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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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21 **Abstract**

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

38  
39 *Keywords:* *Sepia apama*; Cuttlefish; Spencer Gulf; South Australia; Desalination; Brine  
40

## 41 **1. Introduction**

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

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

63 The impact of desalination brine on the marine environment takes place mainly  
64 at the point source, in the vicinity of the brine discharge pipe. Even though the brine  
65 contains natural marine ingredients, without prior mixing, its high specific weight  
66 causes it to sink to the sea floor forming a stratified system with the brine forming a  
67 bottom layer (Jibril and Ibrahim, 2001; Einav et al., 2002; Fernández-Torquemada et al.,  
68 2005). As the plume sinks, its effects potentially could extend over a range of hundreds  
69 of meters. Desalination discharge alters the amount of dissolved oxygen in the water if  
70 there is insufficient mixing, water temperature is increased due to the heat treatment  
71 within the process and turbidity can be increased at the outlet point (Gleick et al., 2006;  
72 Raventos et al., 2006). Desalination brine also may contain many contaminants and  
73 hazardous wastes (Gleick et al., 2006). These include anti-fouling agents, chlorine and  
74 acid which are unavoidably needed in large scale plants to treat the feed water and

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

79 Although heavy metals and toxic chemicals can be detrimental to marine  
80 organisms, salinity is one of the most important physio-chemical factors to which they  
81 are exposed (D'Aniello et al., 1989). Marine organisms exist in osmotic balance with  
82 their environment and the osmotic stresses acting on different species depend upon  
83 individual adaptations and salinity tolerances within specific habitats. The repercussions  
84 of high salinity levels on marine ecosystems and organisms can take a variety of forms.  
85 Animals which are not adapted to such conditions often move away from the affected  
86 area (Young and Potter, 2002). Species richness and density can also decline where  
87 extreme salinities are prominent (Bayly, 1972; Vega-Cendejas and Hernández de  
88 Santillana, 2004). Increases in the concentration of salts may result in the dehydration of  
89 cells, and the inability to hypoosmotically regulate leading to a decrease of turgor  
90 pressure and mortality, especially in larvae, eggs and juveniles (Cintron, 1970; Aladin,  
91 1991; Einav et al., 2002; Young and Potter, 2002).

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

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

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

131

## 132 **2. Methods**

### 133 *2.1 Study site and study species*

134

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

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

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

160

## 161 2.2 *Abundance of clutches*

162

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

173

## 174 2.3 *Water quality*

175

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

181 Surface water samples for analysis of nutrients and water chemistry were taken  
182 (approximately 15 cm below surface) via 20 ml plastic sterilised syringes ( $n=6$ ) at each  
183 of the 9 sites. Samples for nutrient analysis ( $n=3$ ) were then filtered through 0.45  $\mu\text{m}$   
184 glass fibre filters into 15 ml sample containers and stored frozen prior to analysis.  
185 Nutrient samples were then analysed for concentrations of dissolved ammonia ( $\text{NH}_3/4^+$ ),  
186 oxidised nitrogen ( $\text{NO}_x$ ), and orthophosphate (OP) on a Lachat FIA (Flow Injection  
187 Analysis) Automated Ion Analyser.

#### 188 *2.4 Water chemistry*

189 Samples for water chemistry ( $n=3$ ) were also filtered through 0.45  $\mu\text{m}$  glass  
190 fibre filters but placed into acid washed 30 ml sample containers containing 500  $\mu\text{L}$  of  
191 nitric acid ( $\text{HNO}_3$ ) [70%] and refrigerated for trace element analysis. These samples  
192 were analysed by the National Measurement Institute (NMI) for trace elements  
193 (Calcium (Ca), Magnesium (Mg), Potassium (K), Strontium (Sr), Barium (Ba), Iron  
194 (Fe), Zinc (Zn), Manganese (Mn) and Copper (Cu)). A Perkin Elmer 6000 DRC  
195 (Dynamic Reaction Cell) Inductively Coupled Plasma-Mass Spectrometer (ICP-MS)  
196 was used to detect the concentrations within each sample. High resolution ICP-MS was  
197 used to determine Zn concentrations to remove interference of molecular ions  
198 originating from NaCl, S, Mg, K, and Ca. Lutetium and indium were used as internal  
199 standards to correct for ICP-MS drift. Cu and Mn were omitted from further analyses  
200 because the readings were below detection limits.

#### 201 *2.5 Environmental parameters*

202  
203 A YSI 6600 Multi-parameter Water Quality Meter (CTD sonde) was used to  
204 obtain data on depth, temperature, salinity, pH and dissolved oxygen (DO) at each site.  
205 The sonde was slowly lowered from the side of a boat and took recordings from the  
206 surface to the bottom for 90 seconds with 3-7 second intervals between each reading.

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

210

## 211 *2.6 Embryo growth experiment*

212

### 213 *2.6.1 Egg collection*

214 *Sepia apama* eggs were collected from Stony Point (site 4; 32°59'5"S,  
215 137°43'1"E) in the upper Spencer Gulf, South Australia (Fig. 1b), during July 2007.  
216 Newly laid eggs that were soft, bright white and opaque in appearance were collected  
217 (Hall and Fowler, 2003). Eggs were gently prised off the underside of rocks by hand  
218 and placed into mesh catch bags. Eggs ( $n=120$ ) were then transferred to a plastic bucket  
219 underwater to prevent dehydration and transported to the University of Adelaide in an  
220 insulated 20 L foam box whilst being aerated.

221

### 222 *2.6.2 Experimental set up*

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

231

### 232 *2.6.3 Seawater and brine collection*

233 Seawater was collected from the South Australian Research and Development  
234 Institute (SARDI), Aquatic Sciences Centre, West Beach, South Australia. The seawater  
235 originated from 1 km off shore at a depth of 10 m off the metropolitan coast of Adelaide  
236 in Gulf St Vincent. The water was passed through a settlement tank and primary sand  
237 filter before storage at the facility. The water was collected from SARDI on a weekly  
238 basis and stored at constant temperature ( $\sim 18^\circ\text{C}$ ) in a 2000 L tank in the aquarium room  
239 at the University of Adelaide.

