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Tolerances of juveniles and early life stages of the pipi, *Donax deltoides*, to elevated salinity produced by mixing of Coorong and sea water

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EXECUTIVE SUMMARY

With severely reduced water flows in the Murray River over the last 5 years, freshwater flow from the Lower Lakes to the Murray estuary and Coorong lagoons has been negligible (<1 000 $GL.y^{-1}$). As a consequence, water in the South Lagoon of the Coorong is, depending on season, four to six times as saline as seawater, leading to substantial ecological impacts.

It is unlikely that freshwater flows will be available to the Coorong over the next 12-24 months, and salinity will further increase if there are no other management interventions. A proposed intervention is to pump the hypersaline water out of the South Lagoon into the adjacent ocean, drawing in less saline seawater through the Murray mouth and reducing the salinity of the entire system.

If hypersaline water is pumped out of the South Lagoon of the Coorong into the adjacent ocean, it will have potential negative impacts on marine life. The SA MDB NRM board has convened a panel of experts to assess what these consequences might be. The highest priority identified was the pipi (*Donax deltoides*), also known locally as Goolwa cockle, which is abundant in the region, and is the target of a commercial fishery located along the Coorong coast to the north-west of the proposed discharge.

This project assessed the impacts of water from the Coorong South Lagoon on early life stages and juveniles of the pipi. While we focus here on salinity, elevated salinities were achieved by mixing water collected from the Coorong South Lagoon with seawater, and thus the tests are actually whole effluent toxicity (WET) tests. Early life stages were most affected by elevated salinity, with virtually no embryonic development to trochophore or D-stage larvae occurring at a salinity of 50 psu, and reduced or significantly slower development at 45 psu. D-larvae grew less and suffered greater mortality over 6 days at 50 psu than at up to 45 psu, although survival was still around 90%. Juvenile pipis also suffered greater mortality over 14 days at a salinity of 50 psu than at ≤ 45 psu, and appeared to feed less, although there was no significant difference in final size. Salinities of up to 40 psu had no significant impact on either early life stages or juveniles.

1. INTRODUCTION

The Coorong, Lower Lakes, and Murray Mouth (CLLAMM) system, to the south-east of Adelaide, South Australia SA (Figure 1) is one of Australia's largest and most important wetland systems, recognised as a Wetland of International Importance under the Ramsar convention (Phillips and Muller 2006), and as an "icon site" by the Living Murray program of the Murray-Darling Basin Authority (MDBC 2006). The Coorong connects to the sea via a narrow channel at the Murray mouth and extends 140km along the coast, separated from the ocean over most of its length by the narrow coastal dunes of the Younghusband Peninsula. A barrage system separates the freshwater Lower Lakes (Alexandrina and Albert) from the Coorong and Murray mouth (MDBC 2006). The Coorong has a strong salinity gradient from North to South, with three distinct regions: The Murray mouth region, where salinity ranges from near fresh to marine depending on flow over the barrages; the North Lagoon, which ranges from estuarine to moderately hypersaline; and the South Lagoon, which connects to the North Lagoon via a narrow and shallow channel at Parnka Point, and is moderately to extremely hypersaline (MDBC 2006; Brookes *et al.* 2009).

Flow over the barrages is regulated based on the water level of the Lower Lakes, and so is determined primarily by inflow from the River Murray (MDBC 2006). Water extraction from the Murray-Darling Basin has decreased average annual flow to the Murray mouth by 61% since 1895, and with several years of regional drought, flows over the barrages have been greatly reduced since 2002 (< 1 000 $GL.y^{-1}$, compared with 10 000-15 000 $GL.y^{-1}$ historically), with no flow since 2006 (CSIRO 2008; Brookes *et al.* 2009). As a consequence, salinity has steadily increased in the Coorong, to the point where, depending on the season, the South Lagoon is four to six times as saline as seawater. This in turn has led to substantial ecological impacts, with many species formerly common in the South Lagoon now restricted to the North Lagoon. The South Lagoon water also has elevated nutrient and turbidity levels (Brookes *et al.* 2009).

Given the sustained drought conditions and current low water levels in the Lower Lakes, and other upstream storages, it is unlikely that significant freshwater flows will be available to the Coorong over the next 12-24 months (MDBA 2009), and thus salinity will continue to increase if there are no other management interventions. One intervention being considered is to pump hypersaline water out of the South Lagoon into the adjacent ocean, drawing in less saline sea water through the Murray mouth

and reducing the salinity of the entire system (Commonwealth of Australia 2008; Aurecon 2009).

