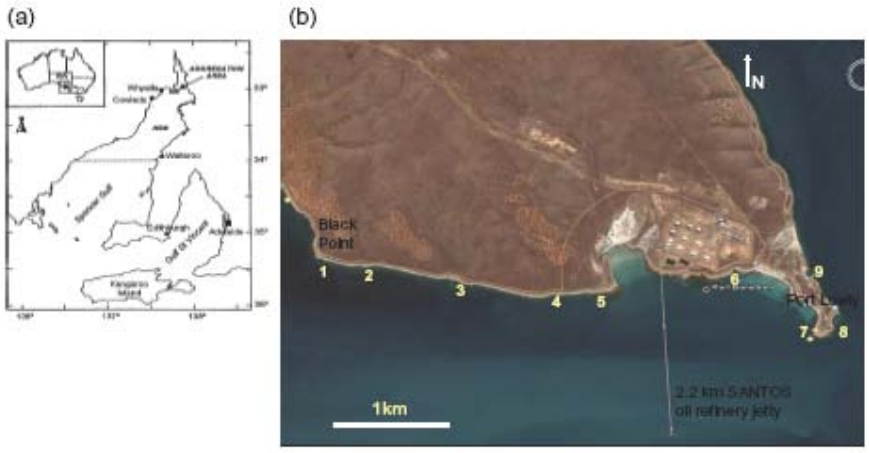


Fig 1

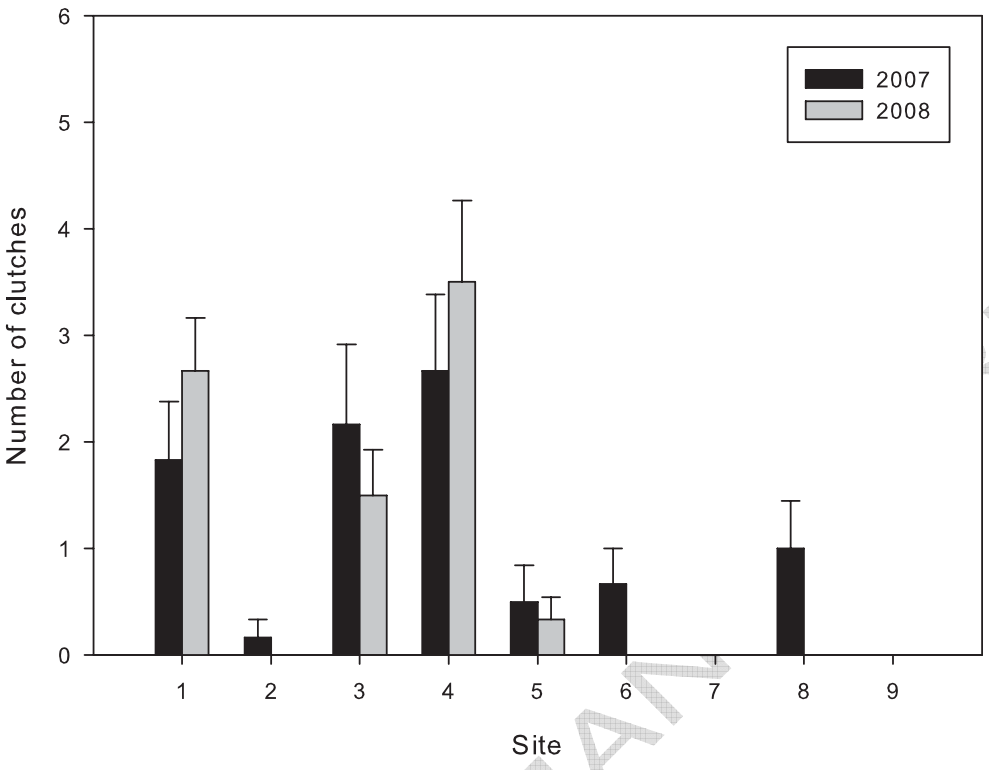


Fig 2

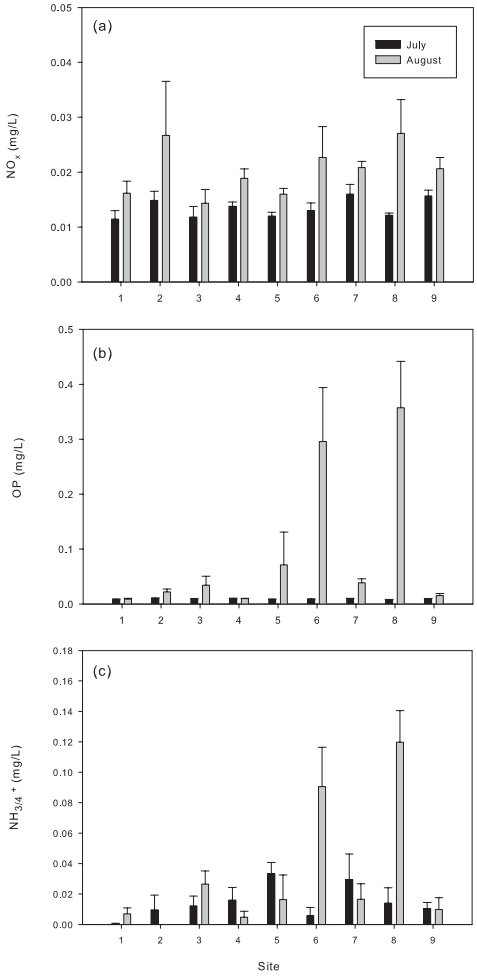