240

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

250

#### 251 *2.6.4 Experimental design*

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

262

#### 263 *2.6.5 Tank water quality*

264 Within the controlled temperature room a flow through system with a biological  
265 filter was not feasible. Water changes within treatments were therefore done manually.  
266 pH was maintained at 7.8-8.2. To maintain water quality for the requirements of  
267 cuttlefish ( $\text{NH}_4 < 0.5 \text{ mg/L}$ ,  $\text{NO}_2 < 0.2 \text{ mg/L}$  and  $\text{NO}_3 < 50 \text{ mg/L}$ ) (Hanley et al., 1998;  
268 Minton, 2004) the levels of these three parameters were tested using an Aquarium  
269 Pharmaceuticals (API) liquid test kit. Nutrient tests were conducted mostly during the  
270 two consecutive days after a water change. However during the initial two weeks of the  
271 experiment nutrient analyses were conducted prior to a water change. Water changes  
272 (50-75 %) were conducted every 3-5 days to maintain water quality and levels of trace  
273 elements which are needed for cephalopod development (Hanley et al., 1998). Water  
274 was changed within specific treatments when significant levels of nutrients within these

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

279

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

283

#### 284 *2.6.6 Length and weight of hatchlings*

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

292

#### 293 *2.6.7 Field samples*

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

298

#### 299 *2.7 Statistical analyses*

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

307

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

313

### 314 **3. Results**

315

#### 316 *3.1 Abundance of clutches*

317

318 The number of clutches of eggs showed a significant difference among sites, but  
319 similar patterns were seen for both years (Fig. 2;  $F_{8,8} = 12.9$ ,  $P < 0.001$ ). Several sites  
320 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  
321 and 4 had significantly greater numbers of clutches than all other sites. Where clutches  
322 were present, between 1 and 21 clutches were found per site.

323

#### 324 *3.2 Field water quality and chemistry*

325

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

333

334 Zinc showed no significant difference among sites or times (Fig. 4g, Table 3).  
335 Concentrations of Ba and Fe were significantly greater during August than July, but did  
336 not vary among sites (Fig. 4a and d, Table 3). For the remaining four elements (Sr, Ca,  
337 K, Mg) a significant interaction between site and time was found largely because for  
338 July there were no significant differences among sites, whereas for August some sites  
339 differed (Fig. 4b, c, e and f). Concentrations of Sr, Ca, K and Mg at site 8 were  
340 significantly lower than all other sites with the exception of site 6 (Sr, Ca, K, Mg) and 9  
341 (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  $\pm$  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  $<0.35^\circ\text{C}$  mean difference among sites; August maximum  
349 difference  $1.07^\circ\text{C}$ ). Mean water temperature in August ( $14.62 \pm 0.05^\circ\text{C}$ ) was  $2.21^\circ\text{C}$   
350 greater than in July ( $12.40 \pm 0.04^\circ\text{C}$ ). Salinity was also constant across all sites during  
351 both months. The mean salinity across all sites within the breeding ground was  $38.77\text{‰}$   
352 ( $\pm 0.05$ ). The highest average salinity was recorded at site 9,  $39.42\text{‰}$  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  $6.06\text{ mg/L}$  (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  $8.37 (\pm 0.02)$  compared to August which  
359 was  $8.48 (\pm 0.02)$ . The maximum depth of the sites varied from  $\sim 2\text{ m}$  to just under  $6\text{ m}$ ,  
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  $\text{NH}_4$  and  $\text{NO}_3$  were within optimum concentrations  
368 ( $\text{NH}_4 < 0.5\text{ mg/L}$  and  $\text{NO}_3 < 50\text{ mg/L}$ ) throughout the experimental period within all  
369 treatments. Levels of  $\text{NO}_2$  were elevated ( $>0.2\text{ mg/L}$ ) 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  $3.42\text{‰}$ , 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  $7.8\text{--}8.2$  for 55 treatment). Water temperature increased within all  
374 treatments by  $4.50^\circ\text{C} (\pm 0.50^\circ\text{C})$  and closely matched the measured temperatures

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

377

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

382

### 383 *3.4.2 Hatching success*

384 Hatching success was similar between the 39 ‰ (control, no brine) and 40 ‰  
385 treatments, but then decreased for 45 ‰. In the 50 ‰ and 55 ‰ treatments there was  
386 total mortality of eggs ( $F_{4,5} = 340.18, P < 0.0001$ ; Fig. 6a). A logistic regression was  
387 fitted to the data [percent survival =  $\exp(B_0 + B_1 \times \text{salinity}) / (1 + \exp(B_0 + B_1 \times \text{salinity}))$   
388 where  $B_0 = 30.256 \pm 5.979$ , and  $B_1 = -0.666 \pm 0.132$ ]. Thus, there was ~ 7 % decrease  
389 in survival for every 1 ‰ increase in salinity. It was also noted that one embryo in the  
390 45 ‰ was malformed. For those treatments where individuals survived through to  
391 hatching, the minimum average time to hatching was 99 days. There was no difference  
392 between the length of time to hatching among the three treatments ( $F_{2,59} = 0.3323, P =$   
393  $0.738$ ; Fig. 6b).

394

### 395 *3.4.3 Length and weight of hatchlings*

396 There was a significant difference between the mantle lengths and weights of the  
397 hatchlings (Mantle length:  $F_{3,68} = 9.514, P < 0.001$ ; Fig 7a; Weight:  $F_{3,68} = 9.501, P <$   
398  $0.001$ ; Fig 7b). Field-collected specimens were significantly larger and heavier than  
399 any of the treatment individuals. Of the laboratory treatments, individuals from 45 ‰  
400 were significantly smaller in length and weight than those from 39 ‰ and 40 ‰.

401

402 **4. Discussion**

403

404 The primary egg-laying area for *S. apama* lies between Point Lowly and Black  
405 Point in the upper Spencer Gulf (Hall and Hanlon, 2002). Within this region certain  
406 areas had a greater number of clutches. Differences in cephalopod egg abundance  
407 between sites within a particular region are common. A previous study found that the  
408 number of *S. apama* eggs varied among areas within a single site of the breeding  
409 aggregation, although the difference was not statistically significant (Hall and Fowler,  
410 2003). Spatial variability of egg abundance on a small spatial scale (within a 1 km) has  
411 also been found for squid species (Moltschaniwskyj and Pecl, 2003). The differences  
412 between sites may be attributed to the fine-scale variability of substrate within the  
413 breeding aggregation. The study area in the upper Spencer Gulf is made up of a hard  
414 substrate which constitutes a conspicuous and finite area. The area with the highest  
415 number of clutches maintained a clear slaty bed rock which was the most suitable for  
416 egg-laying (Gostin et al., 1984; Hall and Fowler, 2003). Knowledge of primary egg-  
417 laying sites can contribute to a more informed decision as to where an intake and  
418 discharge pipe for seawater desalination should be placed.