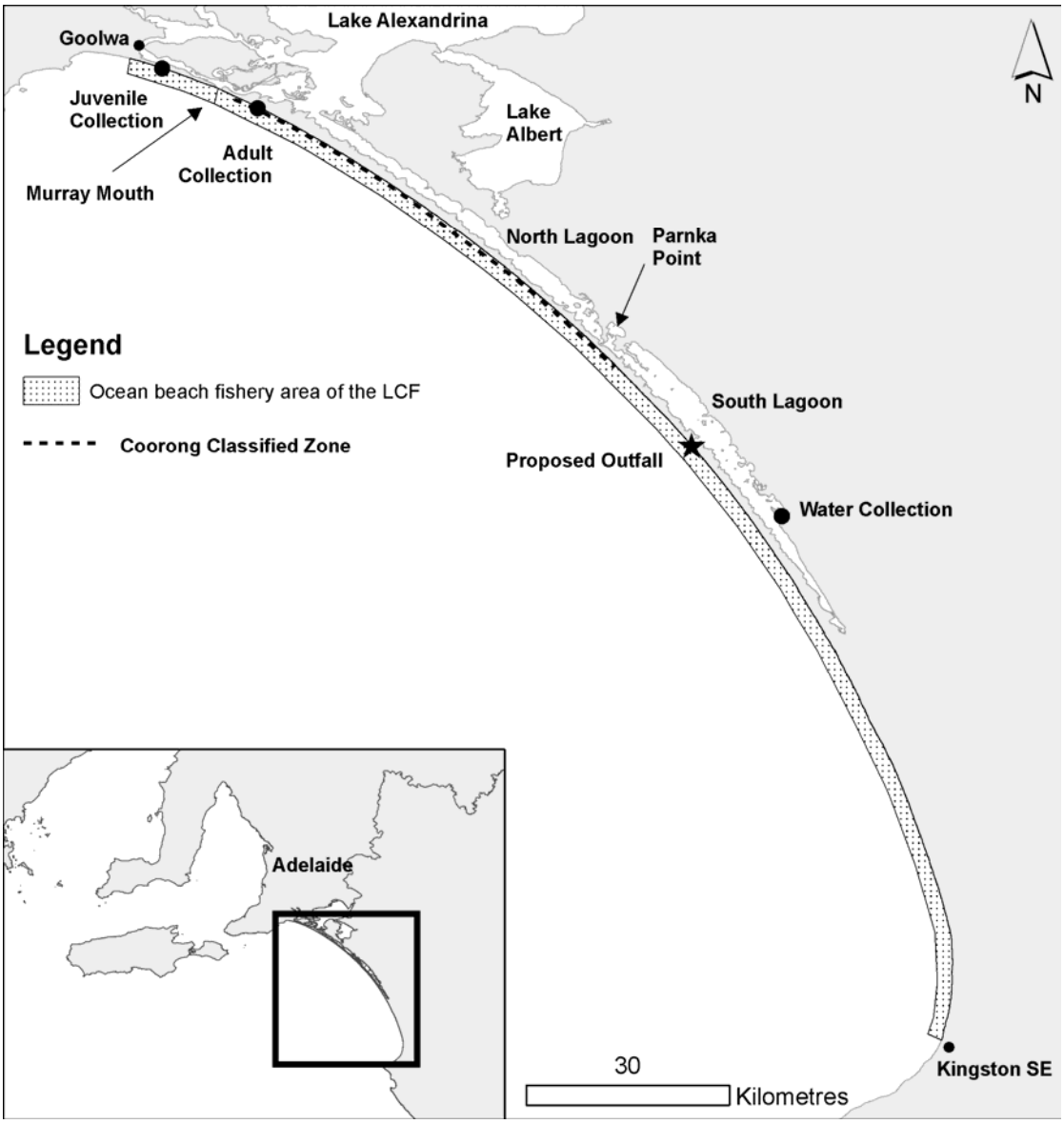

Figure 1. The Coorong region, showing the North and South Lagoons, Murray Mouth and Lower Lakes. The ocean beach fishery area of the Lakes and Coorong Fishery (LCF), the Coorong classified area, collection sites for pipis and water used in the experiments, and location of the proposed outfall for South Lagoon pumping are also shown. Inset shows location of the area relative to Adelaide, South Australia.

Preliminary hydrodynamic modelling (Aurecon 2009) indicates that the discharge water will rapidly mix with the receiving water, with the plume being diluted to within 1 psu of background values within ~2 km. As well as elevating local salinity, the plume will contain high levels of nutrient and turbidity. The discharge water will potentially have negative impacts on marine life in close proximity to the discharge point, hence,

the SA MDB NRM board has convened a panel of experts to assess what these consequences might be. The highest priority identified was the pipi (*Donax deltoides*), also known in South Australia as Goolwa cockle, which is abundant in the region, and an important species for the Lakes and Coorong Fishery (Sloan 2005).

The Lakes and Coorong fishery (LCF) is a multi-species fishery that includes the CLLAMM region as well as the ocean beaches from Kingston in the south-east to Goolwa, extending to 3 nm offshore (Sloan 2005; Figure 1). Since 1999-2000, pipis have comprised >40% of the total LCF catch by weight, and up to 65% of the gross value of production (Econsearch 2009). Collection of pipis is also allowed by licensed Marine Scalefish and Rocklobster fishers, but since 2002-03 the LCF has been responsible for over 90% of the South Australian pipi catch (Ferguson and Mayfield 2006). Pipis are collected for use as bait for finfish or for human consumption. Those collected for human consumption must be collected from within the South Australian Shellfish Quality Assurance Program (SASQAP) "Coorong classified area" of the LCF, which extends from 2 km south of the Murray Mouth for 60 km south-east along the Younghusband Peninsula (Sloan 2005; Ferguson and Mayfield 2006; Figure 1). The proposed outfall for pumping lies within the LCF, approximately 15 km south-east of the southern end of the Coorong classified area (Figure 1).

Pipis are common, often forming dense aggregations, on high energy ocean beaches from southern Queensland to the Eyre Peninsula, SA (Murray-Jones and Steffe 2000; Sloan 2005). They bury up to ~10 cm (less for juveniles) in the sand and are found predominantly subtidally, but may occur up the beach to the high tide zone (King 1985; Murray-Jones and Johnson 2003; Ferguson and Mayfield 2006). The population along the Coorong beaches is thought to be one of Australia's largest (Sloan 2005); in this region, pipis have been found to comprise over 70% of macrobenthic infauna numerically, and around 85% of the biomass (Ferguson and Mayfield 2006).

Large fluctuations in pipi numbers have been observed in SA and NSW and are believed to be linked to natural fluctuations in mortality and recruitment (Murray-Jones and Steffe 2000; Murray-Jones and Johnson 2003; Ferguson and Mayfield 2006). In NSW pipis have been found to be sequential spawners, with reproduction and recruitment occurring throughout the year (Murray-Jones and Steffe 2000), and it appears that the SA population also has an extended spawning season, but with a peak in September-October (King 1985; Murray-Jones and Johnson 2003; Ferguson

and Mayfield 2006). As with all donacid bivalves, pipis are dioecious broadcast spawners. The larval period is thought to last 6-8 weeks, and recruitment may depend on prevailing currents as well as larval mortality (King 1985; Murray-Jones and Johnson 2003). Around the time of the peak spawning in SA, the predominant longshore current flows to the north-west, adults to the south-east of the Coorong are therefore likely to be the primary source of recruits to the beaches further north (King 1985; Ferguson and Mayfield 2006).

Physiological tolerances of the pipi have rarely been investigated. Low salinity following a large freshwater outflow from the Murray mouth is believed to be responsible for a mass mortality of pipis (and other bivalves) in 1984 (Clarke 1985). Pipis respond to low salinity (<25 psu) by withdrawing the foot and siphons and closing the shell, and mortality may result from starvation, muscle fatigue, or from animals being dislodged and stranded up the beach by tidal movement due to the lack of anchorage usually afforded by the extended foot (Clarke 1985; King 1985; Murray-Jones and Johnson 2003). In aquarium experiments, adult pipis were found to survive at between 20 and 45 psu, with 100% mortality occurring within 7 days at salinities ≤15 and ≥50 psu (Nell and Gibbs 1986). Although the animals closed their shells and were inactive at these salinities, mantle fluid osmolarity reflected that of the treatment salinity (as it did also within the tolerance range), indicating that some fluid exchange was occurring and that the animals were not effective osmoregulators (Nell and Gibbs 1986). No investigations of the tolerance of juveniles or early life stages of the pipi have been reported; however, in other bivalves, embryos, larvae and spat generally have a narrower range of salinity tolerance than adults (e.g. Nell and Holliday 1988; Tan and Wong 1996; Madrones-Ladja 2002; Soria *et al.* 2007; Verween *et al.* 2007).

