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Experimental and field evidence suggests extreme salinity tolerances in *Coxiella* gastropods from Australian salt lakes

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Abstract This study aimed to determine salinity tolerances in *Coxiella* gastropods from Australian salt lakes and whether different species exhibit characteristically different tolerances. Controlled gradual accumulation experiments were conducted to estimate both the maximum and minimum salinity levels at which 50% of individuals (IC50) remained active for 25 populations representing six species. All studied species showed remarkable euryhalinity and were tolerant of very high levels of salinity, some more than others, while minimum salinity tolerance varied little among populations and species. The experimental trends in salinity tolerances were consistent with the salinity distributions of species in the field, although the former were typically broader than latter. The findings suggest that *Coxiella* comprises some of the most salt tolerant gastropods globally.

Keywords Halophilic · IC50 · Halophile · Australia · Dose–response · Experiment

Introduction

The distribution of a species from microhabitat to large geographical areas is influenced by a range of factors, including its physiological capabilities (Gaston, 2003). Experimentally determining the physiological tolerances of a species provides information about its fundamental niche, defined as the total range of abiotic conditions that influence the physiology of a species (Devictor et al., 2010). Comparing experimentally determined tolerances with the realised niche, i.e. the actual observed range in which that organism occurs in the field, allows for an understanding of the main factors constraining a species' distribution and can be used to predict a species response to environmental change (Bozinovic et al., 2011).

A key biological challenge associated with living in an aqueous environment is osmoregulation (Bradley, 2009) as all aquatic organisms must be able to cope with osmotic stress (Kosicka et al., 2020). Salinity tolerance is difficult to define but the working definition used here is the minimum and maximum levels of osmotic pressure from the external environment that an organism can withstand before its internal

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cells burst or desiccate under hypoosmotic or hyperosmotic conditions, respectively (Deaton, 2009). Much work has been done on estimating the effect of salinity on freshwater or halotolerant taxa (sensu Lawrie et al., 2021), especially in the past 20 years because of concerns about the increasing salinisation of freshwater ecosystems across the globe (Cañedo-Argüelles, 2020). The results suggest that these taxa are often severely impacted by even slight salinity increases (Cañedo-Argüelles, 2020), although some taxa are more tolerant than others (Kefford et al., 2004b, 2012).

The salinity tolerances of halophilic taxa (sensu Lawrie et al., 2021) from salt lakes (enclosed bodies of water with salinity > 3 g/L; Williams, 1964) are also of interest in part because these organisms are salt-adapted and some species are capable of surviving extreme salinities (Hammer, 1986). Salinity can vary considerably within and especially between lakes and over a broad scale appears to be a key driver of community structure in salt lakes (Williams et al., 1990; McEvoy & Goonan, 2003). Understanding the salinity tolerances of halophiles is also becoming important from a conservation perspective because the salinity of salt lakes is increasing due to the increased rates of evaporation and regionalised reductions in rainfall associated with climate change (Williams, 1998; Saccò et al., 2021). Experimental estimates of salinity tolerance are available for a range of halophilic taxa, including gastropods (Davis, 1981; Williams & Mellor, 1991; Filippov & Komendantov, 1996), water beetles (Dytiscidae) (Céspedes et al., 2013), water boatmen (Corixidae) (Carbonell et al., 2012) and crustaceans (Croghan, 1958; Ellis & Williams, 1970; Geddes, 1981; Ismail et al., 2010). The results suggest that halophilic organisms typically display extraordinary euryhalinity, although eventually their salinity tolerances are exceeded resulting in mortality. However, there are many gaps in our knowledge, and it would be useful to determine how tolerances vary among closely related taxa.

Globally very few gastropods inhabit salt lakes and few salt lakes contain gastropods (Hammer, 1986). However, two of the three Tomichiidae genera (Salvador et al., 2022), *Tomichia* and *Coxiella* occur in salt lakes (Davis, 1981; Williams & Mellor, 1991). The former consists of seven described species from South Africa (Brown, 1994), which collectively occur in a broad range of habitats and includes two species

(*Tomichia ventricosa* (Reeve, 1842) and *Tomichia tristis* (Morelet, 1889)) that occur solely or partly in saline vleis (shallow lakes; Davis, 1981). *Coxiella* contains 15 species, all of which are endemic to Australia (Lawrie et al., 2023). The genus is highly unusual because all species occur in salt lakes and most are only found in these lakes (Lawrie et al., 2021, 2023). Collectively, *Coxiella* species are widespread and common in permanent and seasonal salt lakes in southern Australia, including Tasmania, although most species are endemic to southern Western Australia (Lawrie et al., 2023). *Coxiella* and *Tomichia* are probably Gondwanaland relics (Davis, 1981; Kameda & Kato, 2011). *Coxiella* appears to have undergone a radiation within Australian salt lakes however, it is unclear whether this radiation has been accompanied by physiological or ecological diversification, e.g. in association with lakes with different salinity profiles.

Current field data suggest that collectively *Coxiella* species occur over a wide salinity range (0.3–130 g/L) (Geddes et al., 1981; Timms, 1983; Williams et al., 1990; Pinder et al., 2005). However, there is little reliable published information about the salinity distributions of different *Coxiella* species, especially in Western Australia, in part due to confusion in species identifications (Pinder et al., 2002; Timms, 2009a). Field observations of *Coxiella* from South Australia suggest that some species (*Coxiella glauerti* Macpherson, 1957) have higher salinity tolerances than others (*Coxiella striata* (Reeve, 1842)) (Timms, 2009b; Timms et al., 2014).