Fig 3

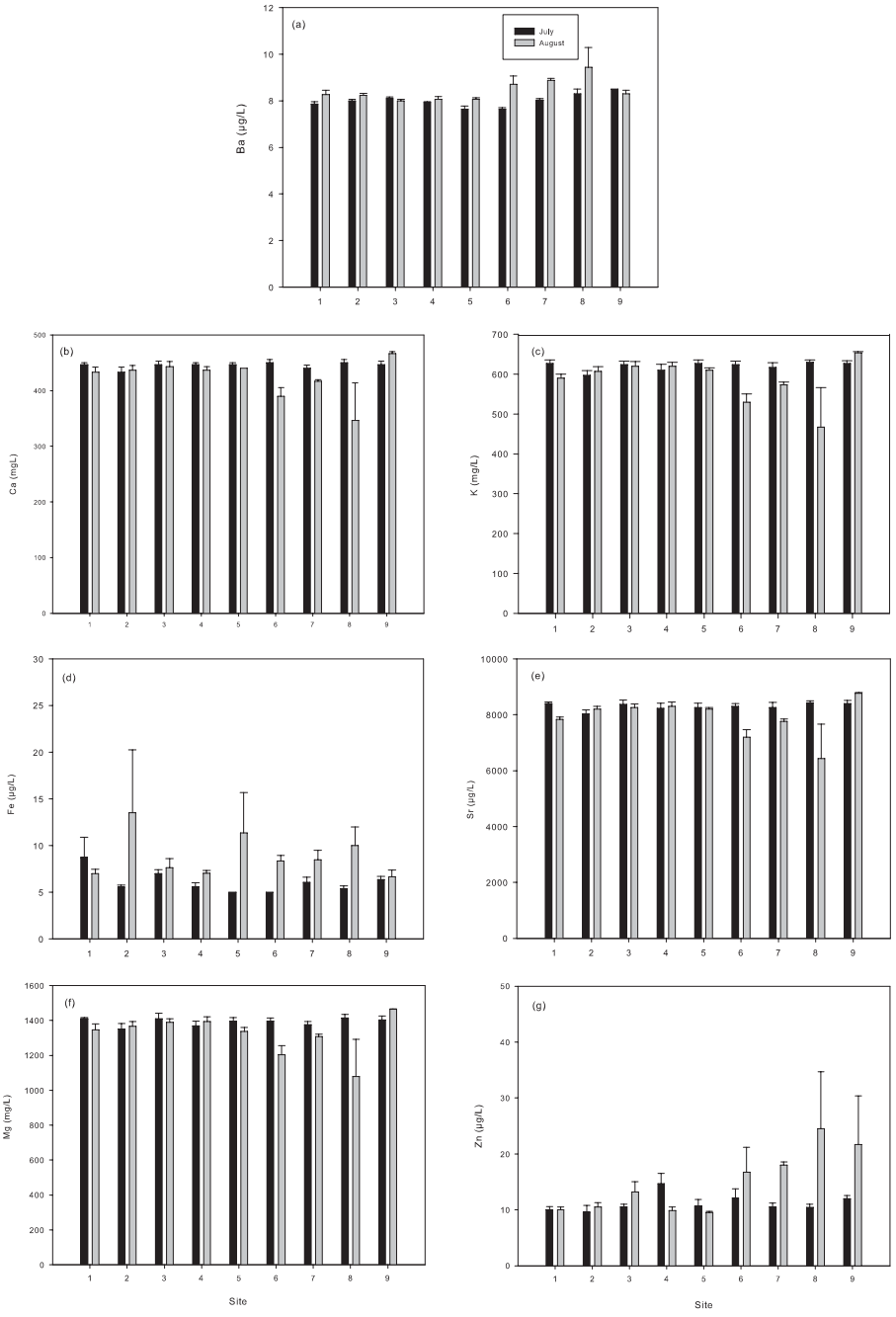

(a)
July
August
Ba (µg/L)
(b)
Ca (mg/L)
(c)
K (mg/L)
(d)
Fe (µg/L)
(e)