The current study aims to investigate the salinity tolerance of early life stages and juveniles of the pipi, in particular with regard to increased salinity caused by mixing of Coorong water with oceanic saltwater. Although we focus here primarily on salinity, elevated salinity treatments were achieved by the addition of Coorong water, thus the tests are actually whole effluent toxicity (WET) tests.

2. METHODS

Four trials were conducted on various life stages of the pipi, using wild collected juveniles and early life-stages obtained by spawning wild collected adults. The trials, of which details are given in following sections, were:

1. Fertilisation to trochophore development
2. Embryonic development to D-larvae
3. Survival and growth of D-larvae
4. Survival and growth of juveniles

2.1. Salinity treatments

Five treatment salinities were used in all trials: 36 (typical oceanic salinity), 38, 40, 45, and 50 psu. Treatment salinities were obtained by mixing ambient salinity seawater, drawn from the Adelaide Metropolitan coast off West Beach, with either Millipore de-ionised water, or hypersaline Coorong South Lagoon water. Since ambient salinity seawater was collected from Gulf St Vincent, which has a higher salinity than the open ocean (Bye and Kämpf 2008), dilution with de-ionised water was required to obtain the 36 and 38 psu treatments. Ambient salinity was measured immediately prior to mixing each batch of treatment water and ranged between 38.2 and 39.4 psu over the course of the experiment.

Coorong water was collected from the South Lagoon immediately to the north of the Salt Creek boat ramp on 18th September 2009 (Figure 1). Salinity of the collected Coorong water was 107.3 psu.

At least 24 hours prior to the start of each trial and water exchange, the required ratios of ambient seawater to Coorong or de-ionised water to make each treatment salinity were determined based on the current ambient salinity, and sufficient quantities of each treatment salinity were mixed in 200 L tubs. Ambient saltwater and Coorong water were passed through 5 µm filters before use to remove plankton and other particulates. Tubs containing treatment salinity water were located in a controlled environment room to maintain stable water temperature.

2.2. Experimental aquaria

All trials except the fertilisation-trochophore trial were carried out in cylindrical, conical-base 20 L fibreglass tanks housed in a controlled environment room. Juveniles were placed in down-wellers with a base of 750 µm mesh, which were suspended in the experimental tanks. A schematic diagram of the down-wellers used is shown in Figure 2. Air lifts provided a water flow through the down-wellers of between 1.2 and 1.9 L.min^{-1}. For larval trials, air was introduced from the base of the conical tanks to circulate water and suspend larvae. A total of 25 tanks, five of each

treatment salinity, were employed for each trial, with salinities randomly assigned to tanks.

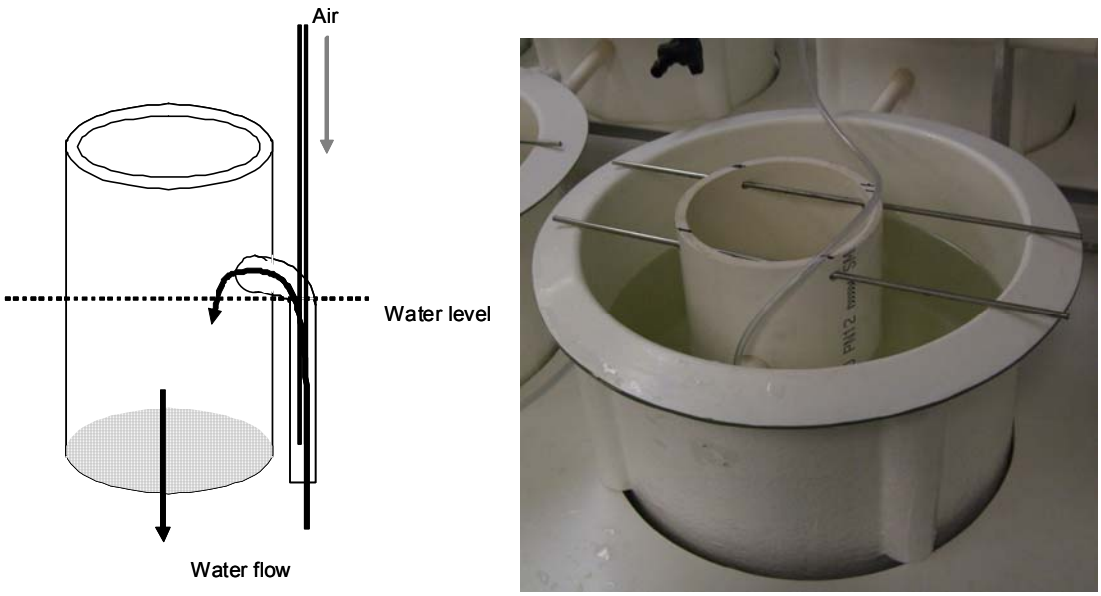

Figure 2. Diagram of down-weller design and operation (left) and a down-weller installed into one of the 20 L experimental tanks (right).

2.3. Adult pipi collection and spawning

Adult pipis in spawning condition were collected from the Younghusband Peninsula, approximately 5 km south-east of the Murray mouth (Figure 1) on the 9th October 2009 and placed in flow-through tanks in the SARDI Aquatic Sciences aquaculture facility at West Beach, Adelaide. Spawning was stimulated using temperature manipulation on the 15th October 2009 and took place in ambient salinity seawater (38.6 psu). On the initiation of spawning, males and females were rinsed and placed into separate tubs of ambient salinity water. Spawning was allowed to continue in these tubs for ~1 hr until females ceased spawning. Fertilisation for the embryonic development to D-larvae, and D-larvae growth and survival, trials was carried out at ambient salinity and 19 °C by mixing gametes in 20 litres to a concentration of approximately 540 eggs.ml^{-1} and 2700 sperm.ml^{-1}.