The salinity tolerances of one *Coxiella* species, *C. striata*, have been experimentally tested. Results from Williams & Mellor (1991) suggested that *C. striata* is an osmoconformer and that 50% of the individuals could tolerate salinities between 3.8 and 125.5 mS/cm. O'Dwyer & Murphy (2021) suggested that individuals of *C. striata* from 'constant' environments (permanent water and stable salinity) tend to have lower survival under both increasing salinity and temperature stress compared to those individuals from salt lakes that were environmentally unstable (ephemeral and fluctuating salinity). Whether these results are applicable to other *Coxiella* species is not known and no experiments comparing the salinity response of multiple species have been conducted. From a conservation perspective, understanding how salinity tolerances vary amongst *Coxiella* species is needed to identify those taxa that are potentially most

vulnerable to changing field salinities. The data will also be useful for understanding whether the evolutionary radiation of this group was accompanied by ecological diversification.

This study experimentally tested the upper and lower salinity tolerances of individuals from multiple populations in six *Coxiella* species. The hypothesis is that salinity tolerance is unequal amongst *Coxiella* species. These results are compared with field records to understand how well the experimental results correspond with the salinity distributions of these species in nature.

Materials and methods

Sample collections

The experiment used individuals of *Coxiella* collected between September and December in 2021 from a total of 25 sites (hereafter called populations), representing a total of six species (Fig. 1; Table 1). The six species were selected on the basis that they have overlapping geographic distributions and field records suggest that three of them (*Coxiella exposita* (Iredale, 1943), *Coxiella glabra* Macpherson, 1957, *Coxiella striatula* (Menke, 1843)) are generally found at lower salinities than the other three (*Coxiella pyrrhostoma* (Cox 1868), *C. glauerti* and *C.* n. sp. 2). The number of populations tested per species ranged from two to six (Table 1) and was mainly determined by the number of known populations with enough snails to run the experiments. For some populations, repeated collections were needed to obtain sufficient snails (Table 1). Where possible, populations from throughout the known distribution of each species were represented in the experiments to help document the extent of any intraspecific variation in salinity tolerance. Snails were collected from each

Fig. 1 **a–c** Examples of salt lakes inhabited by *Coxiella*. **c** Shows the Esperance 22 site in the foreground and E23 site in the background (see discussion for relevance). **d** Example of *C. glauerti* forming 'rafts' of individuals in preparation for desiccation

Table 1 Details of collection sites/populations (site code) for individuals of six *Coxiella* species used in the tolerance experiment

Species	Population	N° collections	Latitude	Longitude
C. exposita	Cranbrook 2 (CR2)	2	− 34.257	117.634
	Varley 3 (V3)	1	− 32.708	119.360
	Wagin 3 (W3)	3	− 33.426	117.379
C. glabra	Goomalling 1 (G1)	2	− 31.136	116.875
	Lake Monger 1 (LM1)	2	− 29.544	116.705
	Morawa 2 (MOR2)	2	− 29.406	115.883
	Three Springs 2 (TS2)	1	− 29.575	115.822
	Wongan Hills 1 (WH1)	2	− 30.747	116.762
	Wongan Hills 6 (WH6)	2	− 30.720	116.793
C. glauerti	Esperance 22 (E22)	1	− 33.474	122.355
	Esperance 30 (E30)	1	− 33.543	122.432
	Esperance 33 (E33)	1	− 33.508	122.409
	Esperance 8 (E8)	2	− 33.498	122.401
C. n. sp. 2	Esperance 23 (E23)	1	− 33.473	122.353
	Esperance 7 (E7)	2	− 33.540	122.431
C. pyrrhostoma	Esperance 21 (E21)	1	− 33.455	122.017
	Esperance 3 (E3)	2	− 33.482	121.697
	Esperance 4 (E4)	2	− 33.516	121.876
	Marchagee 5 (MA5)	1	− 30.199	116.370
	Stirling 1 (STR)	3	− 34.307	118.028
C. striatula	Esperance 2 (E2)	3	− 33.819	121.886
	Frankland 1 (F1)	3	− 34.417	117.252
	Green Head 2 (GH2)	2	− 29.987	114.987
	Jurien Bay 3 (JB3)	2	− 30.209	115.008
	Muir 2 (MU2)	3	− 34.370	116.704

N° collections is the number of times the site was sampled to obtain the snails used in the experiment. Species names are based on Lawrie et al. (2023) taxonomic assessment of *Coxiella*

site by hand or sieving mud, transported back to the laboratory and placed in collection tanks.

Holding conditions

The collection tanks were held in a temperature-controlled room at 20 °C ± 1 °C, with a 12-h light/dark regime. This temperature was chosen because experimental data of Williams & Mellor (1991) suggested that *C. striata* had 100% survival over 5 days at this temperature and 20 °C is well within the range of temperatures at which *Coxiella* is active in the field (Supplemental data). The tanks were aerated and maintained at 39–53 mS/cm with a combination of reverse osmosis (RO) water and Red Sea Salt. This salinity range was chosen because it was well within the tolerable range for all species as indicated by field records (Supplemental data). Since ionic ratios of Australian salt lakes are typically similar to those

of the ocean, with only a few exceptions (Bayly & Williams, 1966; Geddes et al., 1981), Red Sea Salt, which is designed to replicate marine water, was used in these experiments. This product has previously been used in experiments with *C. striata* (O'Dwyer & Murphy, 2021). Snails were fed weekly using Tetra Fin flakes and API algal wafers. Snails were acclimatised to the conditions in the collection tanks for between two and seven days before being included in the experiment.

Experimental design

The experiment tested the response of snails from each population to progressive increases or decreases in salinity. Each experiment had two treatments increasing salinity ('upper') and decreasing salinity ('lower') and a control (constant salinity). For each population, 30 snails were randomly assigned

to each treatment and to the control. The experiment was conducted in 350 mL plastic boxes. Thirty snails from the same population in the same treatment were held in the same box, but snails for each population and each treatment were held in separate boxes.