419

420 The benthic eggs are exposed to water surrounding them during the austral  
421 winter. Values for nutrients and environmental variables near Point Lowly were  
422 generally considered moderate to good according to the ANZECC guidelines  
423 (ANZECC, 2000). Several sites (6 and 9) did exceed trigger values for nutrients  
424 (ammonia and orthophosphate) at one sampling time. Caution will therefore be  
425 required to ensure that the brine discharge does not lead to elevated nutrient  
426 concentrations. Although it is difficult to find guidelines for many of the trace elements  
427 for Australian waters, trace elements are likely to be increased in brine, which if not  
428 dispersed may lead to elevated levels in the vicinity of giant Australian cuttlefish eggs.  
429 Salinity is already elevated in upper Spencer Gulf due to the lack of freshwater input  
430 and inverse estuary nature of the gulf (Nunes Vas et al. 1990). Salinity was lower on  
431 average during this sampling period (38.78 ‰) compared to mean salinities of 40 ‰  
432 recorded in March, 1984, 41 ‰ in August, 1975 and 42.6 ‰ in July, 1976 (Johnson,  
433 1981; Nunes and Lennon, 1986). The brine is expected to have double the  
434 concentration of salts (70-80 ‰), higher temperature and turbidity than ambient  
435 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

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

472 The inhibition of normal metabolic activity also caused the embryos in higher  
473 salinities to be smaller than hatchlings in the ambient salinity treatment. Previous  
474 research has shown that abiotic factors such as salinity have the potential to decrease the  
475 nutritional condition of developing larvae, as indicated by their length and weight  
476 (Folkvord et al., 1996). The decrease in mantle size as an effect of salinity has been  
477 noted previously and described as a malformation (Paulij et al. 1990). Also, correlations  
478 with size of hatchlings and hatching success have been determined for cephalopod  
479 embryos grown in low salinities (Palmegiano and Dapote, 1983; Fagundez and Robaina,  
480 1992; Cinti et al., 2004; Sen, 2004; Sen, 2005; Villanueva et al., 2007); however this is  
481 the first study which indicates a decrease in weight and mantle length in salinities  
482 greater than 42 ‰. The yolk reserves of individual eggs are the only energy source for  
483 development of the embryo; the smaller the hatchling, the greater the physical  
484 constraints imposed on the functional morphology of organs responsible for swimming  
485 and food capture, therefore once hatched survivability may also be decreased (Boyle  
486 and Boletzky, 1996).

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

494 Desalination brine concentrates not only salts, but metals and trace elements  
495 during the process of extracting fresh water (Talavera and Quesada Ruiz, 2001).  
496 Although *S. apama* eggs may already be exposed to heavy metals and trace element  
497 concentrations, due to the breeding aggregations proximity to major industry, increased  
498 concentrations of trace elements may have been a cause of mortality in the experiment  
499 as concentrations of some trace elements were far greater than those found within  
500 waters near the breeding aggregation. Metals retard embryos from hatching at  
501 concentrations equal to or lower than those causing mortality and the effects of metals  
502 on embryos are often increased as a function of exposure duration (Macdonald et al.,  
503 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

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

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

561

## 562 **5. Conclusion**

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

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

579

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585

### 586 **References**

587

588 ANZECC 2000. Australian and New Zealand guidelines for fresh and marine water  
589 quality. Australian and New Zealand Environment and Conservation Council and  
590 Agriculture and Resource Management Council of Australia and New Zealand.  
591 Canberra.

592

593 Aladin, N.V., 1991. Salinity tolerance and morphology of the osmoregulation organs in  
594 Cladocera with special reference to Cladocera from the Aral Sea. *Hydrobiologia* 225,  
595 291-299.

596

597 Arnal, J.M., Sancho, M., Iborra, I., Gozalvez, J.M., Santafe, A., Lora, J., 2005.  
598 Concentration of brines from RO desalination plants by natural evaporation.  
599 *Desalination* 182, 435-439.

600

601 Barker, R., van Koppen, B., Shah, T., 2000. 'A global perspective on water scarcity and  
602 poverty: Achievements and challenges for water resources management.' International  
603 Water Management Institute (IWMI). Colombo, Sri Lanka.

604

605 Bayly, I.E., 1972. Salinity tolerance and osmotic behaviour of animals in athalassic  
606 saline and marine hypersaline waters. *Annual Review of Ecology and Systematics* 3,  
607 233-268.

608

609 Boyle, P.R., Boletzky, S.V., 1996. Cephalopod populations: Definition and dynamics.  
610 *Philosophical Transactions of the Royal Society of London. Biological Sciences* 351,  
611 985-1002.

612

613 Bustamante, P., Teyssie, J.L., Fowler, S.W., Cotret, O., Danis, B., Miramand, P.,  
614 Warnau, M., 2002. Biokinetics of zinc and cadmium accumulation and depuration at