Sr (µg/L)
(f)
Mg (mg/L)
(g)
Zn (µg/L)
Site
Site

Fig 4

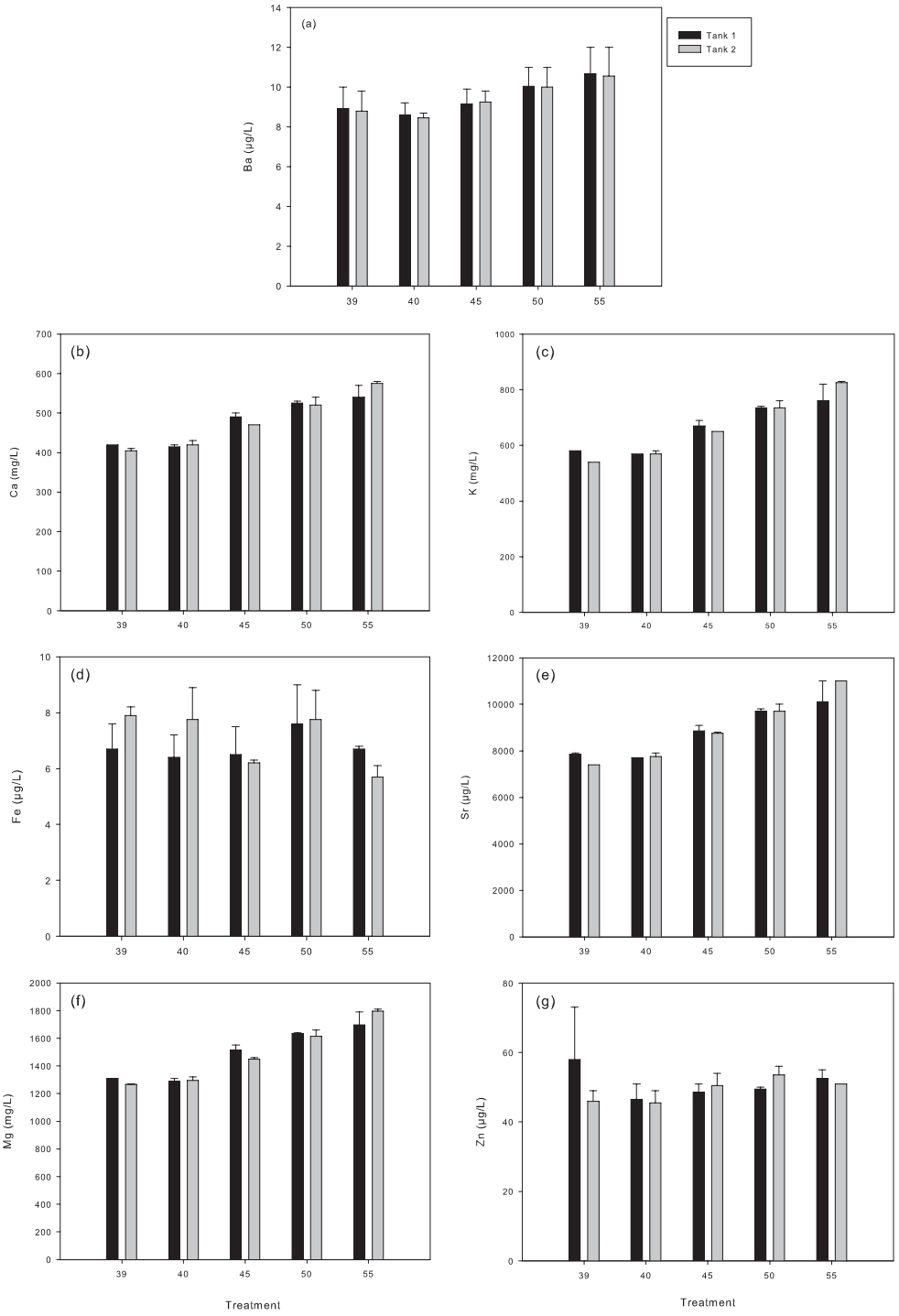
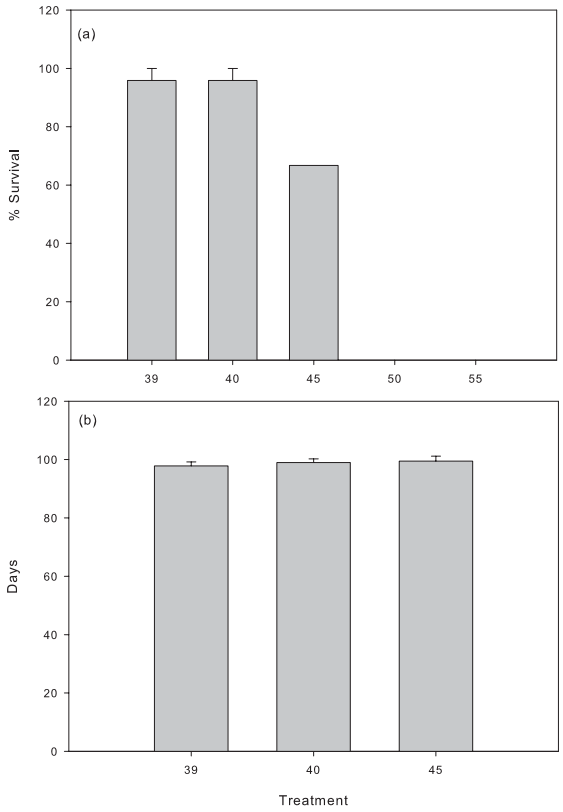