Each treatment and the control started at a salinity of 53 mS/cm ($\pm$ 7 mS/cm), with daily water changes used to increase salinity in the upper treatment or reduce salinity in the lower treatment or keep the salinity in the control at 53 mS/cm ($\pm$ 7 mS/cm). This salinity was selected to minimise the osmotic shock to individuals after their transfer from the collection tanks. The salinity of treatment tanks was changed gradually to replicate salinity changes that salt lakes experience due to evapoconcentration (increasing salinity) or rainfall events (decreasing salinity). Salinity was increased or decreased by an average of 6.3 and 6.8 mS/cm per 24 h, respectively. Below ~9 mS/cm in the lower treatment, conductivities of ~5 mS/cm, ~2 mS/cm and ~0.1 mS/cm were tested. All conductivity data presented here have been corrected to 25 °C.

During the experiment, snails were held at each salinity level for a 24-h period on the basis that it took 20–28 h for the body tissues of a congeneric species (*C. striata*) to become isosmotic with the surrounding water when specimens were switched from high to low salinity conditions or vice versa (Williams & Mellor, 1991).

To estimate experimental variability, the experiment was repeated three times with fresh sets of snails in each repeat. All populations and treatments within a single experimental run were assayed at the same time. The different experimental runs were conducted at different times between October 2021 and January 2022. Each repeat was started within one to two weeks of the end of the previous one. Where possible, every population was included in every repeat, but only two repeats were possible for CR2, TS2, WH6, LM1, and E33 due to insufficient snails. Similarly, there were not enough snails to include the lower treatment for W3 in the third run.

Field collections

Data on the conductivity of selected field sites inhabited by the six *Coxiella* study species are presented for comparison with the experimental results (see Supplemental data). Conductivity at these sites was measured using a YSI probe. Whether the snails at the site were active (moving) or inactive (opercula shut) was also recorded. The data were collected between 2017 and 2021 and include repeated measurements from some lakes (see Supplemental data). The identities of the species collected at these sites have been confirmed via genetic data in Lawrie et al. (2023)

Data analysis

The point at which snails became inactive (sealed themselves into their shells by closing their operculum) was measured because once retracted it was considered unlikely for an individual to regain mobility and therefore this point represented the salinity at which individuals could no longer biologically function. Estimates and associated 95% confidence intervals of the salinity at which 50% of tested individuals became inactive (hereafter called IC50) were calculated using the R package medrm, using the metadrm function, which conducts a two-stage meta-analysis to fit a hierarchical dose–response model (Gerhard & Ritz, 2015). This package combines the automated nonlinear regression modelling framework of the package drc (Ritz et al., 2015) with the nonlinear mixed estimation framework of the package nlme (Pinheiro et al., 2015) to produce dose–response estimates for repeated measures experimental designs. IC50 estimates were calculated using a log logistic model for binomial data with curves grouped by population using data from the repeated experimental runs, with the dependant variable 'inactive' (i.e. the proportion of inactive/dead specimens at a salinity level) explained by the independent variable 'salinity' (mS/cm) and with the correlation between repeated observations from the same experimental unit factored into the model. This modelling was done independently on the lower and upper tolerance datasets. A Kruskal–Wallis test (Hollander et al., 1973) was used to test for a significant difference in the median IC50s for each species for the lower and again for the upper salinity experiments, with post-hoc pairwise comparisons examined using Dunn's test (Dunn, 1964) and the Bonferroni method used to adjust P-values for multiple comparisons (Hochberg, 1988). All statistical significance was determined using an alpha of $P < 0.05$.

Results

Controls

Most snails (80% or more) remained active in 68% of the controls (Supplemental data). The lowest percentage of active snails in any control was 50% for population V3 at the end of the 2nd run (Supplementary data).
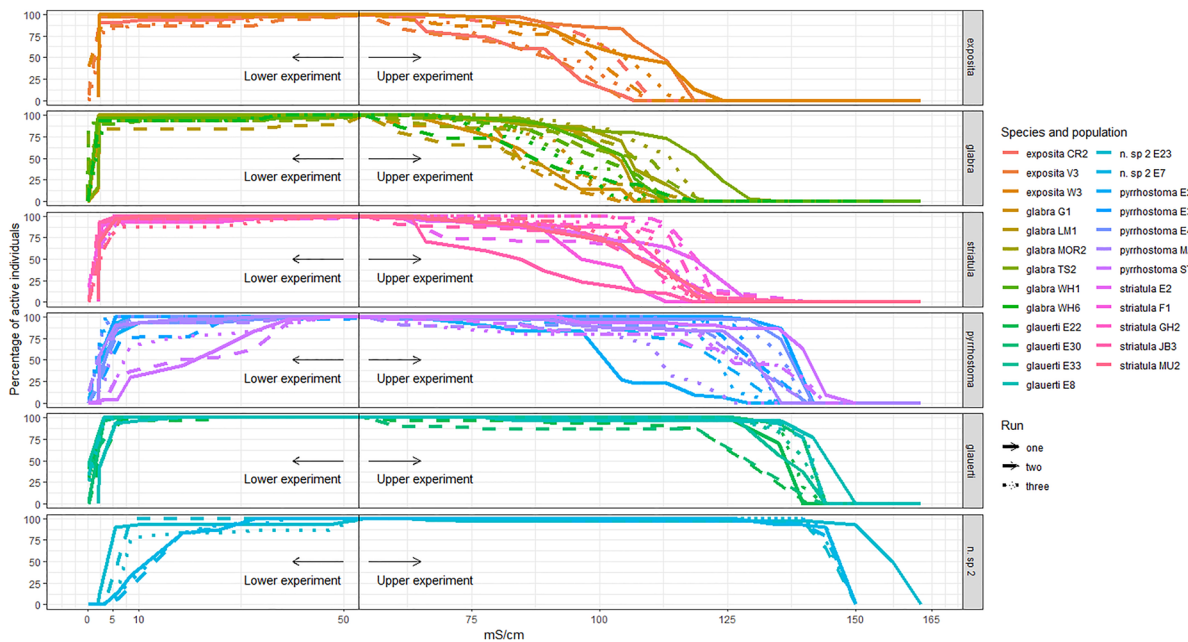
Salinity range