2.4. Fertilisation to trochophore development

The fertilisation to trochophore development trial was carried out in 25 1.8 L jars (5 replicates per salinity treatment), which were placed in a flow-through water bath to maintain temperature at 19°C. Eggs were added to treatment jars to a concentration of 3 eggs ml^{-1}, followed 0.5 hr later by sperm to concentration of 15 ml^{-1}. Successful

development to trochophore was assessed after 17 hours by examining live samples under the microscope and counting the numbers of trochophore larvae and undeveloped eggs in a sample of at least 100 eggs/larvae. Trochophore larvae could easily be distinguished from undeveloped eggs due to their ciliated band, and most were motile. Development success was scored as the percentage of trochophore larvae. Development to trochophore clearly indicates successful fertilisation, however, it could not be determined whether undeveloped eggs had been fertilised or not.

2.5. Embryonic development to D-larvae

Embryos obtained by fertilisation at ambient salinity were placed into the 20 L treatment salinity tanks at 4.75 ml^{-1} one hour after fertilisation, and provided with gentle aeration. Temperature was maintained at 20°C. At 39 hours post fertilisation, larvae were collected by draining aquaria onto 20 µm mesh, and preserved with 10 % Bennett's solution (1:1 propylene glycol and formalin). Development was scored by examining preserved larvae under the microscope and counting the proportion of larvae with shell development (post-trochophore), and the proportion of well-formed straight-hinge D-larvae (see Figure 3).

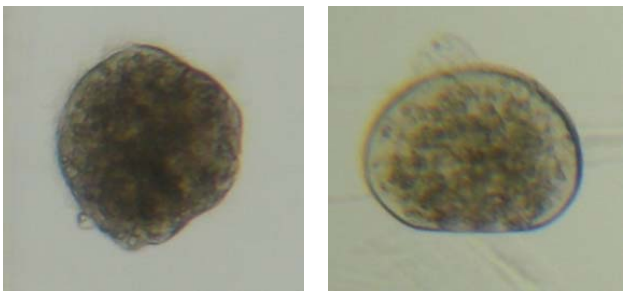

Figure 3. Pipi larvae showing (left) development beyond trochophore stage, and (right) well-formed (straight-hinged) D-larva.

2.6. Survival and growth of D-larvae

Approximately 8.5 million embryos were placed into a 2 000 L hatchery tank at ambient salinity and maintained at 20°C for 43 hours to allow development to D-stage. D-larvae (average initial size ± s.d. 115.8 ± 6.7 µm) were placed into the 20 L treatment salinity tanks at 2 ml^{-1}, with gentle aeration provided. Initial size was determined by measuring 50 larvae using an eyepiece micrometer on a compound microscope. Temperature was maintained at 20°C. Larvae were fed a 1:1:1 mix of *Chaetoceros calcitrans, Isochrysis galbana* and *Pavlova lutheri.* An initial feed of

20 000 cells.ml^{-1} was provided, with 30 000 cells.ml^{-1} added after water changes, which were completed every second day, and 15 000 cells.ml^{-1} on alternate days. During water changes, larvae were drained onto screens and rinsed with seawater of the appropriate treatment salinity. After six days maintenance at treatment salinities, when larvae were 8 days old, tanks were drained onto 70 µm screens with all larvae resuspended in a beaker. Live specimens were observed under a compound microscope and the proportion of empty shells determined as a measure of mortality. Fifteen live (non-empty) larvae from each tank were measured using an eye-piece micrometer to determine final size.

2.7. Juvenile survival and growth

Juvenile pipis were collected from Goolwa Beach, approximately 7 km north-west of the Murray mouth, on the 9th September 2009 (Figure 1). Juveniles were placed in flow-through tanks in the SARDI Aquatic Sciences aquaculture facility at West Beach, Adelaide, and maintained at ambient salinity and 18°C for 21 days prior to the start of the experiment. The juvenile growth and survival trial was carried out over 14 days from 30th September to 14th October 2009. Each tank was stocked with 100 juvenile pipis, with average initial size of 4.57 ± 0.78 mm (mean ± s.d). Initial size was determined from photographs of 15 juveniles per tank using *ImageJ* (U.S. National Institute of Health, Bethesda, MD) image analysis software to determine Feret (maximum calliper) diameter, which corresponds to the longest dimension across the shell.

Temperature for the juvenile trial was maintained at 19°C. Juveniles were fed a 1:1:1 mix of *Chaetoceros muelleri, C. calcitrans* and *Pavlova lutheri* at 50 000 cells.ml^{-1}, with feed added at the start of the trial and every second day following water changes. At each water change, and on days 7, 9 and 13, any gaping shells were counted as a measure of mortality. At the end of the experiment (day 14) juveniles were removed from tanks, examined under a magnifying lamp and counted. Mortality was then determined as the proportion of juveniles with empty or gaping shells. All live juveniles remaining from each tank were photographed and final size was determined by image analysis as per initial size.

2.8. Statistical analyses

Results for each trial were analysed using PASW Statistics (ver 17, SPSS Inc). Proportion data follow a binomial distribution, hence, data for % mortality in juvenile

and D-larvae trials, % development in fertilisation-trochophore and embryonic development trials, and % settlement were arcsine transformed prior to analysis (Zar 1996). A better arcsine transformation where the total count is small is to use a proportion of 1/4n, where n= total count, rather than zero (Zar 1996), therefore, this transformation was selected. For each set of trial results a one-way analysis of variance (ANOVA) was used to compare salinity treatments. Post hoc tests with Bonferroni corrections applied were carried out where significant differences were found to determine groupings.

3. RESULTS

3.1. Fertilisation to trochophore development

More than 90% of embryos developed to the trochophore stage at salinities of 36, 38 and 40 psu, while 74.5 ± 0.12% developed at 45 psu and less than 1% (0.82 ± 0.02%) at 50 psu (Figure 4). ANOVA showed that salinity had a significant impact on development to trochophore ($F_{4,24}$=155.8, p<0.001), with % development at 45 psu being significantly less than at 36-40 psu, while % development at 50 psu was significantly less than in all other treatments (Table 1).

