Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Supplementary Information

Assisted natural recovery of hypersaline sediments: salinity thresholds for the establishment of a community of bioturbating organisms

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Methods A: general chemical methods

Sediment characterisation

Quantitative x-ray powder diffractometry (XRD, Philips PW 1771/00 diffractometer) was used to characterise the initial 400 psu sediments using Cu K_{α} radiation, x-ray-tube at 1 kW and a Spellman DF3 generator (40 kV and 30 mA). Sediment was dried and crushed before being analysed with a diffraction angle of two theta ranging from 4 to 70° at a step size of 0.02°. Estimated quantities of crystalline material were quantified directly from the x-ray powder diffraction traces using the PANalytical X'Pert Highscore Plus Version 2 software.

Elemental analysis of the sediments (metals and major ions) was made on dried and crushed samples following a low-pressure microwave assisted (MARS 5, CEM) aqua-regia digestion of the samples (Belzunce-Segarra et al., 2015). As the sediments were not significantly contaminated (i.e. metal concentrations were well below the sediment quality guideline values (Simpson and Batley, 2016)), these particulate analyses targeted the major 'saltwater ions' Na^+, K^+, Mg^{2+}, Ca^{2+}, and SO_4^{2-}. For QC, 10% of the samples were blanks and all were measured in duplicate, with the mean result reported, with certified reference materials (CRMs) for metals (ERM®-CC018, European Reference Material), with recoveries falling between 90 – 105%. For the target seawater ions, sediment digests were validated against in-house calcium recovery values of the sediment CRM, with 100 – 105% recoveries. To validate the performance of the instrumental analyses, spike recoveries were performed on selected digest diluents, with recoveries within 90 – 100% for all ions of interest. Blanks were less than practical quantitation limits (PQLs) and duplicates were typically within 20% for target elements. Acid-volatile sulfide (AVS) in sediments was determined colorimetrically as per Simpson (2001). Total organic carbon (TOC) analysis was conducted using a high temperature CO_2 evolution method, in which dried and crushed samples were acid-treated to remove inorganic carbonates followed by combustion (LECO furnace) in the presence of strong oxidants/catalysts and infrared detection of CO_2.

Pore water-brines and metal analyses

Brine densities and total dissolved solid (TDS) concentrations were determined gravimetrically using pre-weighed 5 mL polycarbonate vials, which were then heated to 90^{o}C for 48 h (low temperature used to minimise the loss of organic carbon fractions). Estimations of porewater turbidity were undertaken at 750 nm using a UV-VIS spectrophotometer (LKB Biochrom Ultraspec 2E, glass cuvette, 1 cm path length).

Water samples taken for analyses of seawater elements (Na, K, Mg, Ca, chloride and sulfate) and metals were filtered (0.45 µm cellulose nitrate, 25 mm Minisart Sartorius) and acidified to 2% HNO_3 (v/v, Tracepur, Merck) before storage (<4^{o}C). Analyses were made by ICP-AES (Varian 730-ES), where porewater brine solutions were diluted as necessary (up to 100-fold) to be within the concentration range of the standards (1 – 10,000 mg L^{-1}; QCS-27, High-Purity Standards in deionised water). To validate the performance of the instrumental analyses, spike recoveries were performed on selected diluents, with recoveries within 90 – 110% for all ions of interest. For QA/QC, 10% of the samples were blanks and all were measured in duplicate, with the mean result reported. Blanks were less than practical quantitation limits (PQLs) and duplicates were typically within 20% for metals.

The quantification of bromide (Br^-), chloride (Cl^-) and sulfate (SO_4^{2-}) was done by ion chromatography with direct conductivity detection (IC-CD) (Metrohm 838 IC-CD). QC for the IC method was undertaken by using spike recovery where recoveries were between 90 – 110%.

Physicochemistry

Salinity measurements were made using a Mettler Toledo Seven2Go S3 conductivity meter fitted with an InLAB® 73X series conductivity probe. If salinities exceeded the probe's calibration range, solutions were diluted 5- to 10-fold with RO water and the salinities remeasured, and then multiplied by the dilution factor. Dissolved oxygen (DO) and pH were measured using WTW (Wissenschaftlich-Technische Werstätten) instruments (Multi 3410 with FDO® 925 probe and pH320 with SenTix 41 pH electrode) calibrated as per manufacturer's instructions. Dissolved ammonia was measured using a rapid test kit (API Fish Care, LR8600).

Methods B: test media preparation

Sediments

For whole-sediment bioassays, treatments with porewater salinities ranging from 50 to 400 psu were prepared by washing the Dry Creek sediment with RO water. For each wash, approximately 1 kg of sediment was suspended in the RO water by vigorous shaking by hand for several minutes, followed by rolling for 3 h. Sediments were left to settle at 4°C for > 24 h and the overlying waters decanted off before the remaining slurry was centrifuged (9700 g). The salinities of the extracted porewater-brines were measured before storage in polyethylene bottles and then the sediments were reconstituted back into their respective containers, mixed well and then stored at 4°C.

Pore water-brines

As filtering most of the porewater-brines was near-impossible due to their high viscosities, they were extracted by centrifuging (6,000 - 9,000 g, 2 × 30 min bursts) the original and RO-washed Dry Creek sediments, and the dark, viscous brines collected via decantation. The brine extracted from the initial, un-manipulated sediment had a salinity of approximately 400 psu. To prepare for the porewater brine bioassays (50, 100 and 200 psu), the brines were centrifuged for 1 h (9,000 g) to remove most of the suspended material, and then diluted with RO water to produce waters covering the desired salinity range (40, 60, 65, 70, 80 and 300 psu). Diluted brines were mixed for 1 h (magnetic stirrer) prior to bioassay commencement to ensure homogeneity. To compare the effect of salt composition to toxicity, an additional artificial brine solution of salinity of 50 psu was prepared by dissolving artificial sea salts (Instant Ocean ® Sea Salt) in seawater until the salinity was reached.