The salinity ranges for all populations of every species were broad, with high levels of activity observed between 10 and 75 mS/cm (Fig. 2). The minimum salinity at which at least one individual was active was broadly similar for all populations, both within and between species, ranging from 0.1 to 5.7 mS/cm (Table 2). The extent of the variation in the upper salinity limit among the populations of a species ranged from 2.1 mS/cm in *C. glauerti* to 25 mS/cm in *C. glabra* (Table 2). The population most tolerant of high salinity was E23 of *C.* n. sp. 2, which had active individuals at 157.2 mS/cm. In contrast, the highest

value recorded for the least tolerant population, i.e. population W3 in *C. exposita*, was 118.8 mS/cm (Table 2).

Salinity tolerance—variation within species

Different populations in four of the six *Coxiella* species (*C. exposita*, *C. striatula*, *C. glauerti* and *C.* n. sp. 2) showed similar upper IC50 estimates and broadly overlapping confidence limits (Fig. 3; Table 2). However, *C. glabra* and *C. pyrrhostoma* showed more variation. In the case of *C. glabra*, the upper IC50 estimates for TS2 (107.8 mS/cm) and MOR2 (104.9 mS/cm) were relatively high compared to those for G1 (87.3 mS/cm), WH6 (88.3 mS/cm) and LM1 (91 mS/cm); the estimate for WH1 (97.3 mS/cm) was intermediate between these two groups (Fig. 3; Table 2). Nevertheless, there was overlap in the 95% confidence limits for all populations of this species except for G1 vs TS2. For *C. pyrrhostoma*, individuals from E3 (133.4 mS/cm), E4 (131.7 mS/cm) and STR (129.7 mS/cm) had higher upper IC50 estimates than those from E21 (116 mS/cm) and MA5 (116.3 mS/cm; Fig. 3; Table 2).

Fig. 2 Line plot of the percentage of active individuals for each *Coxiella* species in each run across the measured salinity range. Vertical black bar indicates starting salinity for both the lower and upper experiment with data on the left and right from the lower and upper treatments, respectively. Results for first (solid), second (dashed) and third (dotted) runs are indicated by different line types

Table 2 Summary of experimental results of upper and lower salinity tolerance for 25 populations of six *Coxiella* species

| Species	Lower IC50	Min	Upper IC50	Max	Repeats	Field mS/cm
C. exposita	1.9		95.5			
CR2	3 (0–6.5)	2	92.1 (80.9–103.3)	104.3	1/2	7.2–9.2
V3	2 (0–4)	0.2	95.5 (86.2–104.8)	113.1	3/3	15.3
W3	1.2 (0.3–2.1)	**0.1**	99 (89.9–108.2)	**118.6**	2/3	1–22.5
C. glabra	0.6		94.1			17.5–30.5
G1	0.4 (0–1.3)	**0.1**	87.3 (78.8–95.9)	104.3	3/3	19.8–32.1
LM1	0.8 (0–2.6)	0.2	91 (81.7–100.3)	113.7	2/3	32.1
MOR2	0.2 (0–1)	**0.1**	104.9 (95.8–113.9)	113.4	3/3	40.8
TS2	0.9 (0–1.9)	**0.1**	107.8 (96.4–119.2)	**129.3**	2/2	22.6–45
WH1	0.4 (0–1.2)	**0.1**	97.3 (88.6–105.9)	107.6	3/3	49.9–63.8
WH6	1 (0–2.8)	**0.1**	88.3 (76.6–100.1)	113.7	2/2	7.2–9.2
C. striatula	1.9		106.3			
E2	1.9 (1–2.9)	0.1	106.3 (96–116.6)	124.3	3/3	32.1–32.8 (91.5 NA)
F1	0.9 (0–2.5)	0.1	113 (106–119.9)	119.3	3/3	17.1–36.7
GH2	2.7 (0.2–5.1)	0.1	108.8 (99.7–117.8)	119.2	3/3	34.1–58.6
JB3	1.9 (1–2.9)	0.1	103.9 (95.7–112.1)	119.3	3/3	36.8–48.7
MU2	2.3 (1.1–3.4)	0.1	105.9 (96.8–115.1)	119.2	3/3	27.8–34.7
C. pyrrhostoma	2.8		129.7			
E21	2.4 (0.1–4.6)	**0.1**	116 (108–124)	126.3	3/3	45.8
E3	2.1 (1.1–3)	**0.1**	133.4 (125.2–141.7)	135.5	3/3	89.1 (161.7 NA)
E4	3.4 (0.9–5.9)	2.1	131.7 (122.4–140.9)	139.7	3/3	100.2–132
MA5	2.9 (0.8–4.9)	0.2	116.3 (106.3–126.2)	129.3	3/3	158.9 (NA)
STR	12.3 (5.2–19.4)	3.2	129.7 (116.7–142.7)	**144.2**	3/3	58.8–67.4 (130.2 NA)
C. glauerti	0.3		138.3			
E22	0.3 (0–1.1)	**0.1**	135.2 (128.2–142.1)	139.7	3/3	62.2
E30	0.3 (0–1.4)	**0.1**	138.7 (131.6–145.7)	140.8	3/3	27.6
E33	0.1 (0–0.9)	**0.1**	138 (130–145.9)	139.7	2/2	127.3 (NA)
E8	0.5 (0–1.3)	**0.1**	140.6 (132.7–148.5)	**141.8**	3/3	63.7 (143.2 NA)
C. n. sp 2	8.6		146.4			
E23	5.7 (4.3–7.2)	**2.3**	146.5 (136.3–156.7)	**157.2**	3/3	105.3
E7	11.5 (6.2–16.7)	5.7	146.3 (138.6–153.9)	144.2	3/3	104.6 (167.7 NA)