- 615 different stages in the life cycle of the cuttlefish *Sepia officinalis*. Marine Ecology  
616 Progress Series 231, 167-177.  
617
- 618 Cinti, A., Baron, P.J., Rivas, A.L., 2004. The effects of environmental factors on the  
619 embryonic survival of the Patagonian squid *Loligo gahi*. Journal of Experimental  
620 Marine Biology and Ecology 313, 225-240.  
621
- 622 Cintron, G., 1970. Some consequences of brine pollution in the Bahia Fosforescente,  
623 Puerto Rico. Limnology and Oceanography 15, 246-249.  
624
- 625 Cronin, E.R., Seymour, R.S., 2000. Respiration of the eggs of the giant cuttlefish *Sepia*  
626 *apama*. Marine Biology 136, 863-870.  
627
- 628 D'Aniello, A., D'Onofrio, G., Pischetola, M., Denucci, J.M., 1989. Effect of pH, salinity  
629 and  $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$ ,  $\text{K}^{+}$  and  $\text{SO}_4^{+2}$  ions on hatching and viability of *Loligo vulgaris* embryo.  
630 Comparative Biochemistry and Physiology 94, 477-481.  
631
- 632 Edgar, G.J., Barrett, N.S., Morton, A.J., 2004. Biases associated with the use of  
633 underwater visual census techniques to quantify the density and size-structure of fish  
634 populations. Journal of Experimental Marine Biology and Ecology 308, 269-290.  
635
- 636 Einav, R., Harussi, K., Perry, D., 2002. The footprint of the desalination processes on  
637 the environment. Desalination 152, 141-154.  
638
- 639 Epifanio, C.E., Srna, R.F., 1975. Toxicity of ammonia, nitrite ion, nitrate ion, and  
640 orthophosphate to *Mercenaria mercenaria* and *Crassostrea virginica*. Marine Biology  
641 33, 241-246.  
642
- 643 Fàgundez, S.B., Robaina, G., 1992. Effects of temperature, salinity, and photoperiod in  
644 the embryonic development of the squid *Sepioteuthis sepioidea* (Blainville, 1823).  
645 Memoria de la Sociedad de Ciencias Naturales La Salle 52, 93-103.  
646
- 647 Fernández-Torquemada, Y., Sánchez-Lizaso, J.L., González-Correa, M., 2005.  
648 Preliminary results of the monitoring of the brine discharge produced by the SWRO  
649 desalination plant of Alicante (SE Spain). Desalination 182, 395-402.  
650
- 651 Folkvord, A., Ystanes, L., Johannessen, A., Moksness, E., 1996. RNA: DNA ratios and  
652 growth of herring (*Clupea harengus*) larvae reared in mesocosms. Marine Biology 126,  
653 591-602.  
654
- 655 Giménez, L., Anger, K., 2001. Relationships among salinity, egg size, embryonic  
656 development, and larval biomass in the estuarine crab *Chasmagnathus granulata* Dana,  
657 1851. Journal of Experimental Marine Biology and Ecology 260, 241-257.  
658
- 659 Gleick, P., Cooly, H., Wolff, G., 2006. With a grain of salt: An update on saltwater  
660 desalination. In: 'The world's water 2006-2007.' The biennial report on freshwater  
661 resources.' (Ed. HC P. H. Gleick, D. Katz, E. Lee, J. Morrison, M. Palaniappan, A.  
662 Samulon and G. Wolff.) pp. 51-89. (Island Press: Washington, DC).  
663

- 664 Gostin, V.A., Hails, J.R., Belperio, A.P., 1984. The sedimentary framework of Northern  
665 Spencer Gulf, South Australia. *Marine Geology* 61, 111-138.  
666
- 667 Hall, K., Fowler, A.J (eds). 2003. The fisheries biology of the cuttlefish *Sepia apama*  
668 Gray, in South Australian waters. Final report to FRDC (Project No. 98/151). SARDI  
669 Aquatic Sciences, Adelaide, 289pp.  
670
- 671 Hall, K.C., Hanlon, R.T., 2002. Principal features of the mating system of a large  
672 spawning aggregation of the giant Australian cuttlefish *Sepia apama* (Mollusca:  
673 Cephalopoda). *Marine Biology* 140, 533-545.  
674
- 675 Hanley, J.S., Shashar, N., Smolowitz, R., Bullis, R.A., Mebane, W.N., Gabr, H.R.,  
676 Hanlon, R.T., 1998. Modified laboratory culture techniques for the European cuttlefish  
677 *Sepia officinalis*. *Biological Bulletin* 195, 223-225.  
678
- 679 Hanna, R.G., Muir, G.L., 1990. Red sea corals as biomonitors of trace metal pollution.  
680 *Environmental Monitoring and Assessment* 14, 211-222.  
681
- 682 Hashim, A., Hajjaj, M., 2005. Impact of desalination plants fluid effluents on the  
683 integrity of seawater, with the Arabian Gulf in perspective. *Desalination* 182, 373-393.  
684
- 685 Jibril, B.E-Y., Ibrahim, A.A., 2001. Chemical conversions of salt concentrates from  
686 desalination plants. *Desalination* 139, 287-295.  
687
- 688 Johnson, J. E., 1981. Hydrological data for Upper Spencer Gulf 1975-1978. Department  
689 of Fisheries, South Australia. No. 3.  
690
- 691 Macdonald, J.M., Shields, J.D., Zimmer-Faust, R.K., 1988. Acute toxicities of eleven  
692 metals to early life-history stages of the yellow crab *Cancer anthonyi*. *Marine Biology*  
693 98, 201-207.  
694
- 695 Mandelli, E.F., McIlhenny, W.F., 1971. 'A study of the effect of desalination plant  
696 effluents on marine benthic organisms.' United States Department of the Interior, 803.  
697
- 698 Minton, J.W., 2004. The pattern of growth in the early life cycle of individual *Sepia*  
699 *pharaonis*. *Marine and Freshwater Research* 55, 415-422.  
700
- 701 Miramand, P., Bustamante, P., Bentley, D., Kouéta, N., 2006. Variation of heavy metal  
702 concentrations (Ag, Cd, Co, Cu, Fe, Pb, V, and Zn) during the life cycle of the common  
703 cuttlefish *Sepia officinalis*. *Science of the Total Environment* 361, 132-143.  
704
- 705 Moltschaniwskyj, N.A., Pecl, G.T., 2003. Small-scale spatial and temporal patterns of  
706 egg production by the temperate loliginid squid *Sepioteuthis australis*. *Marine Biology*  
707 142, 509-516.  
708
- 709 Nunes, R.A., Lennon, G.W., 1986. Physical property distributions and seasonal trends  
710 in Spencer Gulf, South Australia - an inverse estuary. *Australian Journal of Marine and*  
711 *Freshwater Research* 37, 39-53.  
712