Test organism handling

The epibenthic amphipod *Melita plumulosa* (Zeidler, 1989) and harpacticoid copepod *Nitocra spinipes* (Boeck, 1865) are endemic to the estuaries of south-eastern Australia, and were obtained from previously established laboratory cultures, maintained as described by Spadaro and Simpson[26,27]. The burrowing amphipod *Victoriopisa australiensis* (Chilton, 1923; 2-3 cm body length) gastropod *Pyrazus ebeninus* (mud whelk; Bruguière, 1792; 3-4 cm shell length) and polychaete *Neanthes succinea* (Frey & Leuckart, 1847; 3-4 cm body length) were collected via sieve (2 - 4 mm mesh). Individual burrowing mangrove crabs *Parasesarma erythrodactyla* (Hess, 1865; 4-5 cm leg span) were collected by hand and plastic spatula. The bivalve was sourced *Plebidonax deltoides* (Lemarck, 1818; 5-6 cm shell length) was sourced from the Younghusband Peninsula, South Australia via sieve. All benthic organisms were stored within maintained and aerated cultures of their native sediments in a temperature-controlled laboratory (21 ± 1.0 °C) for two weeks prior to their use in tests. Organisms (except the crab) were fed

a mixture of sera Micron Powdered Food (containing approximately 50% Spirulina and 16% Krill) and a mixture of in-house cultured algal species (temperate *Ceratoneis closterium*, Temperate *Tetraselmis sp.* and Tropical *Tisochrysis lutea*). Crabs were fed a mixture of John West Tuna (*Katsuwonus pelamis*) chunks and Aqua One 10mm Vege wafers. Crab cultures also contained a matrix of bark and mangrove vegetation collected from their collection site for shelter and food, and underwent 6-h tide cycles three times per week. Cysts of the shrimp *Artemia salina* (Linnaeus, 1758) were sourced from the Great Salt Lake, Utah USA by Aquasonic Pty Ltd. Nauplii of A. salina were prepared by cyst reactivation in fresh seawater (31 ± 1.3 psu, 23 ± 1 ^{o}C) under fluorescent light with gentle aeration and used after 48-h of acclimatisation. Nauplii were fed with fed with sera Micron Powdered Food (containing approximately 50% Spirulina and 16% Krill) every 24-h.

Methods C: bioassays

The bioassay endpoints differed between species, and included behaviour, e.g. Avoidance and movement behaviour (mollusc: gastropods and bivalve), endurance (time to death, using most test organisms), and reproduction/development (crustacean: amphipod, and shrimp. Brief summaries of the test are provided below. At the start of tests, porewaters were extracted from sediments and salinity measurements were made on these pore waters and repeated for the porewater brines that had been stored. In all tests, physicochemical measurements were made periodically (dissolved oxygen (DO), temperature, salinity, pH, ammonia and seawater ions).

Avoidance tests – measuring the threshold for recolonisation

120-h Pore water-brine bioassays

Brine avoidance tests were undertaken using the gastropod *P. ebeninus* (mud whelk) and bivalve *P. deltoides* by observing the time taken for either reorientation in brine or burial in brine-saturated sand. For the gastropod bioassay, two individuals were placed corneous-operculum up into 250 mL beakers containing 150 mL of test solution (salinities of 30 – 400 psu), and the time taken for them to flip over was measured. Following 96-h exposure, the gastropods were transferred to seawater (31 ± 1.3 psu) and the test repeated to compare post-exposure recovery. The bivalve burial bioassay was conducted using a single bivalve, placed in a 250 mL beaker containing 200 g of clean sand saturated with the test solution (seawater or brine of varied salinity), and the time taken to bury was measured. Three replicates were conducted for each test for both species.

120-h Sediment bioassays

Sediment avoidance tests were undertaken with the amphipod, *M. plumulosa*, and the gastropod, *P. ebeninus,* following the approach described by Ward et al. (2013), with the following changes. The chamber design for the avoidance tests are shown in Figure S5 of the SI. For both organisms, sediments with three salinities were tested, 30 ± 2.0 (control), 50 and 100 psu. Each treatment was prepared in triplicate, with two sediments tested separated by a polycarbonate barrier (within the chamber). The control sediment was placed on the left-hand side, and a more saline sediment placed on the right-hand side. In the control treatment, the control sediment was placed on each side of the barrier. The overlying water was initially 31 ± 1 psu and increased in the tests that contained a portion of hypersaline sediment, because the overlying water salinity was the same on both sides of the exposure chamber. For the amphipod test, 30 adult organisms (8-12 mm body length) were placed on the right-hand side (treatment) of a barrier and no organisms on the left (control), the organisms were left to adjust for 5 min, the barrier was removed for 120-h (avoidance test termination), and the numbers of amphipods on each side of the barrier were counted. For the gastropod tests, four organisms (2-3 cm shell length) were placed on the midline between the two sediments (no initial barrier) and after 120-h a barrier was added. The number of gastropods on each side of the barrier were then counted, and the number buried on each side also quantified. For the gastropod, the test was then repeated to reduce the variability resulting from these being slow-moving organisms.

Endurance tests with amphipod, copepod, polychaete worm, and crabs in porewater-brines

Organism endurance, assessed by considering the length of time an organism can survive unfavourable conditions, was undertaken in porewater brine solutions with salinities ranging from 40 - 400 psu. Five organisms were assessed: copepod (*N. spinipes*), amphipod (*M. plumulosa*), amphipod (*V. australiensis*), polychaete (*N. succinea*) and crab (*P. erythodactyla*). These were added to test chambers (3 to 4 replicates). The time taken to death was recorded to evaluate a threshold limit. The test conditions are shown in Tables S4 and S8. If no effect was observed (after 68-h for copepod and amphipod, and after 120-h for crab and polychaete tests), treatments were terminated.

Amphipod reproduction and survival tests

The survival and reproduction of the amphipod *M. plumulosa* was assessed in 10-d renewed whole-sediment bioassays as per Spadaro and Simpson (2016a) using sediments with porewater salinities of 30 ± 2 (control), 50, 60, 70, 80, 100, 200, 300 and 400 psu. In brief, 40 g of test sediment was added to 250 mL beakers covered with 150 mL of fresh seawater and left to equilibrate for 1-day, the waters were exchanged, and organisms added, and the number of juveniles and embryos counted after 10 days. Sediments were renewed on day 5, and waters were exchanged on days 3, 5 and 7 with periodical physicochemical measurements taken.