Lower IC50 and Upper IC50 are the IC50 estimates, with associated upper and lower 95% confidence intervals in parentheses for populations and median for the species. Min and Max are the minimum and maximum salinities at which at least one individual remained active for a 24-h period, respectively. Repeats is the number of repeats conducted for the lower/upper experiment. Field mS/cm is the salinity (or salinity range for multiple collections) of the water body when snails used in the experiment were collected. NA not active. Bolded values represent the lowest (in the lower experiment) and highest (in the upper experiment) salinity that at least one individual tolerated in each population of each species

Different populations in four of the six species (*C. exposita*, *C. glabra*, *C. striatula*, and *C. glauerti*) displayed almost no difference in their IC50 responses to decreasing salinity (Fig. 3). However, this was not the case for *C. pyrrhostoma* for which the lower IC50 estimate for the STR population (12.3 mS/cm) was higher than that for the other populations (Fig. 3; Table 2). Also, in *C.* n. sp. 2, the lower IC50 estimate

for E7 population was noticeably higher than that for the E23 but their 95% confidence limits still overlapped (Fig. 3; Table 2).

Salinity tolerance—variation between species

The experiment provided evidence of significant differences in the upper salinity tolerance
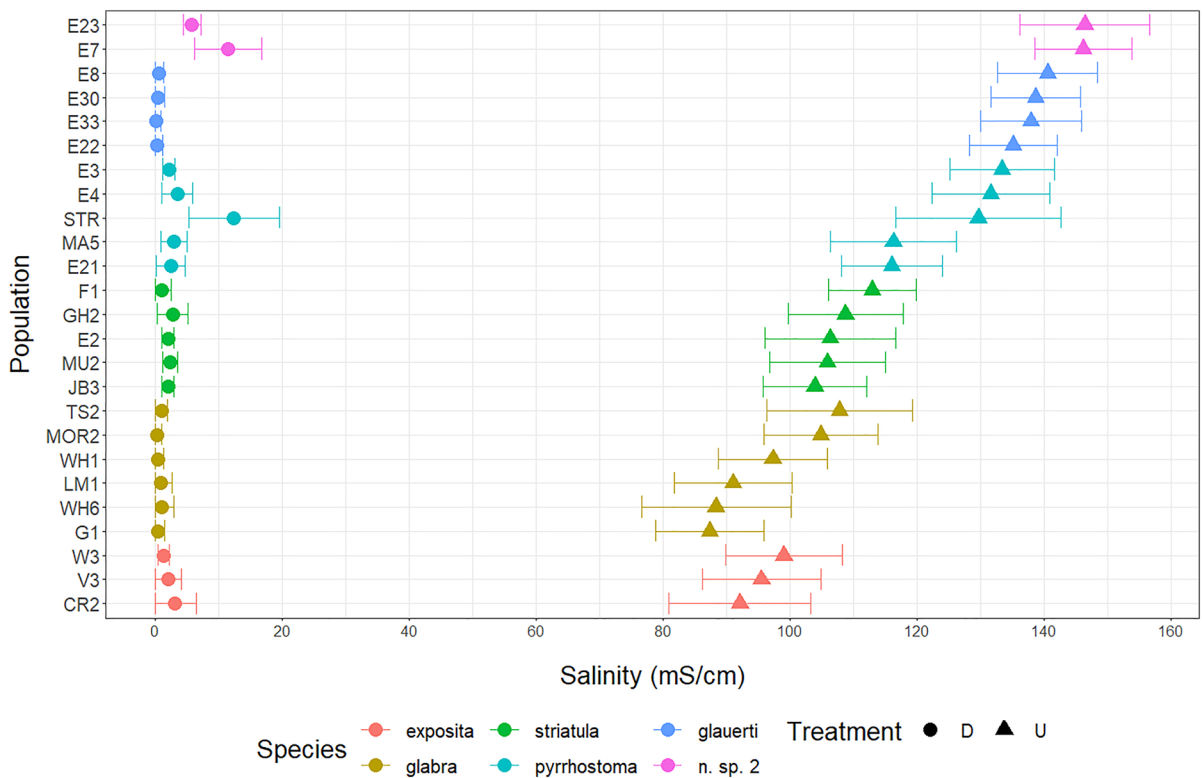
Fig. 3 IC50 estimates and associated 95% confidence intervals for all *Coxiella* populations tested in both the lower (circles) and upper (triangles) salinity experiments

of some *Coxiella* species ($\chi^2 = 21.48$, $df = 5$, *p*-value < 0.001). Estimates of the upper IC50 limits for *C. exposita* (median = 95.5 mS/cm) and *C. glabra* (median = 94.1 mS/cm) were significantly different than those for both *C. glauerti* (median = 138.3 mS/cm) and *C. n. sp. 2* (median = 146.4 mS/cm; Table 3; Fig. 3). The upper salinity tolerances of *C. striatula* (median = 106.3 mS/cm) and *C. pyrrhostoma* (median = 129.7 mS/cm) were intermediate between these two species groups, with *C. striatula* more closely aligned with the low salinity species and *C. pyrrhostoma* having some populations more closely aligned with the low salinity species (MA5, IC50 = 116.3 mS/cm; E21, IC50 = 116 mS/cm) and others with the high salinity species (E3, IC50 = 133.4 mS/cm; E4, IC50 = 131.7 mS/cm; STR, IC50 = 129.7 mS/cm; Table 3; Fig. 3). The maximum upper IC50 values recorded for a species' population increased in the following order—*C. exposita* (W3, 99 mS/cm),

C. glabra (TS2, 107.8 mS/cm), *C. striatula* (F1, 113 mS/cm), *C. pyrrhostoma* (E3, 133.4 mS/cm), *C. glauerti* (E8, 140.6 mS/cm) to *C. n. sp. 2* (E23, 146.5 mS/cm; Fig. 3; Table 2).