Table 1. Pairwise comparisons of % development to trochophore over five salinity treatments with Bonferroni corrections applied. Results shown in bold are significant at the 0.05 level

Comparison	Mean difference	p
36 vs 38	-0.012	1.000
36 vs 40	0.001	1.000
36 vs 45	**0.226**	**0.012**
36 vs 50	**1.221**	**<0.001**
38 vs 40	0.123	1.000
38 vs 45	**0.238**	**0.008**
38 vs 50	**1.232**	**<0.001**
40 vs 45	**0.225**	**0.013**
40 vs 50	**1.219**	**<0.001**
45 vs 50	**0.994**	**<0.001**

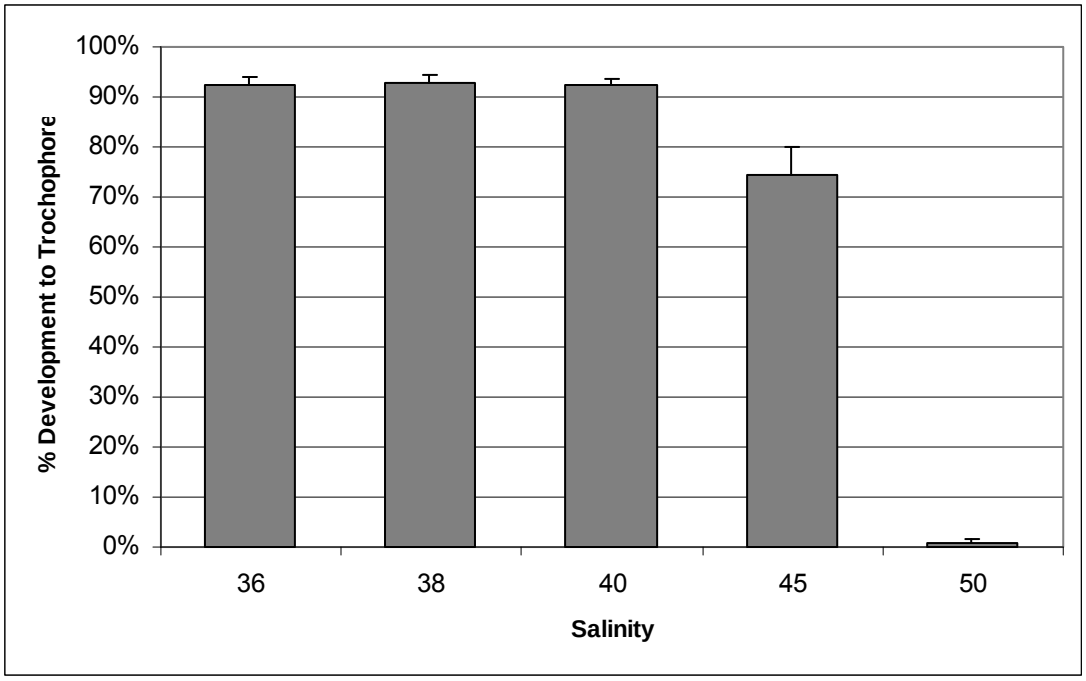

Figure 4. Percentage of eggs developing to trochophore larvae after 17 hours at five treatment salinities, following fertilisation at treatment salinity. Error bars represent s.e. (n=5).

3.2. Development to D-stage

Over 90% of larvae had developed beyond the trochophore stage by 39 hours post-fertilisation at 36-40 psu, but progressively fewer well-formed D-larvae were found with increasing salinity (Figure 5). Less than 50% (45.7 ± 4.6%) of larvae had developed beyond the trochophore stage at a salinity of 45 psu and only 3.2 ± 2.2% were well-formed D-larvae, while at 50 psu, only 3.4 ± 2.0% had developed past the trochophore stage, with around half of these (1.7 ± 1.7%) being well-formed D-larvae. ANOVA showed a significant impact of salinity on both the proportion of larvae above trochophore stage ($F_{4,24}$=170.3, $p<0.001$) and proportion of well-formed D-larvae ($F_{4,24}$=53.8, $p<0.001$). A significantly lower proportion of larvae had developed past trochophore stage at a salinity of 45 psu than at 36-40 psu, and significantly less again at 50 psu (Table 2). The proportion of well-formed D-larvae was significantly lower at 45 and 50 psu than at the other salinities, while the difference in % well-formed larvae between 36 and 40 psu treatments approached significance (Table 3).

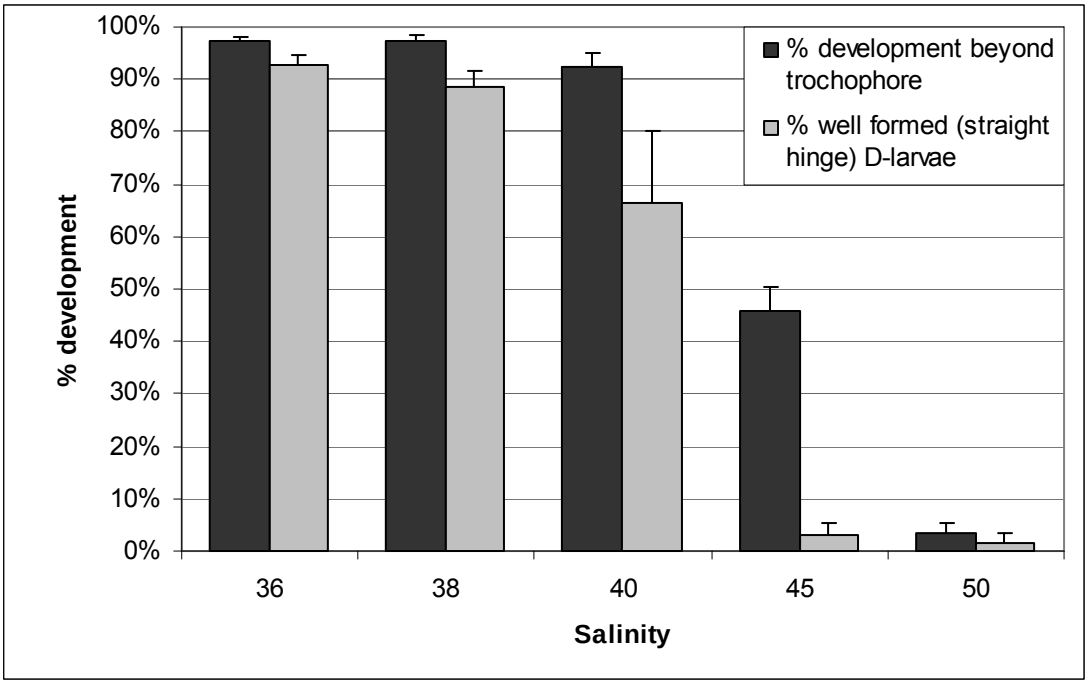

Figure 5. Percentage of larvae developing beyond trochophore stage and % of well-formed (straight-hinge) D-larvae after 38 hr at five treatment salinities, following fertilisation at ambient salinity. Error bars represent s.e. (n=5).