- 713 Nunes Vas, R.A., Lennon, G.W., Bowers, D.G., 1990. Physical behaviour of a large,  
714 negative or inverse estuary. *Continental Shelf Research* 10, 277-304.  
715
- 716 Palmegiano, G.B., Dapote, M.P., 1983. Combined effects of temperature and salinity on  
717 cuttlefish (*Sepia officinalis* L) hatching. *Aquaculture* 35, 259-264.  
718
- 719 Paulij, W.P., Bogaards, R.H., Denuce, J.M., 1990. Influence of salinity on embryonic  
720 development and the distribution of *Sepia officinalis* in the Delta area South Western  
721 part of the Netherlands. *Marine Biology* 107, 17-24.  
722
- 723 Porter, J.W., Lewis, S.K., Porter, K.G., 1999. The effect of multiple stressors on the  
724 Florida Keys coral reef ecosystem: A landscape hypothesis and a physiological test.  
725 *Limnology and Oceanography* 44, 941-949.  
726
- 727 Raventos, N., Macpherson, E., Garcia-Rubies, A., 2006. Effect of brine discharge from  
728 a desalination plant on macrobenthic communities in the NW Mediterranean. *Marine*  
729 *Environmental Research* 62, 1-14.  
730
- 731 Sen, H., 2004. A preliminary study on the effects of salinity on egg development of  
732 European squid (*Loligo vulgaris* Lamarck, 1798). *Israeli Journal of Aquaculture*  
733 *Bamidgeh* 56, 95-101.  
734
- 735 Sen, H., 2005. Incubation of European squid (*Loligo vulgaris* Lamarck, 1798) eggs at  
736 different salinities. *Aquaculture Research* 36, 876-881.  
737
- 738 Sherwood, J.E., Stagnitti, F., Kokkinn, M.J., Williams, W.D., 1991. Dissolved oxygen  
739 concentrations in hypersaline waters. *Limnology and Oceanography*. 36, 235-250.  
740
- 741 Talavera, P., Quesada Ruiz, J., 2001. Identification of the mixing processes in brine  
742 discharges carried out in Barranco del Toro Beach, south of Gran Canaria (Canary  
743 Islands). *Desalination* 139, 277-286.  
744
- 745 URS Australia., 2002. 'Summary report: Introduction to desalination technologies in  
746 Australia.' A report prepared for the Department of Agriculture, Fisheries and Forestry -  
747 Australia (AFFA), Australian Capital Territory.  
748 [http://www.environment.gov.au/water/publications/urban/pubs/desalination-](http://www.environment.gov.au/water/publications/urban/pubs/desalination-summary.pdf)  
749 [summary.pdf](http://www.environment.gov.au/water/publications/urban/pubs/desalination-summary.pdf) (accessed 1 October 2008).  
750
- 751 Vega-Cendejas, M.E., Hernández de Santillana, M., 2004. Fish community structure  
752 and dynamics in a coastal hypersaline lagoon: Rio Lagartos, Yucatan, Mexico.  
753 *Estuarine Coastal and Shelf Science* 60, 285-299.  
754
- 755 Villanueva, R., Moltchanivskyj, N.A., Bozzano A., 2007. Abiotic influences on  
756 embryo growth: statoliths as experimental tools in the squid early life history. *Reviews*  
757 *in Fish Biology and Fisheries* 17, 101-110.  
758
- 759 York, R., Foster, M., 2005. 'Issues and environmental impacts associated with once  
760 through cooling at California's coastal power plants.' California Energy Commission,  
761 Sacramento, CA.  
762

763 Young, G.C., Potter, I.C., 2002. Influence of exceptionally high salinities, marked  
764 variations in freshwater discharge and opening of estuary mouth on the characteristics  
765 of the ichthyofauna of a normally-closed estuary. *Estuarine, Coastal and Shelf Science*  
766 55, 223-246.  
767  
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769

770 Table 1

771 Locations of study sites between Black Point and Point Lowly, upper Spencer Gulf, South Australia,

772 showing latitude and longitude (decimal degrees). See Fig. 1*b* for figure of sites.

773

774

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

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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 <sub>x</sub> )	Ammonia (NH <sub>3/4</sub> <sup>+</sup> )	Orthophosphate (OP)
Source of variation	<i>d.f.</i>	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<sub>x</sub> data were *ln* (x) transformed; Cochran's *C* test was non-  
 782 significant for NO<sub>x</sub> and NH<sub>3/4</sub><sup>+</sup>, 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	<i>d.f.</i>	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 ( $\pm$  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 (‰)		Dissolved O <sub>2</sub> (mg/L)		pH		Depth (m)	
	July	August	July	August	July	August	July	August	July	August
1	12.49 ( $\pm$ 0.01)	14.75	38.80 ( $\pm$ 0.01)	38.58	7.11 ( $\pm$ 0.13)	6.06 ( $\pm$ 0.12)	8.39	8.48	2.58	5.24
2	12.47 ( $\pm$ 0.01)	14.84	38.84 ( $\pm$ 0.01)	38.59	7.58 ( $\pm$ 0.06)	6.19 ( $\pm$ 0.06)	8.43	8.48	3.81	3.01
3	12.32 ( $\pm$ 0.01)	-	38.81 ( $\pm$ 0.01)	-	7.86 ( $\pm$ 0.16)	-	8.40	-	3.18	-
4	12.45	14.73	38.67	38.63	9.50 ( $\pm$ 0.09)	8.52 ( $\pm$ 0.11)	8.38	8.49	4.72	2.78
5	12.63 ( $\pm$ 0.01)	14.48	38.64	-	8.78 ( $\pm$ 0.23)	7.40 ( $\pm$ 0.11)	8.39	8.47	2.54	2.24
6	12.28	15.00	38.85 ( $\pm$ 0.01)	38.58	6.72 ( $\pm$ 0.11)	8.97 ( $\pm$ 0.03)	8.37	8.55	1.96	3.79
7	12.32	-	38.81 ( $\pm$ 0.02)	-	6.61 ( $\pm$ 0.17)	-	8.37	-	2.42	-
8	12.28	-	38.94	-	8.19 ( $\pm$ 0.07)	-	8.36	-	5.69	-
9	12.40	13.93	39.42	38.86	9.45 ( $\pm$ 0.09)	9.41 ( $\pm$ 0.04)	8.21 ( $\pm$ 0.01)	8.42	5.90	4.08