The magnitude of difference between species median lower IC50 estimates was not as large compared to the upper salinity tolerances, but some species still demonstrated significantly different tolerances ($\chi^2 = 20.12$, $df = 5$, $P = 0.001$). *Coxiella glauerti* (median = 0.3 mS/cm) and *C. glabra* (median = 0.6 mS/m) were the most tolerant of low salinities and recorded IC50 values of < 1 mS/cm (Fig. 3; Table 2). *Coxiella n. sp. 2* was the least tolerant to low salinity (median = 8.6 mS/cm) and showed significantly less tolerance than *C. glabra* and *C. glauerti* (Table 3; Fig. 3). *Coxiella pyrrhostoma* also displayed significantly less tolerance to low salinities than *C. glabra* and *C. glauerti* (Table 3).

Table 3 Results of a Dunn's test comparing IC50 estimates for pairs of *Coxiella* species for the upper (bottom diagonal) and lower (top diagonal) salinity experiments

	exposita	*glabra*	*glauerti*	n. sp. 2	*pyrrhostoma*	*striatula*
exposita		1.76	2.18	− 1.12	− 0.78	0.26
		0.59	0.22	0.99	0.99	0.99
		1.3	1.6	6.7	0.9	0
glabra	− 0.03		0.65	**− 2.77**	**− 3.00**	− 1.74
	0.99		0.99	**0.04**	**0.02**	0.61
	1.4		0.3	**8**	**2.2**	1.3
glauerti	**− 2.88**	**− 3.37**		**− 3.10**	**− 3.33**	− 2.20
	0.03	**< 0.001**		**0.01**	**< 0.001**	0.21
	43.3	**44.7**		**8.3**	**2.5**	1.6
n. sp. 2	**− 2.85**	**− 3.16**	− 0.47		0.54	1.45
	0.03	**0.01**	0.99		0.99	0.99
	50.9	**52.3**	7.6		5.8	6.7
pyrrhostoma	− 2.17	− 2.58	0.91	1.22		1.20
	0.22	0.07	0.99	0.99		0.99
	32.2	35.6	9.1	16.7		0.9
striatula	− 1.09	− 1.28	2.09	2.16	1.25	
	0.99	0.99	0.28	0.23	0.99	
	10.8	12.2	32.5	40.1	23.4	

Z statistics (top number), p values (middle number) and magnitude of difference in mS/cm between median IC50 estimates (bottom number). Statistically significant pairwise comparisons after Bonferonni correction are bolded

Field vs experimental data

A total of 151 field records of salinity from 68 different lakes were available for analysis; 136 of these records were for active snails and the remainder for inactive ones (Supplemental data). Most records, 37, 35 and 33, were for *C. striatula*, *C. glabra* and *C. pyrrhostoma,* respectively, while *C.* n. sp. 2 and *C. glauerti* had the fewest with 8 and 14, respectively (Fig. 4). Overall, active specimens for each of the six species were recorded from a very broad salinity range, i.e. from 1 to 132 mS/cm (Fig. 4).

The patterns of active species' salinity distributions in the field corresponded to those observed in the tolerance experiments. For example, *C.* n. sp. 2, *C. glauerti* and *C. pyrrhostoma* were active in salt lakes that were consistently more saline than those occupied by *C. exposita*, *C. glabra* and *C. striatula* (Fig. 4). Also, as in the experimental results, there was considerable overlap in the field salinities of *C. exposita*, *C. glabra* and *C. striatula* and, among these species, *C. exposita* was generally found at the lowest salinities (median = 19.1 mS/cm) and *C. striatula* at the highest (median = 39.3 mS/cm). The field data suggest that *C. pyrrhostoma* has the broadest salinity range (5.1–132 mS/cm; median = 78.5 mS/cm), notwithstanding that field data for *C. glauerti* and *C.* n.

sp. 2 are limited. *Coxiella* n. sp. 2 is yet to be recorded in salinities < 67.9 mS/cm in the field (Fig. 4), which is noteworthy since this species was least tolerant of low salinities in the experiment.

The highest recorded field salinities for active individuals were lower than the experimentally determined upper IC50 estimates for all tested populations in all species except *C. pyrrhostoma,* for which the four highest field records fall within the estimated 95% confidence interval of the population least tolerant of high salinities (E21, Fig. 4). In addition, inactive individuals were observed in the field at salinities less than or within the IC50 range estimates for populations of *C. exposita*, *C. striatula*, *C. pyrrhostoma* and *C. glauerti* (Fig. 4). The lowest recorded field salinities were higher than experimentally determined lower IC50 estimates for all tested populations in all species except *C. exposita* and *C. pyrrhostoma* (Fig. 4).