Shrimp toxicity tests

The survival of shrimp nauplii and development of brine shrimp eggs were assessed in separate tests. A 24-h survival bioassay was undertaken using 3-d old nauplii of *A. salina*. From a batch of nauplii, 10 organisms were transferred via pipette into microwell plates (Sigma® cell culture plates polystyrene, well area = 9.5 cm^2, 6 wells per plate) containing 5 mL of test solution. Four replicates were tested for each salinity treatment (30, 40, 50, 100, 200, 400 psu). The plates were covered with Parafilm and incubated at 23 ± 1^oC (Labec Refrigerated Cycling Incubator, 12:12-h light:dark cycle, light intensity = 3.5 µmol photons/s/m^2) for 24 h. After 24-h, survival of the nauplii was determined by first chilling organisms in the microwell plates at 4^oC for at least 1 h to suppress the nauplii movement, and then counting the surviving nauplii under light microscopy (Leica dissecting microscope).

A 48-h gastrulation (cyst development) bioassay was undertaken using desiccated shrimp eggs. Twenty eggs were transferred via spatula to Sigma® cell culture plates (polystyrene, well area = 9.5 cm^2, 6 wells per plate) containing 5 mL of test solution. The plates were gently swirled to ensure eggs were submerged into their respective solutions before they were capped and incubated for 48 h at 23 ± 1^oC (Labec Refrigerated Cycling Incubator, 12:12-h light:dark cycle, light intensity = 3.5 µmol photons/s/m^2) with agitation at 24 h. At the completion of test, the plates were chilled at 4^oC for at least 1 h to suppress the nauplii movement, and then the nauplii and remaining dormant eggs were counted by light microscopy (Leica dissecting microscope). Four replicates were tested for each salinity treatment. For quality control (QC) purposes for both amphipod and shrimp bioassays, tests were only deemed valid if survival and reproduction/development in all control replicates for each test was >80%. For the shrimp bioassay, >80% development of dormant cysts to nauplii within the control seawater (30 psu) occurred within between 24 – 36 h.

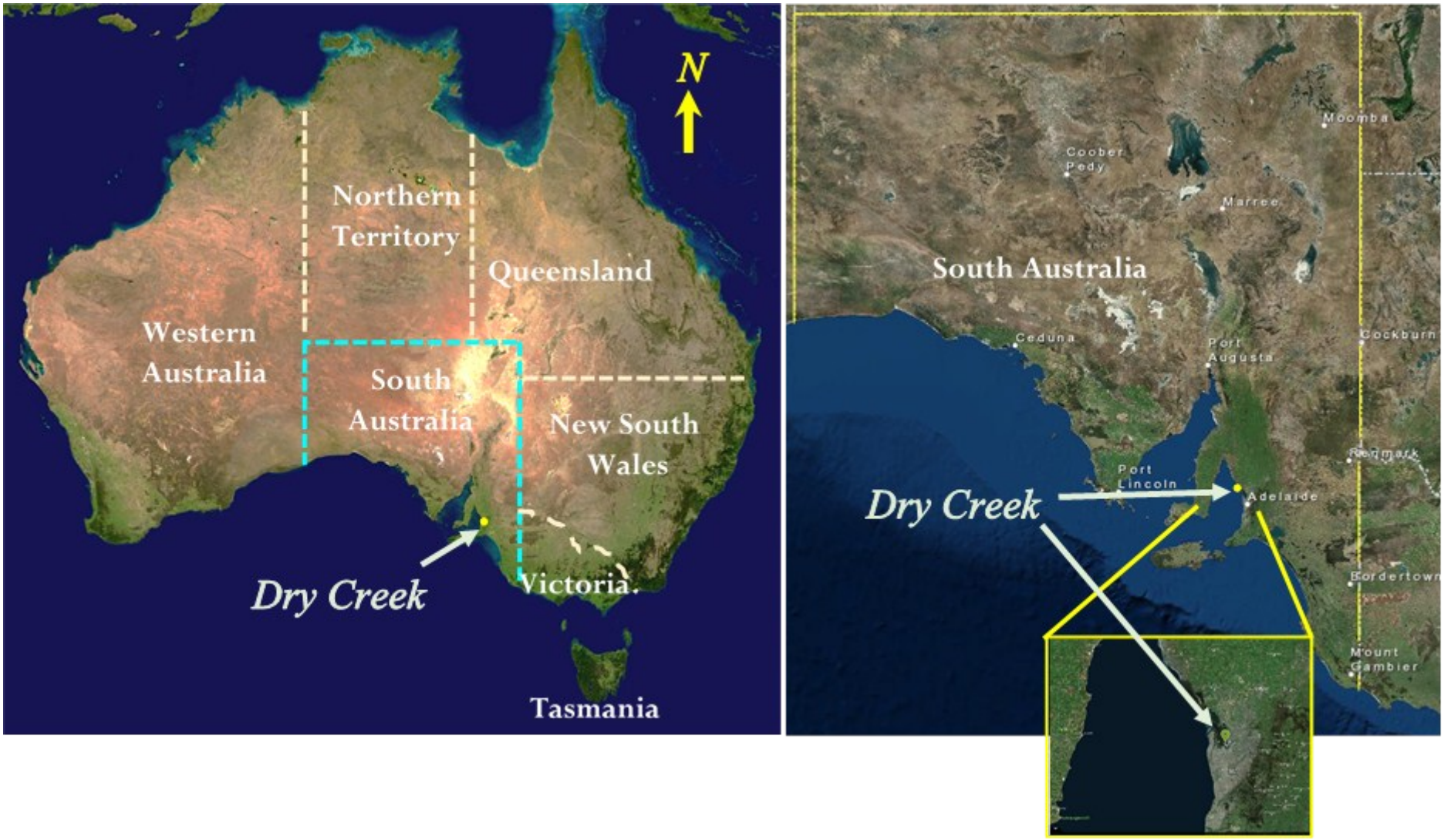


Figure S1. Satellite images depicting locality of dry creek in South Australia.