Table 5

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

Treatment	Salinity (‰), (mean)	Temperature (°C)	pH	NH <sub>4</sub> (mgL <sup>-1</sup> )	NO <sub>2</sub> (mgL <sup>-1</sup> )	NO <sub>3</sub> (mgL <sup>-1</sup> )
39	39.60 ( $\pm$ 0.03) (39.04 – 39.98) $n = 50$	(13.34 – 18.11) $n = 50$	7.8 $\pm$ 0 $n = 6$	0.06 $\pm$ 0.03 (0 – 0.50) $n = 26$	0.18 $\pm$ 0.02 (0 – 0.25) $n = 43$	2.17 $\pm$ 0.59 (0 – 10.00) $n = 23$
40	40.44 ( $\pm$ 0.03) (40.10 – 41.05) $n = 49$	(13.29 – 18.03) $n = 49$	7.8 $\pm$ 0 $n = 6$	0.05 $\pm$ 0.02 (0 – 0.35) $n = 24$	0.18 $\pm$ 0.01 (0.10 – 0.25) $n = 43$	2.70 $\pm$ 0.48 (0 – 5.00) $n = 25$
45	45.28 ( $\pm$ 0.02) (45.06 – 45.62) $n = 51$	(13.24 – 18.07) $n = 51$	7.8 $\pm$ 0 $n = 4$	0.07 $\pm$ 0.02 (0 – 0.35) $n = 24$	0.32 $\pm$ 0.02 (0.10 – 0.50) $n = 45$	5.93 $\pm$ 0.54 (0 – 10.00) $n = 27$
50	50.20 ( $\pm$ 0.04) (49.62 – 50.43) $n = 54$	(13.09 – 18.51) $n = 54$	7.8 $\pm$ 0 $n = 6$	0.03 $\pm$ 0.02 (0 – 0.35) $n = 24$	0.4 $\pm$ 0.03 (0.15 – 1.00) $n = 42$	8.64 $\pm$ 1.19 (0 – 20.00) $n = 22$
55	54.55 ( $\pm$ 0.15) (52.79 – 56.21) $n = 48$	(13.42 – 17.93) $n = 48$	8.0 $\pm$ 0.1 (7.8 – 8.2) $n = 8$	0 $n = 26$	0.49 $\pm$ 0.04 (0.15 – 1.00) $n = 42$	9.58 $\pm$ 1.08 (0 – 20.00) $n = 24$

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	<i>d.f.</i>	MS	MS	MS	MS	MS	MS	MS
Treatment	4	2.931 <i>ns</i>	0.071***	1.582 <i>ns</i>	0.073***	41220.000***	6376750.000***	25.450 <i>ns</i>
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 ( $\pm$  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 ( $\text{NO}_x$ ), (b) orthophosphate (OP) and (c) ammonia ( $\text{NH}_3/4^+$ ) in seawater samples from nine sites within the breeding aggregation during July and August 2007. Shown are mean values ( $\pm$  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 ( $\pm$  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 ( $\pm$  s.e.). Note: units vary among graphs.

Fig. 6. Mean ( $\pm$  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<sup>0</sup>/<sub>00</sub> treatment was a control and contained no brine.

Fig. 7. Mean ( $\pm$  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.