Table 2. Pairwise comparisons of % larval development beyond trochophore over five salinity treatments with Bonferroni corrections applied. Results shown in bold are significant at the 0.05 level

Comparison	Mean difference	p
36 vs 38	-0.004	1.000
36 vs 40	0.105	0.942
36 vs 45	**0.669**	**<0.001**
36 vs 50	**1.253**	**<0.001**
38 vs 40	0.109	0.831
38 vs 45	**0.673**	**<0.001**
38 vs 50	**1.257**	**<0.001**
40 vs 45	**0.564**	**<0.001**
40 vs 50	**1.149**	**<0.001**
45 vs 50	**0.585**	**<0.001**

Table 3. Pairwise comparisons of % well-formed D-larvae over five salinity treatments with Bonferroni corrections applied. Results shown in bold are significant at the 0.05 level

Comparison	Mean difference	p
36 vs 38	-0.074	1.000
36 vs 40	0.346	0.064
36 vs 45	**1.166**	**<0.001**
36 vs 50	**1.215**	**<0.001**
38 vs 40	0.273	0.265
38 vs 45	**1.093**	**<0.001**
38 vs 50	**1.141**	**<0.001**
40 vs 45	**0.820**	**<0.001**
40 vs 50	**1.149**	**<0.001**
45 vs 50	0.048	1.000

3.3. Growth and survival of D-stage larvae

After six days exposure to treatment salinities, no mortality of D-stage larvae was observed in the 36, 38 or 40 psu treatments (ie no empty shells), there were few empty shells found at 45 psu (<2%) and ~10% empty at 50 psu (Figure 6). ANOVA showed that there was a significant difference in the proportion of empty shells ($F_{4,24}$=39.1, p<0.001) and hence in mortality. Post hoc tests confirmed that mortality was greater in the 50 psu treatment than at salinities 36-45 psu (Table 4).

Larvae grown at salinities ≤45 psu reached an average size of at least 155 µm, while those at 50 reached only 127 ± 6.0 µm (Figure 7). ANOVA showed the effect of salinity on final size to be significant ($F_{4,24}$=48.2, p<0.001), with post hoc tests confirming that larval size at 50 psu was significantly smaller than in other treatments (Table 5).

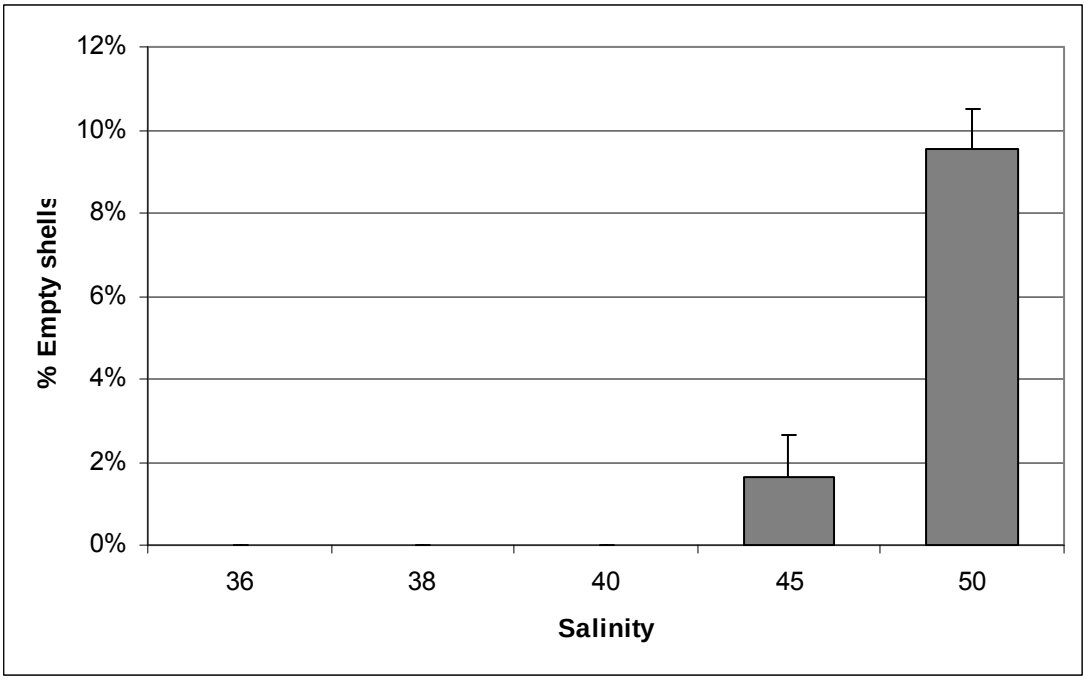

Figure 6. Percentage of empty shells as a measure of mortality in D-larvae cultured at treatment salinities for 6 days. No mortality was observed in 36-40 psu treatments. Error bars represent s.e. (n=5).

Table 4. Pairwise comparisons of % empty shells for D-larvae cultured over five salinity treatments with Bonferroni corrections applied. Results shown in bold are significant at the 0.05 level

Comparison	Mean difference	p
36 vs 38	0.003	1.000
36 vs 40	0.001	1.000
36 vs 45	-0.036	1.000
36 vs 50	**-0.211**	**<0.001**
38 vs 40	-0.002	1.000
38 vs 45	-0.039	0.792
38 vs 50	**-0.214**	**<0.001**
40 vs 45	-0.037	0.934
40 vs 50	**-0.212**	**<0.001**
45 vs 50	**-0.175**	**<0.001**