Figure S2. Satellite (left) and aerial (right) imagery of the Dry Creek solar salt fields

Figure S3. Photographs of the Dry Creek solar salt fields in proximity of where sediments were collected from.

Selected test species relevance

Previous species diversity surveys of a tidal saltmarsh in the region identified twelve macroinvertebrate taxa, which included polychaetes, gastropods, bivalves, amphipods and dipteran (flies). This range was similar to the range of species selected for our study, making ideal for field-comparison. In contrast the species richness within salt ponds at Dry Creek was low (see image and table below, unpublished data), which correlates to the tolerances observed within this work.

Table S1. Species richness from tidal saltmarsh and two evaporative salt ponds (A and B).

Group	Taxa	Tidal saltmarsh	Salty Creek	Saltpond A	Saltpond B
Nematoda	Nematoda	✓	.	.	.
Mollusca	*Salinator fragilis*	✓	✓	.	.
Mollusca	*Marinula xanthosoma*	.	.	✓	.
Crustacea	Eusiridae	✓	.	.	.
Crustacea	Talitridae	.	✓	.	.
Crustacea	*Halonicus* sp.	.	.	.	✓
Crustacea	*Paratemia zietiana*	✓	.	.	.
Diptera	*Ephydra* sp.	.	.	✓	✓
Diptera	*Culicoides* sp.	✓	.	.	.
Diptera	*Tanytarsus barbitarsus*	✓	✓	✓	✓
Diptera	Dolichopodidae	.	.	.	✓
Polychaeta	*Capitella* spp.	✓	.	.	.
Polychaeta	*Simplesetia* sp.	✓	.	.	.
Polychaeta	*Nephtys* sp.	✓	.	.	.

* Source: Environment Protection Authority (South Australia)

Figure S4. Taxa identified from the healthy tidal saltmarsh

Table S2. Summary table of the specifications used for each bioassay undertaken.

Test	Organism	Species	Size	Endpoint	Test Chamber	Duration	Test quantities
Behavioural bioassays							
Sediment	Amphipod	*M. plumulosa*	Adult, 1 – 2 cm	Avoidance	Sistema® 3 L polyethylene container (150 × 100 × 250 mm). Plastic divider (100 mm high) was installed in the centre of the exposure chamber.	120 h	2 cm sediment covered by 5 cm fresh seawater
	Gastropod	*P. ebeninus*	3 – 4 cm	Avoidance	Home Leisure® StoreMAX 4 L polypropylene container (185 × 116 × 325 mm). Plastic divider (116 mm high) was installed in the centre of the exposure chamber.		4 cm sediment covered by 10 cm fresh seawater
Porewater-brines	Gastropod	*P. ebeninus*	3 – 4 cm	Time to flip	250 mL borosilicate beaker	120 h	150 mL of test solution per replicate
	Bivalve	*P. deltoides*	5 – 6 cm	Time to bury	400 mL borosilicate beaker		150 mL of test solution and 8 cm of clean sand per replicate
Endurance bioassays							
Porewater-brines	Amphipod	*V. australiensis*	2 – 3 cm	Endurance (Time to death)	250 mL borosilicate beaker	68 h	150 mL of test solution per replicate
		M. plumulosa	1 – 2 cm		250 mL borosilicate beaker		150 mL of test solution per replicate
	Copepod	*N. spinipes*	~1 mm		Falcon™ polystyrene microplates (2 m^2, 24-well)		2 mL of test solution per replicate
	Crab	*P. erythodactyla*	4 – 5 cm		1 L borosilicate beaker	120 h	200 mL of test solution per replicate
	Polychaete	*N. succinea*	3 – 4 cm		Techno Plas polystyrene petri dish (90 × 140 mm)		25 mL of test solution per replicate
Sub-lethal bioassays							
Sediment	Amphipod	*M. plumulosa*	Adult, 1 – 2 cm	Survival and reproduction	250 mL borosilicate beaker	10 d	Approximately 40 g of test sediment and 240 mL of fresh seawater.
Porewater-brines	Shrimp	*A. salina*	Cysts	Developmental	Sigma® cell polystyrene cell culture plates (9.5 cm^2, 6 wells/plate)	48 h	5 mL of test solution per replicate
			Nauplii	Survival			

Table S3. Summary of physicochemical parameters of test waters

Bioassay	pH	N-NH_3 (mg/L)	Dissolved O_2 (mg/L)	Temperature (^oC)
Sediment-based tests				
Avoidance				
Amphipod (MP)	7.6 - 8.3	0.0 - 2.0	98 - 99	21.1 - 22.5
Gastropod	7.8 - 8.1	0.5 - 1.5	98 - 99	21.1 - 22.5
Sub-lethal tests				
Amphipod (MP)	7.6 - 8.3	1.0 - 4.0	98 - 99	21.2 - 22.0
Pore water-brine based tests				
Behavioural				
Bivalve	7.0 - 8.2	1.5 - 4.0	97 - 99	21.1 - 21.5
Gastropod	7.6 - 8.1	1.5 - 2.5	97 - 100	20.9 - 21.3
Endurance				
Amphipod (MP)	7.2 - 7.9	0.5 - 1.5	98 - 100	21.1 - 21.5
Amphipod (VA)	7.3 - 8.3	0.5 - 2.0	97 - 99	20.5 - 21.9
Copepod	7.2 - 8.2	1.0 - 2.0	97 - 99	21.0 - 21.9
Crab	7.5 - 8.3	0.5 - 4.0	95 - 99	21.1 - 21.5
Polychaete	7.5 - 8.2	1.5 - 4.0	96- 99	21.5 - 21.8
Sub-lethal tests				
Shrimp Survival	7.3 - 8.3	0.0	97 - 100	21.5 - 22.6
Shrimp Development	7.5 - 8.3	0.0 - 0.5	97 - 99	21.2 - 21.9

*Data presented as ranges between treatments throughout the test periods.