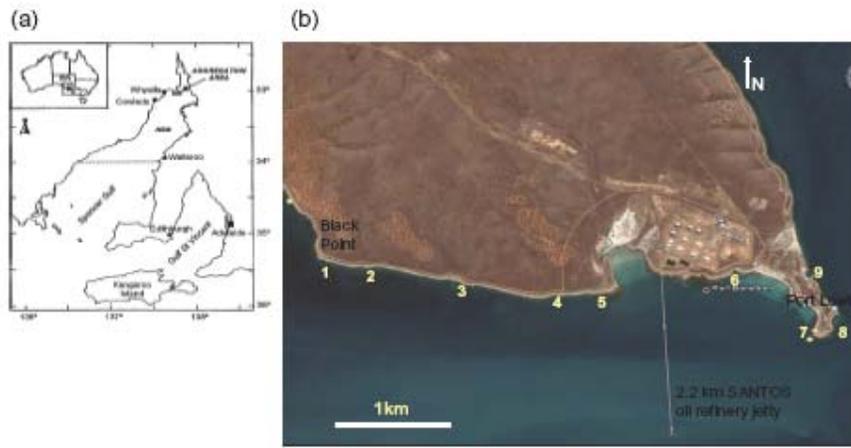


Fig 1

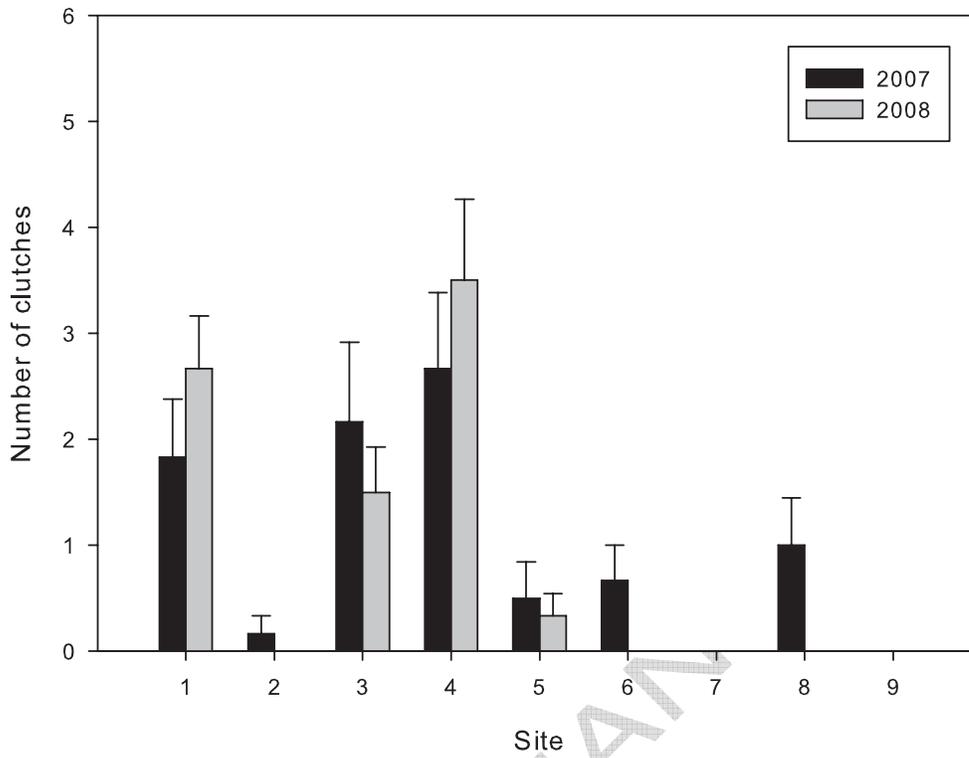


Fig 2

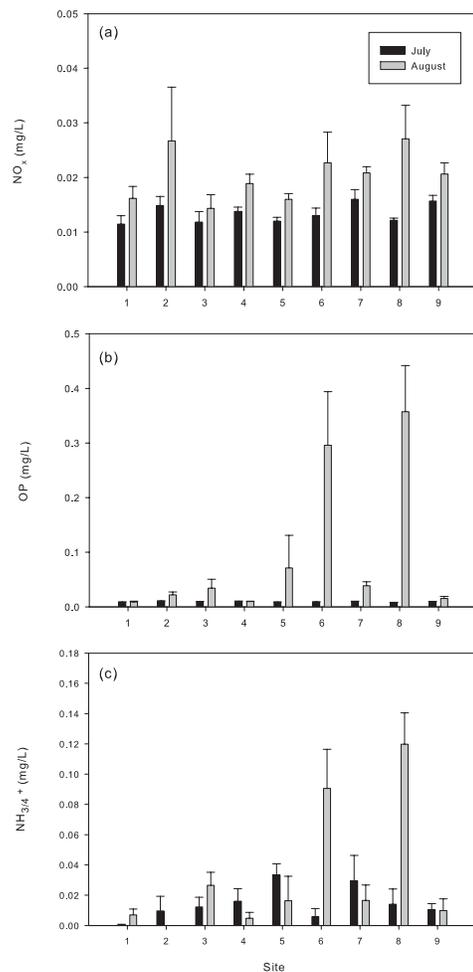


Fig 3

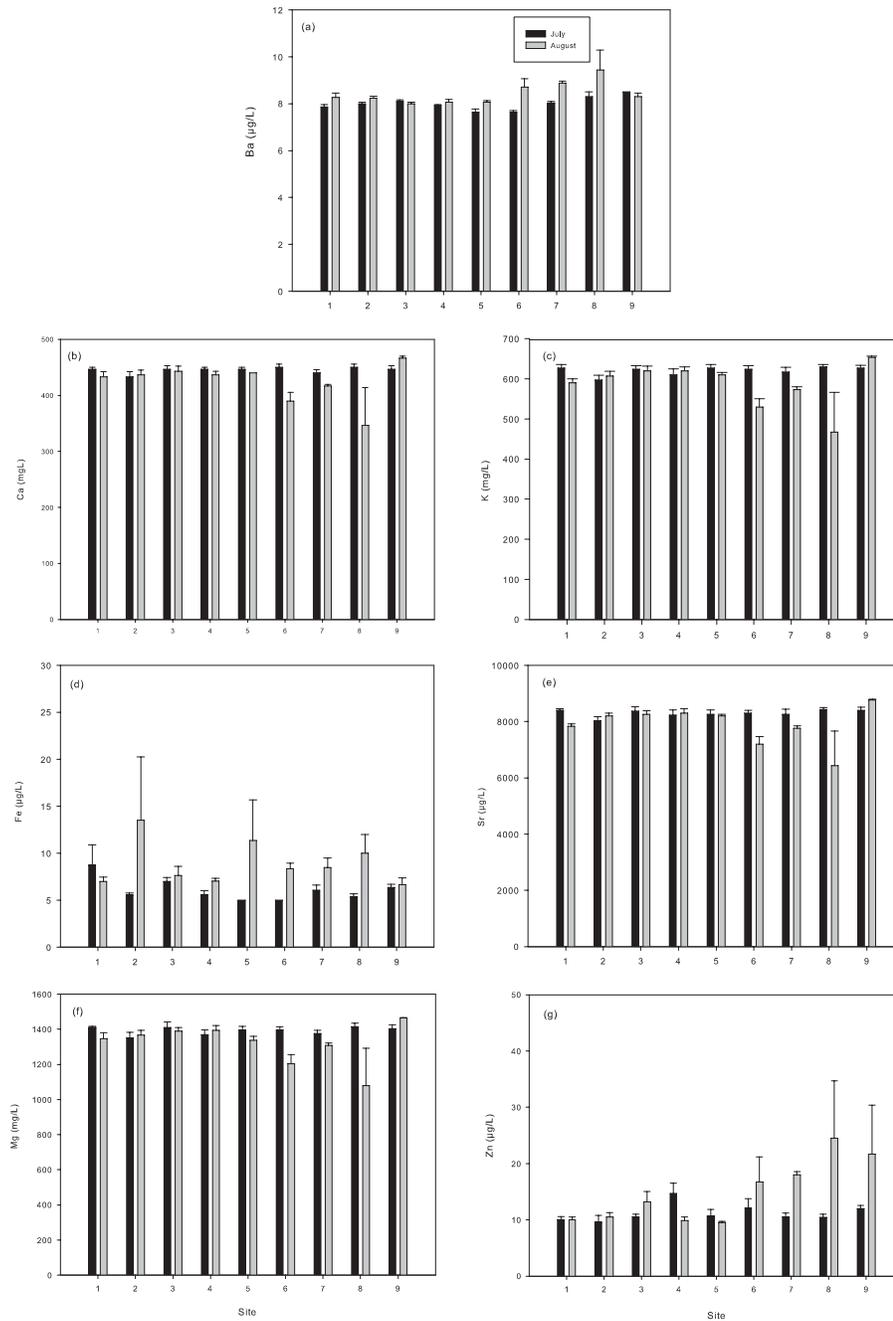