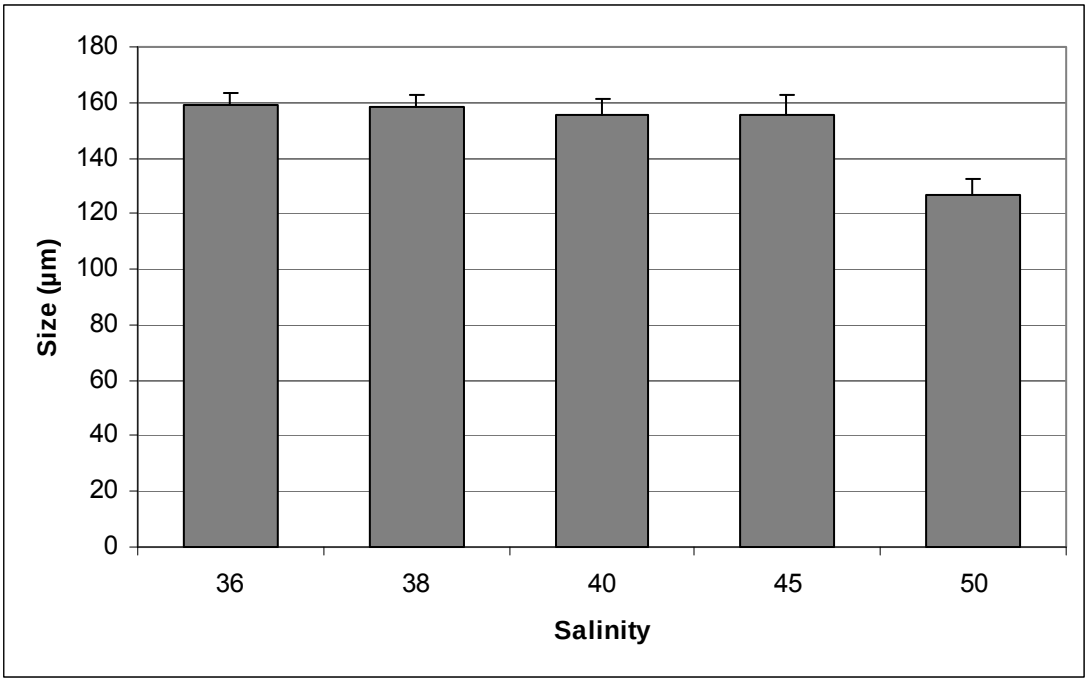

Figure 7. Final size of D-larvae cultured at treatment salinities for 6 days. Error bars represent s.e. (n=5).

Table 5. Pairwise comparisons of final size of D-larvae cultured over five salinity treatments with Bonferroni corrections applied. Results shown in bold are significant at the 0.05 level

Comparison	Mean difference	p
36 vs 38	0.533	1.000
36 vs 40	3.600	1.000
36 vs 45	3.733	1.000
36 vs 50	**32.400**	**<0.001**
38 vs 40	3.067	1.000
38 vs 45	3.200	1.000
38 vs 50	**31.867**	**<0.001**
40 vs 45	0.133	1.000
40 vs 50	**28.800**	**<0.001**
45 vs 50	**28.667**	**<0.001**

3.4. Juvenile survival and growth

Little mortality was observed in any treatment to day 7 of the trial, with an average of 1% mortality or less per tank. On day 8, an average 2.6% mortality was observed in the 50 psu treatment tanks and by day 13 there was 9.4% mortality in this treatment, while other treatments averaged less than 3% mortality. After 14 days, when

juveniles were removed from tanks and examined more thoroughly, it was determined that the counts of mortality based on gaping shells had underestimated true mortality, as empty shells that remained closed were not obvious and thus not counted (Figure 8). Therefore, only the final count of mortality was statistically analysed. ANOVA showed that there was a significant difference in mortality between treatments ($F_{4,24}$=10.8, p<0.001), with post hoc tests confirming that mortality was significantly greater at 50 psu than at 36-45 psu (Table 6).

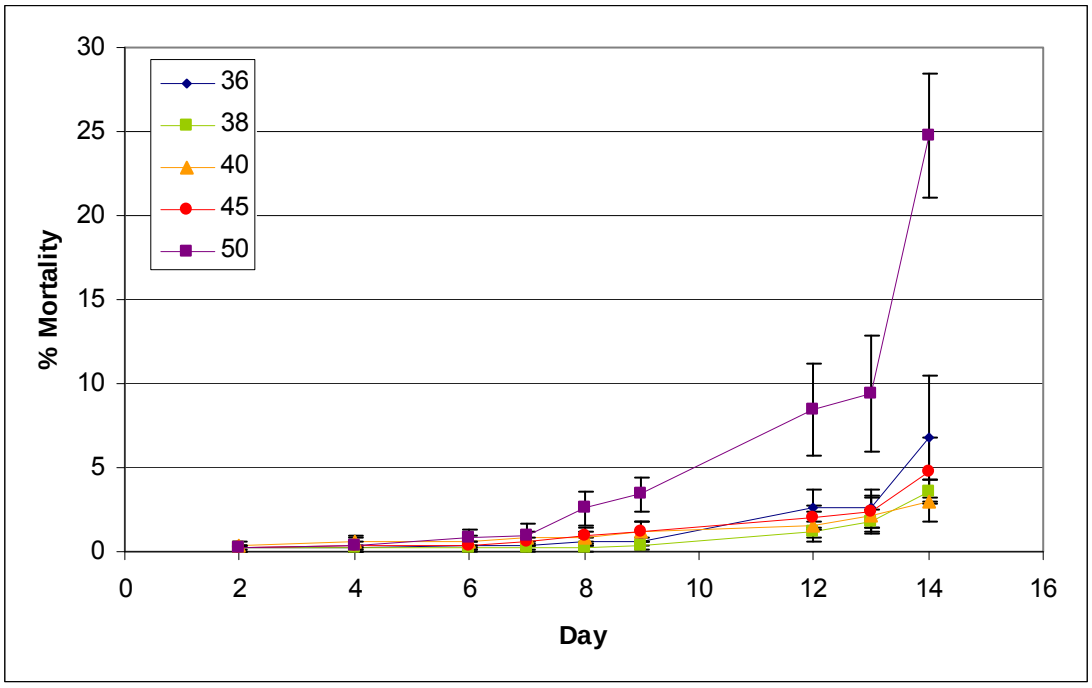

Figure 8 . Mortality, assessed as percentage of gaping shells (days 2 to 13) or percentage of empty and/or gaping shells (day 14), of juvenile pipis maintained at different salinities. Error bars represent s.e. (n=5).