Environmental influence

For all but one *Coxiella* species, there was no relationship between either the upper or lower experimental tolerances and habitat salinity when experimental snails were collected (Fig. 5a, b). However, for the three populations of *C. exposita*, estimates
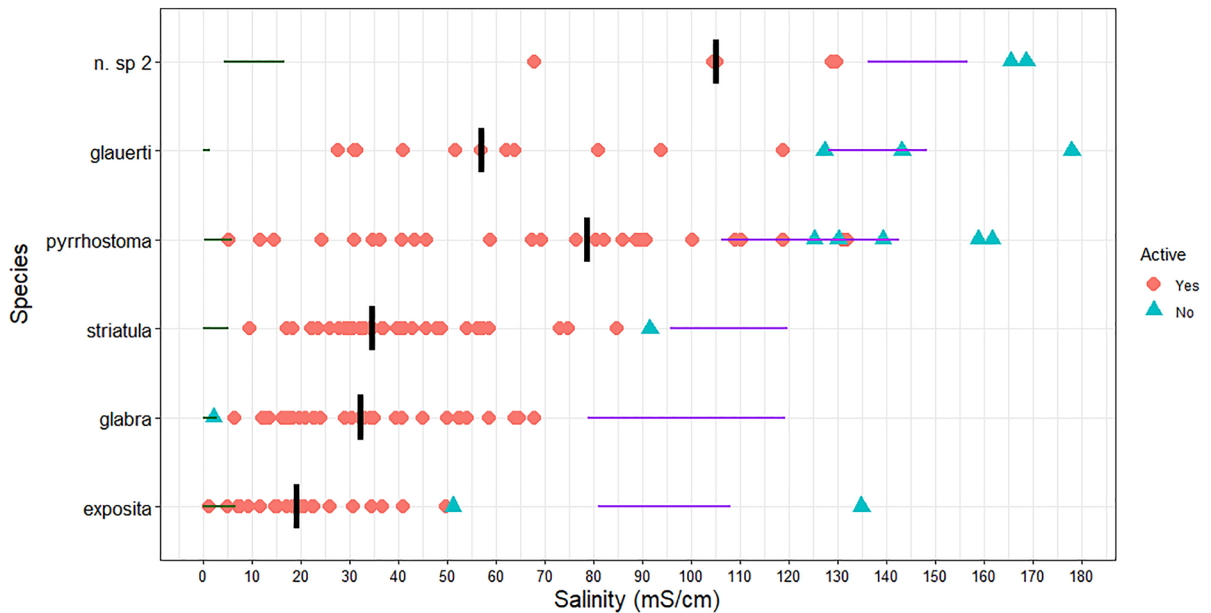
Fig. 4 Field records for active (red circles) and inactive (blue triangles) individuals from all experimentally tested *Coxiella* species. Black rectangle is the median salinity of active occurrence (mS/cm). Dark green and purple bars are the lowest and highest 95% confidence intervals for CR2 (*C. exposita*), WH6 (*C. glabra*), JB3 (*C. striatula*), MA5 (*C. pyrrhostoma*), E22 (*C. glauerti*) and E23 (*C. n. sp. 2*). These populations were selected as they each had the lowest upper IC50 for each of their respective species (see Table 2). Note that Fig. 4 contains all water quality data from all available sites, not just the sites from which individuals were collected for the experiment in Table 1

of the upper salinity tolerance increased with habitat salinity at the time of collection (Fig. 5b).

Discussion

Halophilic gastropods

This study has generated experimental data on the upper and lower salinity tolerances for 25 populations of six *Coxiella* species. Prior to this, data on salinity tolerance in *Coxiella* were only available for five populations of one species, *C. striata* (Williams & Mellor, 1991; O'Dwyer & Murphy, 2021). In the current study, the experimental data were supported by detailed information on the salinity distributions of each species in the field. The results suggest that *Coxiella* species are amongst the most euryhaline and salt tolerant gastropods in the world, notwithstanding the difficulties of comparing estimates across studies that have used different experimental methods. Field records suggest halophilic gastropods are rare but include *Caspiohydrobia* spp. from the Aral and

Caspian seas (Filippov & Riedel, 2009; Andreeva et al., 2020), *Tomichia ventricosa* and *T. tristis* that inhabit saline vleis in South Africa (Davis, 1981) and *Heleobia* spp. that occur in a range of saline environments in South America (Reid et al., 2021). Of these, salinity tolerances have only been experimentally tested in *T. ventricosa*, which can remain active between 0 and 50 ppt for a month (Davis, 1981) and *Caspiohydrobia* spp. (species unknown), which had ~35% survival in tested individuals for two weeks at 110 g/L (Filippov & Komendantov, 1996).