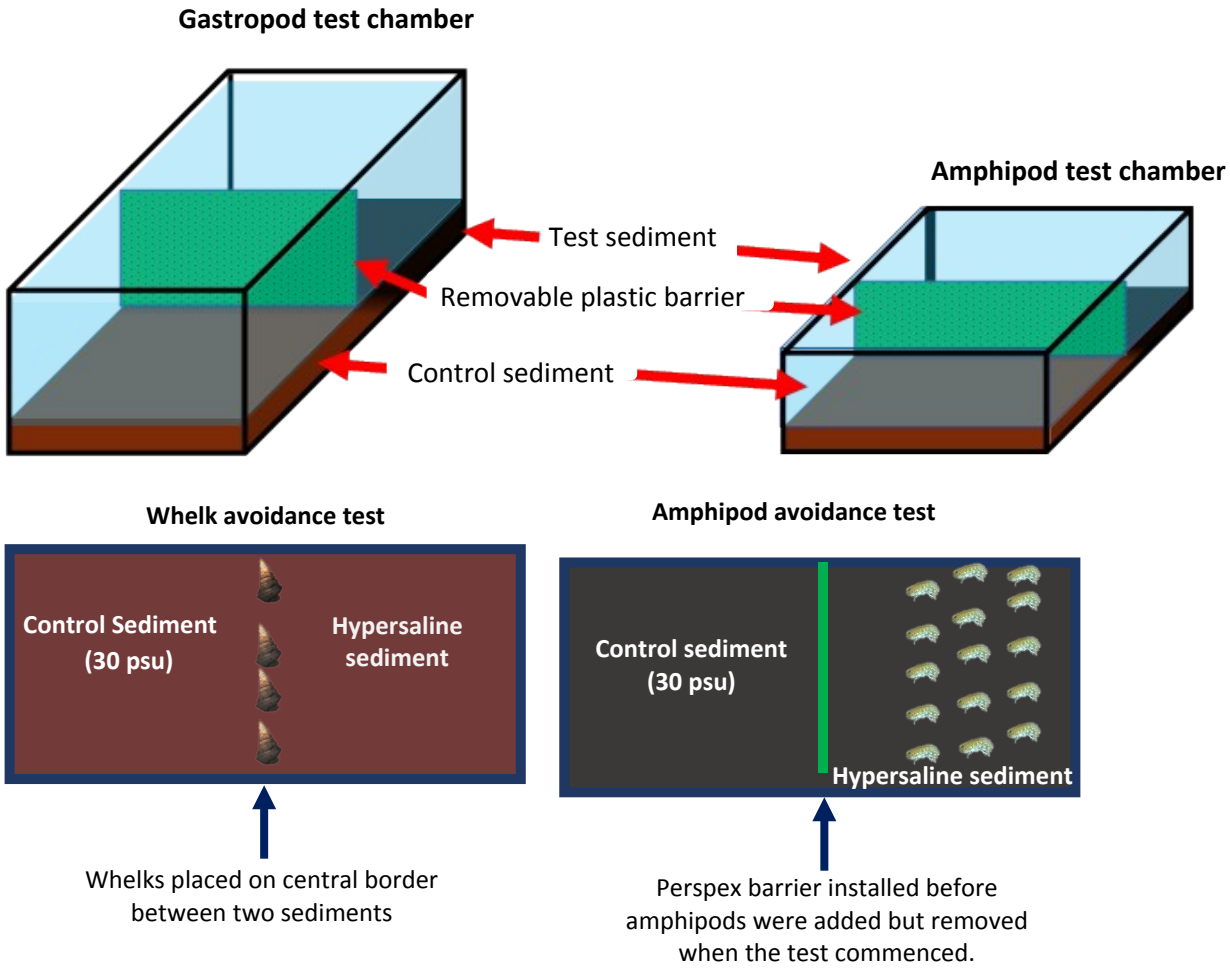
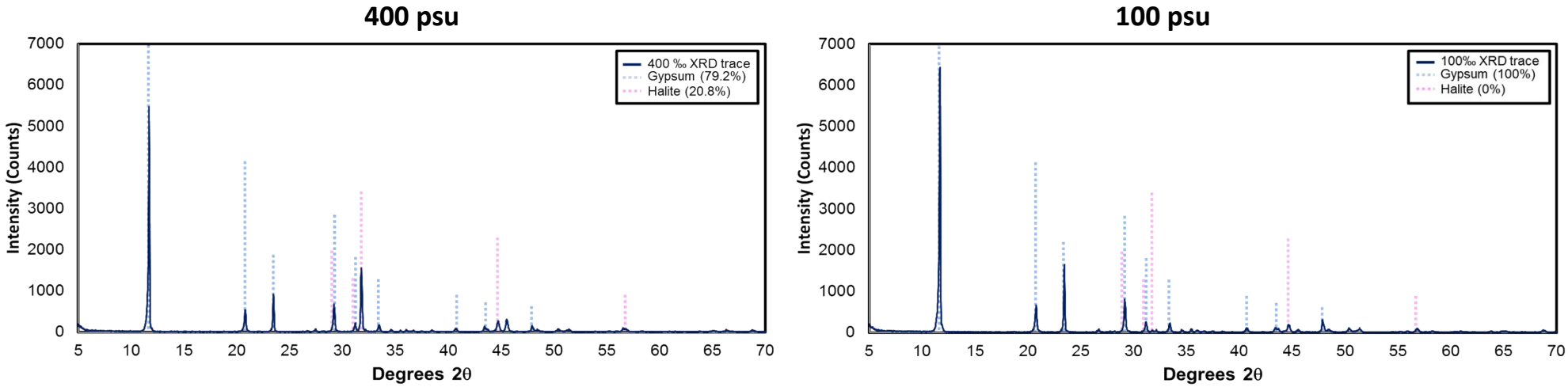


Figure S5 Avoidance test chamber designs and experiment process

Figure S6. XRD powder diffraction pattern (PDF) for the 400 psu and RO-washed 100 psu Dry Creek sediment



Reference peaks obtained from Mindat.Org (2017).