Fig 4

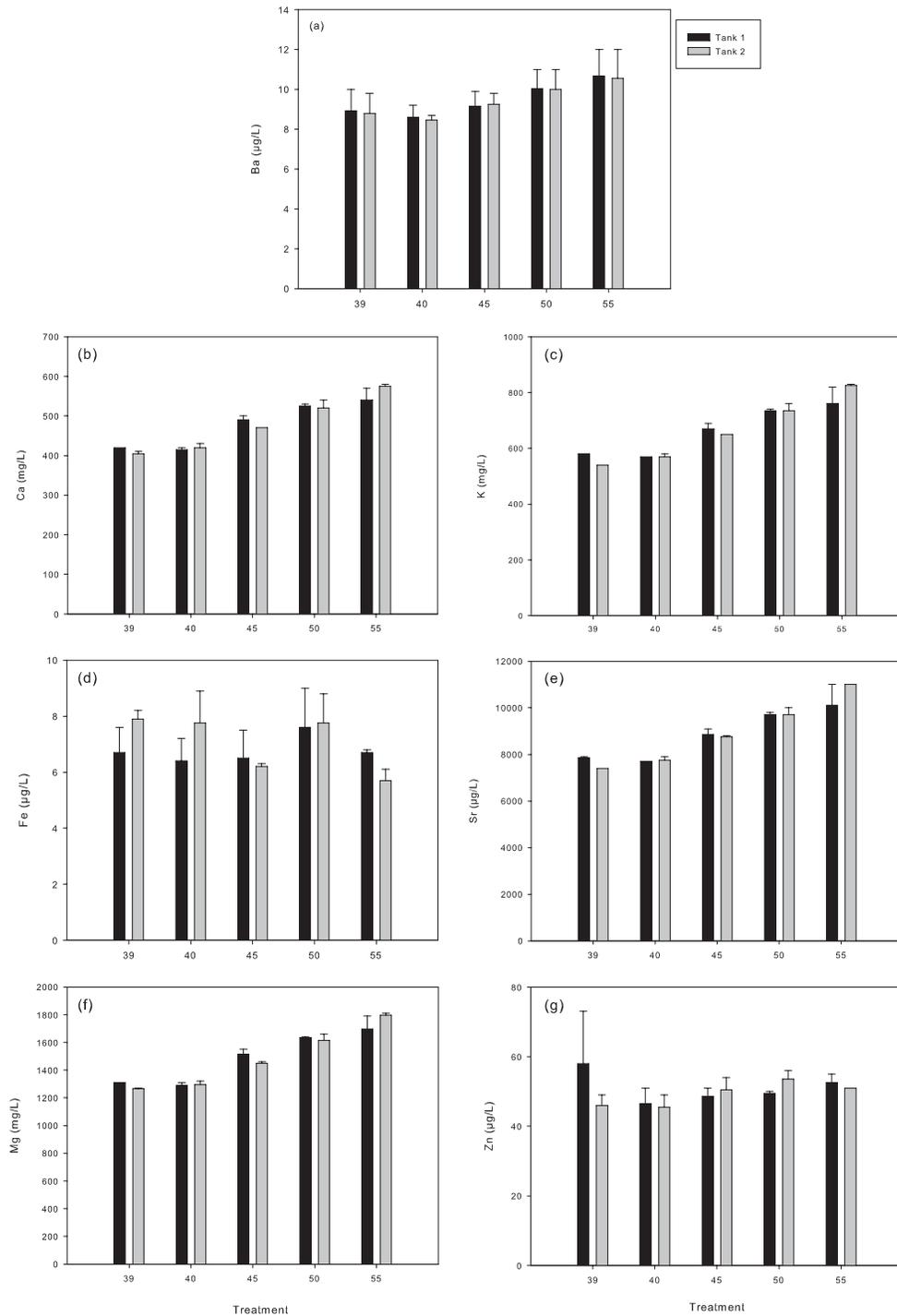
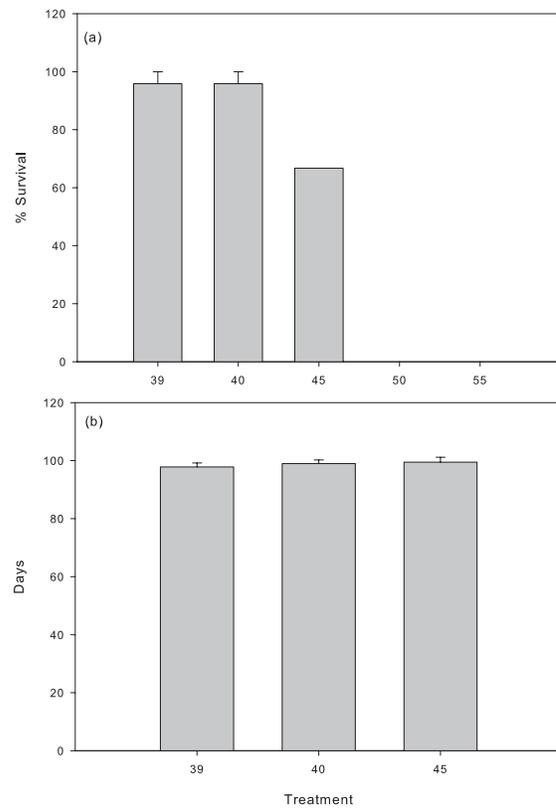


Fig 5

Fig 6



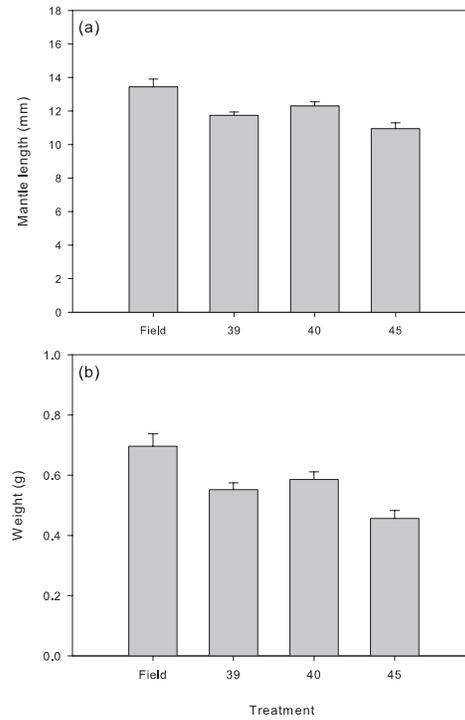


Fig 7