The presence or absence of the gut being visible through the juvenile shell as a dark spot is often used to separate live and dead bivalve spat. This method was not used here as some juveniles were large enough for the shell to be opaque and obscure the gut. In addition, juveniles from the 50 psu treatment were uniformly pale with the gut invisible on most (Figure 9), even those that responded to stimuli by shell closing and hence were clearly alive. However, mortality in this treatment may have been greater, since closed shells were counted as being live unless clearly empty. The absence of a dark spot in juveniles from this treatment indicates that it is likely they were feeding at a reduced rate, but final size was not found to be significantly different between treatments ($F_{4,24}$=0.7, p=0.591). Juveniles grew to an average final

size (mean ± s.d.) of 5.94 ± 1.13 mm, compared to their initial size of 4.57 ± 0.78 mm.

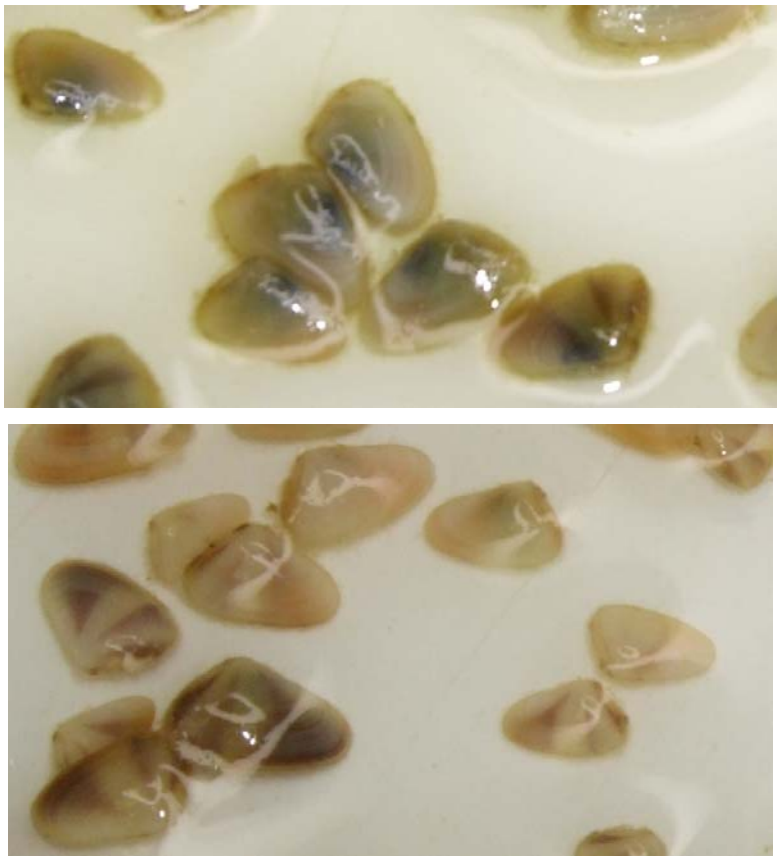

Figure 9. Juveniles maintained at a salinity of 36 (top) and 50 psu (bottom) showing the almost complete absence of colour from the gut of juveniles from the higher salinity treatment.

Table 6. Pairwise comparisons of juvenile pipi mortality over five salinity treatments with Bonferroni corrections applied. Results shown in bold are significant at the 0.05 level

Comparison	Mean difference	p
36 vs 38	0.050	1.000
36 vs 40	0.073	1.000
36 vs 45	0.034	1.000
36 vs 50	**-0.287**	**0.002**
38 vs 40	0.023	1.000
38 vs 45	-0.016	1.000
38 vs 50	**-0.337**	**<0.001**
40 vs 45	-0.039	1.000
40 vs 50	**-0.360**	**<0.001**
45 vs 50	**-0.321**	**0.001**

4. DISCUSSION

Salinity of 50 psu caused some negative effects in all pipi life stages tested. Early life stages were also negatively impacted by 45 psu, but salinities up to and including 40 psu were tolerated by all life stages.

Juveniles appeared to have a similar upper salinity tolerance to that found previously for adults, with no difference in survival at salinities of 36-45 psu, although juveniles survived longer periods than adults at >45 psu. Nell and Gibbs (1986) found that adult pipis survived no more than 7 days at 50 psu; however, in the current trial, juvenile mortality at 50 psu was not notably different to other treatments for the first 8 days, and after 14 days, around 75% still survived. Juveniles appeared to be feeding less at 50 psu, but still showed growth and there was no significant difference in final size between treatments. This indicates that juveniles are likely to be able to tolerate periods of exposure to salinities up to 50 psu with few negative effects, although the effect of any salinity change over a longer period is unknown. Impacts on earlier life stages were, however, more pronounced.

The earliest life stages were most affected by elevated salinity. When fertilisation took place at high salinity, virtually no development to trochophore took place at 50 psu, with significantly reduced development at 45 psu. When embryos were exposed to elevated salinity post-fertilisation, a greatly reduced proportion of larvae continued to develop past the trochophore stage, and fewer fully developed to D-stage in 39 hours. Given the time-frame used for the fertilisation-trochophore trial, undeveloped eggs that remained are unlikely to have developed given more time. In the development to D-larvae trial, larvae that had continued development past trochophore but that had not reached D-stage may have continued to develop and eventually reached D-stage. This continuation of development was observed in the larvae raised at ambient salinity. The larvae that did not develop to or beyond trochophore, however, are not likely to have continued to develop. The combined results of these trials suggest that exposure to a salinity of 50 psu at or soon after fertilisation will lead to almost complete absence of development, and significantly slower development to D-stage for the surviving larvae, while exposure to 45 psu is likely to lead to a reduction of 25-50% in the numbers of larvae developing, along with significantly slower development to D-stage.

Once larvae reach D-stage they appear to be tolerant of exposure to salinities up to 45 psu, even for several days, as larvae showed no significant difference in growth or

survival over 6 days at salinities of 36-45 psu. Significantly greater mortality occurred at a salinity of 50 psu, but around 90% of larvae survived the 6 days exposure. However, growth was greatly reduced. D-stage larvae are also likely to tolerate short exposures to a salinity of 50 psu, but prolonged exposure could lead to more severe impacts. A reduced growth rate has implications for development time, and mortality due to predation will increase if the larval period is extended. Additionally, it is not known whether larvae would recover and resume growing if returned to lower salinity.

Recruitment of juveniles to the Lakes and Coorong Fishery may depend on larval supply from beaches further south, hence, impacts on successful early development in this region could have flow-on effects to the fishery. From this perspective, salinities ≥45 psu, and particularly ≥50 psu, during or soon after spawning events appear to pose the greatest risk.

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