Table S4. Physicochemistry

Salinity (psu)	*Sediment*				
	<63 µm	*TOC*	*Moisture Content*	*AVS*	
		%		*µmol/g*	
29 (Ref)	40 ± 3.0	1.64	0.5 ± 0.0	0.3 ± 0.0	
400 (Undiluted)	20 ± 1.5	0.68	0.4 ± 0.0	5.4 ± 0.4	
	Porewater properties				
Treatment (psu)	**pH**	**Salinity (psu)**	**N-NH_3 (mg/L)**	**Density (g/mL)**	**TDS (g/mL)**	**Turbidity (Abs)**
30 (Seawater)	8.0	30.2	0	1.1 ± 0.1	2.0 ± 0.1	0.006
22 (Brine))	7.3	29.0	0	1.9 ± 0.3	2.0 ± 0.3	0.23
40	6.7	40.2	0	1.0 ± 0.0	2.0 ± 0.0	0.89
50	6.7	50.0	1.0	1.0 ± 0.1	2.0 ± 0.1	0.78
50[(A)]	8.1	50.2	0	1.0 ± 0.1	2.0 ± 0.1	0.13
80	7.5	80	1.0	1.1 ± 0.1	2.1 ± 0.3	2.7
100	8.3	100	2.0	1.6 ± 0.5	2.8 ± 0.8	2.0
200	7.7	201	4.0	1.6 ± 0.1	2.4 ± 0.1	10
300	7,3	310	4.0	2.1 ± 0.1	3.3 ± 0.2	10
400 (Undiluted)	7.5	409	4.0	2.6 ± 0.1	3.5 ± 0.5	10

< 63 µm fraction (n=2, mean ± SD), TOC = total organic carbon (n=1), moisture content (n=2, mean ± SD).

Table S5. Sediment metal concentrations

	TRM CONCENTRATIONS (mg/kg)											
Porewater salinity (psu)	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
Control (30)	6340 ± 100	11 ± 1.3	0.2 ± 0.04	2.8 ± 0.2	14 ± 0.1	16 ± 0.2	13200 ± 406	0.6 ± 0.2	4.9 ± 0.3	29 ± 1.0	21 ± 0.04	87 ± 0.5
50	1050 ± 150	1.1 ± 0.1	0.02 ± 0.01	0.8 ± 0.4	1.8 ± 0.2	1.6 ± 0.4	1390 ± 183	6.0 ± 0.7	0.6 ± 0.1	1.7 ± 0.2	6.3 ± 0.6	5.8 ± 0.6
100	2030 ± 110	0.7 ± 0.2	0.04 ± 0.02	1.1 ± 0.3	3.4 ± 0.04	3.1 ± 0.3	2512 ± 84	11 ± 0.004	1.3 ± 0.1	3.1 ± 0.6	10 ± 0.6	10 ± 0.1
250	268 ± 80	1.1 ± 0.1	0.01 ± 0.01	0.7 ± 0.4	0.4 ± 0.01	0.4 ± 0.5	303 ± 44	2.4 ± 0.4	0.1 ± 0.1	0.7 ± 0.1	4.3 ± 1.7	2.2 ± 0.01
400	445 ± 170	0.6 ± 0.1	0.01 ± 0.01	0.2 ± 0.2	0.6 ± 0.1	0.6 ± 0.2	488 ± 105	3.2 ± 0.6	0.5 ± 0.04	0.4 ± 0.4	3.5 ± 0.8	4.1 ± 0.4
Guideline Value (SQGV)		*1.5*				*65*			*21*	*50*		*200*

TRM = microwave-assisted aqua-regia digested metal fraction, n=2, mean ± SD. The guideline values are from Simpson and Batley.[27]

Table S6. Selected compositions of unwashed and washed Dry Creek sediment and pore water-brines

Sediments						
Treatment	**Porewater salinity**	**Concentrations (g/kg)**				
	(psu)	**Na**	**K**	**Mg**	**Ca**	**S**
Control	30±1.3	3.6 ± 0.1	0.7 ± 0.01	2.8 ± 0.1	27 ± 2.1	1.3 ± 0.2
Wash 3	50 ± 2.5	8.7 ± 0.1	0.5 ± 0.4	1.9 ± 0.1	111 ± 2	60 ± 3.8
Wash 2	100 ± 1.5	18 ± 4	1.1 ± 0.1	3.6 ± 0.4	127 ± 3	62 ± 16
Wash 1	200 ± 1.2	29 ± 4	0.5 ± 0.01	2.1 ± 0.1	105 ± 3	50 ± 9.9
Unwashed	400 ± 1.0	79 ± 5	1.2 ± 0.2	5.0 ± 0.8	91 ± 11	44 ± 7.6

Porewater-brines										
Salinity (psu)	**Density (g mL^{-1})**	**I (mol L^{-1})**	**Dissolved concentrations (g/L)**							
			Na	**K**	**Mg**	**Ca**	**S**	**Cl^-**	**Br^-**	**SO_4^{2-}**
Control 30	1.1 ± 0.1	0.6	8.2 ± 0.04	0.3 ± 0.002	1.1 ± 0.01	0.4 ± 0.01	0.9 ± 0.02	17	0.06	2.5
50	1.0 ± 0.1	1	18 ± 0.6	0.5 ± 0.01	1.5 ± 0.02	0.94 ± 0.09	1.3 ± 0.09	28	0.08	3.4
100	1.6 ± 0.1	1.9	26 ± 0.7	0.7 ± 0.04	0.7 ± 0.04	3.8 ± 0.1	0.98 ± 0.06	56*	0.16*	6.8*
200	1.6 ± 0.1	2.9	31 ± 0.9	0.8 ± 0.01	2.8 ± 0.06	2.4 ± 0.05	2.9 ± 0.03	112*	0.32*	14*
400	2.6 ± 0.1	6.3	78 ± 0.6	3.0 ± 0.04	10 ± 0.1	0.57 ± 0.10	4.8 ± 0.05	224*	0.64*	27*
50(†)	1.0 ± 0.1	1.2	11 ± 0.3	0.3 ± 0.003	1.6 ± 0.02	0.41 ± 0.03	0.9 ± 0.03	47	0.06	5.6