(a)
Tank 1
Tank 2
Ba (µg/L)
(b)
Ca (mg/L)
(c)
K (mg/L)
(d)
Fe (µg/L)
(e)
Sr (µg/L)
(f)
Mg (mg/L)
(g)
Zn (µg/L)
39
40
45
50
55
Treatment

Fig 5

Fig 6



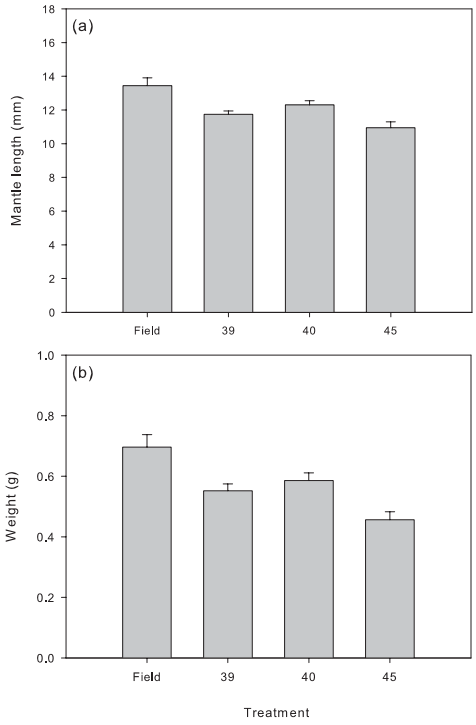


Fig 7