Control sediment: non-hypersaline, uncontaminated sediment; control pore water = seawater (31± 1 psu). Sediment treatments unwashed = original sediment and washes undertaken using RO water. Porewater brines isolated from the Dry Creek sediment initially and following consecutive washes; † represents an artificial brine created from the dissolution of artificial seasalts in seawater, until the salinity of ~50 psu was reached. Additional pore water-brine treatments of 40, 60, 65, 70, 80 and 300 psu were prepared by dilution of the brines with RO water. Detailed dissolved ionic analyses of these dilutions were not undertaken, but analyte concentrations are assumed to decrease linearly. Density was measured gravimetrically. I = ionic strength (calculated using concentrations only for Na^+, Mg^{2+}, K^+, Ca^{2+}, Cl^-, Br^-, HCO_3^-). *=Extrapolated from anion concentrations in 50 psu brine. For both sediment and brine analyses, n=2, mean ± SD. Measurements for total dissolved solids (TDS) and turbidity (light absorbance at 750 nm) are presented within the Supporting Information.

Table S7. Behavioural toxicological effects (avoidance) of salinity to *M. plumulosa* and *P. ebeninus*

Sediment

Treatment		Population residence (%)				Population (%)	
		Amphipod		Gastropod		Gastropod	
Left side	**Right Side**	**Left side**	**Right Side**	**Left side**	**Right Side**	**Buried**	***Flipped***
29 psu	29 psu	44 ± 5.9	56 ± 10	50 ± 6.5	50 ± 6.5	88 ± 5.6	0.0 ± 0.0
29 psu	50 psu	59 ± 2.9	41 ± 2.9	46 ± 7.7	54 ± 7.7	67 ± 11	33 ± 11
29 psu	100 psu	79 ± 4.0	19 ± 2.9	75 ± 9.1	21 ± 10	38 ± 14	67 ± 12

Porewater-brines

Bivalve – time to bury		Gastropod – time to flip		
Salinity (psu)	**Time to bury (min)**	**Salinity (psu)**	**During exposure (min)**	**Post-exposure (seawater recovery) (min)**
30 psu (control)	5.0 ± 0.3	30 psu (control)	2.2 ± 0.2	3.8 ± 0.2
40 psu	23 ± 3.7	50 psu(A)	11 ± 0.6	16 ± 1.1
50 psu $^{(\dagger)}$	39 ± 2.2	65 psu	7.7 ± 0.4	37 ± 1.5
50 psu	0.0 ± 0.0	80 psu	47 ± 0.6	80 ± 2.0
400 psu	0.0 ± 0.0	100 psu	-88 ± 7.5	92 ± 0.9
		200 psu	0.0 ± 0.0	0.0 ± 0.0
		300 psu	0.0 ± 0.0	0.0 ± 0.0
		400 psu	0.0 ± 0.0	0.0 ± 0.0

Sediment bioassays: data = mean ± SE (*M. plumulosa*: n=4, using 30 organisms per replicate) (*P. ebeninus*: n=6, using 4 organisms per replicate).

Porewater-brine bioassays: data = mean ± SE (*P. deltoides*: n=3, 1 bivalve per replicate) (*P. ebeninus*: n=3, 2 gastropods ber beaker).

50 psu$^{(\dagger)}$ = represents an artificial brine created from the dissolution of artificial sea salts in seawater, until the salinity of ~50 psu was reached.

Table S8. Endurance bioassay data and calculated salinity thresholds for survival of organisms[a]

Organism		Copepod	Amphipod		Crab	Polychaete
Species		*N. spinipes*	*M. plumulosa*	*V. australiensis*	*P. erythodactyla*	*N. succinea*
Habitat		epibenthic	epibenthic	endobenthic	epi/endobenthic	endobenthic
Time to death (h)						
Salinity (psu)	**22**	NR	NR	NR	NR	NR
	30	NR	NR	NR	NR	NR
	40	NR	NR	NR	NR	NR
	50 (A)	NR	NR	NR	NR	NR
	50	68 ± 0.0	NR	51 ± 0.1	NR	NR
	60	24 ± 0.6	51 ± 0.0	38 ± 0.1	NR	NR
	65	3.0 ± 0.1	9.0 ± 0.2	3.4 ± 0.5	NR	NR
	70	2.5 ± 0.0	5.0 ± 0.1	1.1 ± 0.1	NR	2.1 ± 0.4
	80	1.0 ± 0.0	1.5 ± 0.1	0.4 ± 0.01	5.0 ± 0.03	1.0 ± 0.07
	100	-	1.0 ± 0.1	0.3 ± 0.01	4.2 ± 0.04	-
	130	0.02 ± 0.01	0.2 ± 0.01	0.3 ± 0.01	3.4 ± 0.03	0.5 ± 0.02
	190	-	-	0.3 ± 0.01	-	-
	200	-	-	0.3 ± 0.01	-	-
	290	0.01 ± 0.001	0.04 ± 0.01	0.2 ± 0.002	1.2 ± 0.02	0.4 ± 0.01
	300	-	-		-	0.3 ± 0.02
	400	0.005 ± 0.002	0.02 ± 0.0	0.2 ± 0.01	0.9 ± 0.01	0.2 ± 0.01
Estimated lethal salinities (psu)						
Time (hours)	**6**	58	61	59	74	71
	12	57	59	58	74	69
	24	55	58	56	73	68
	48	52	55	54	72	68

[a]Organisms were exposed in brine solutions for 68 h (copepods and amphipods), and 120 h (polychaete and crab) with the time taken for mortality timed for each organism in each replicate (n=3-4). Salinity of 30 psu is the seawater control - = Not Tested. (mean ± SE); NR= No Response; † represents an artificial brine created from the dissolution of artificial sea salts in seawater, until the salinity of ~50 psu was reached.

Table S9. Chronic toxicity of *M. plumulosa* and *A. Salina* to sediments and elutriates.

***M. plumulosa* (10-day sediment bioassay)**

Porewater Salinity (psu)	Survival		Ave. offspring/ female	
	(% control)			
30 (control)	100 ± 2.9		100 ± 5.0	
50	79 ± 8.8		80 ± 12	
70	79 ± 8.8		47 ± 4.7	
80	63 ± 3.0		35 ± 18	
100	74 ± 2.9		19 ± 4.0	
200	71 ± 5.1		16 ± 2.8	
300	53 ± 0.0		6.8 ± 3.6	
Calculated EC Values	**Salinity (psu)**	**95 % CI**	**Salinity (psu)**	**95 % CI**
EC_{20}	>65	ND	37	(20, 70)
EC_{50}	>100	ND	65	(50,84)

***A. salina* (48-h brine bioassays)**

Porewater-brine Salinity (psu)	Acute bioassay Survival		Chronic Bioassay Hatched nauplii	
	(% control)			
30	100 ± 0.4		101 ± 3.4	
40 (†)	98 ± 0.5		88 ± 4.4	
40	94 ± 1.5		76 ± 7.7	
50 (†)	97 ± 0.4		79 ± 3.6	
50	92 ± 0.4		54 ± 2.5	
100 (†)	36 ± 0.6		0.0 ± 0.0	
100	21 ± 0.6		2.4 ± 1.7	
200	0.0 ± 0.0		0 ± 0.0	
400	0.0 ± 0.0		0 ± 0.0	
Calculated EC Values	**Salinity (psu)**	**95 % CI**	**Salinity (psu)**	**95 % CI**
EC_{20}	64	(54, 76)	41	(35,49)
EC_{50}	96	(84,110)	54	(49,59)

Data for *M. plumulosa*: n=3, mean ± SE. ND= not able to be determined from the data.
For *A. salina*: n=4. Mean ± SE. Salinity of 30 psu is the seawater control - = Not Tested. (mean ± SE); -NR= No Response; † represents an artificial brine created from the dissolution of artificial seasalts in seawater, until the defined salinity was reached.

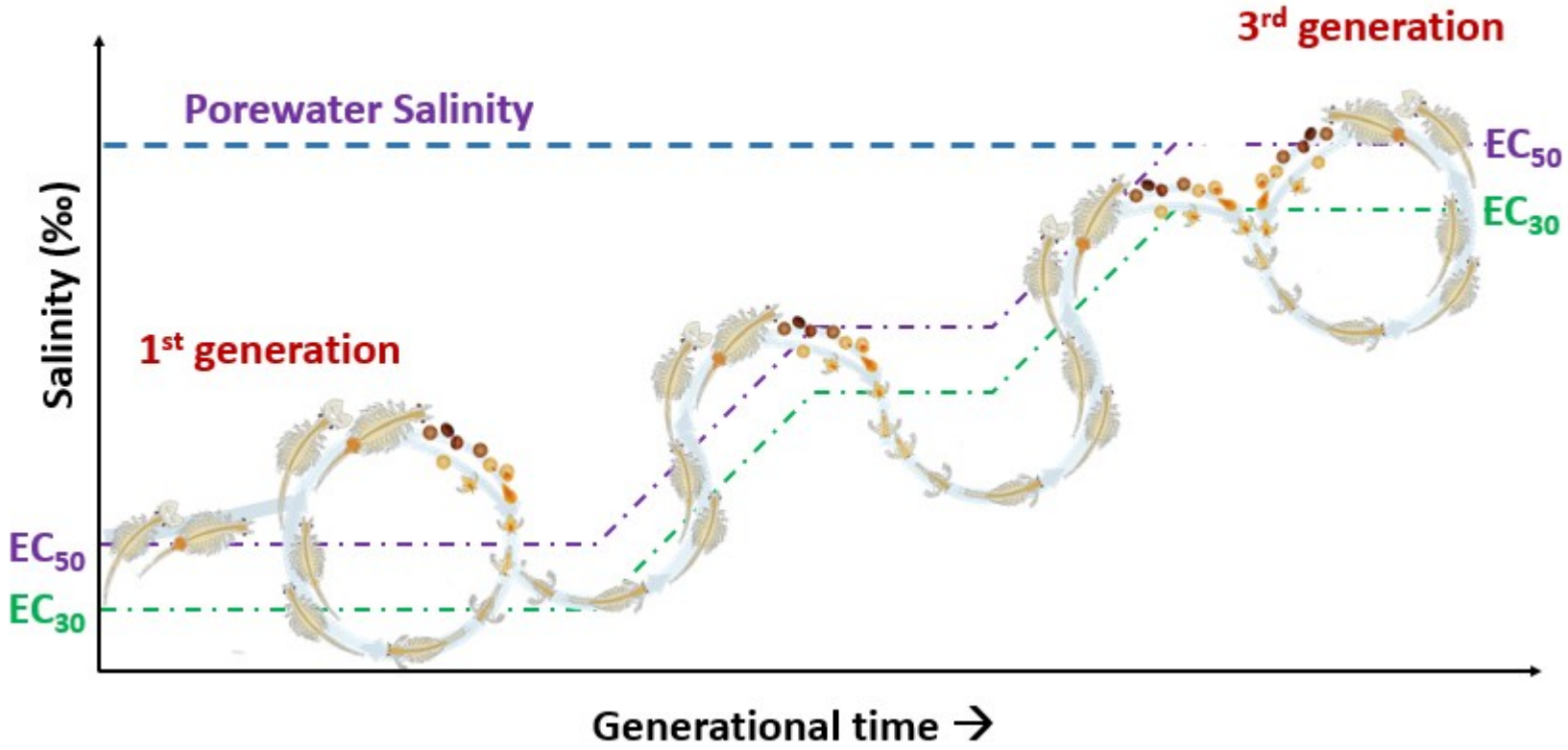


Figure S7. Example of generational adaptation to salinity. Adapted from: http://fishkeepingadvice.com/brine-shrimp/.