Within species variation

The experimentally determined lower salinity limits varied little among conspecific populations, except that these limits were unusually high for single populations of *C. n. sp. 2* and *C. pyrrhostoma*. The majority of species also did not show significant variation in the upper salinity tolerances among their populations. The notable exceptions were populations of *C. pyrrhostoma* and of *C. glabra* which sometimes displayed relatively large differences in maximum
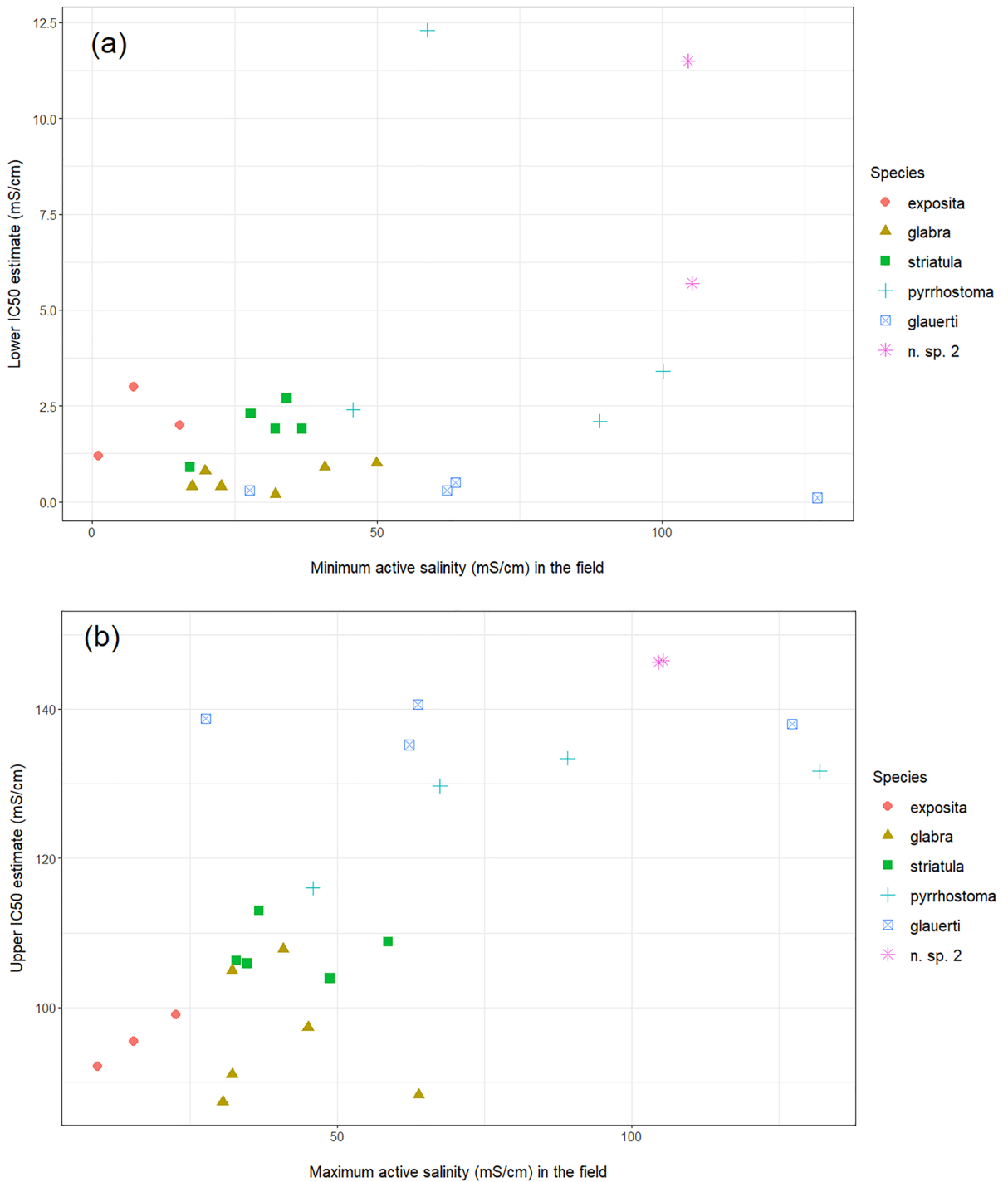
Fig. 5 Scatter plot illustrating the relationship between IC50 estimates and site salinity at the time when the experimental snails were collected for 25 populations of six *Coxiella* species. **a** minimum field salinity versus lower IC50 estimate, and **b** maximum field salinity versus upper IC50 estimate, for each population. Field salinity data are only included when active snails were collected

salinity tolerance. The increased variability observed in these two species may reflect the fact that they were represented in the experiment by, respectively, five and six populations compared to only two to four populations for *C.* n. sp. 2, *C. exposita* and *C. glauerti*. On the other hand, *C. striatula* was represented by six populations but showed little intraspecific variation. Comparing the same number of populations for each species would have been ideal, however, this was not possible due to the small number of known populations of *C.* n. sp. 2, the limited number of Western Australian populations of *C. glauerti* and a low abundance of snails in known populations of *C. exposita*.

O'Dwyer & Murphy (2021) compared the stress tolerance of populations of *C. striata* in lakes with less environmental variance (e.g. permanent water bodies with stable salinities) with those in lakes that experience variable conditions (e.g. ephemeral water bodies with fluctuating salinities). They concluded that the former was less tolerant of environmental change due to stabilising selection while the latter have maintained standing variation in physiological tolerances. The relevance of these findings to this study is not clear because all our sites are temporary and likely to be variable in the sense of O'Dwyer & Murphy (2021). More regular monitoring, both within and between filling cycles, of the salinity and other physicochemical parameters in our study sites is needed to understand whether some of these habitats are inherently more variable than others.

Between species variation

This study is one of few to experimentally compare the salinity tolerances of multiple closely related halophilic taxa not in the genus *Artemia* (Conte & Geddes, 1988; Browne & Wanigasekera, 2000; O'Dwyer & Murphy, 2021). The results suggest that, although all species demonstrated impressive euryhalinity, some *Coxiella* species have different upper salinity tolerances. According to the IC50 estimates, the upper salinity tolerances of *Coxiella* species rank from highest to lowest in the following order *C.* n. sp. 2, *C. glauerti*, *C. pyrrhostoma*, *C. striatula*, *C. exposita* and *C. glabra*. The difference between the most tolerant species (*C.* n. sp. 2, IC50 = 146.4 mS/cm) and the least tolerant species (*C. glabra*, IC50 = 94.1 mS/cm) was substantial. Statistical testing confirms that upper salinity tolerances in *C. glauerti* and *C.* n. sp. 2

were different (higher) from those of *C. exposita* and *C. glabra.* The upper salinity tolerances of *C. striatula* and *C. pyrrhostoma* were intermediate between these two groups but were not significantly different to each other or those of any other species. Obtaining data for additional populations of each species would improve the power of the statistical testing and might reveal further significant differences among species, although it could also just increase the amount intraspecific variation/overlap among species. Most tested species overlapped in their lower salinity limit, but some species seem to tolerate low salinity conditions better than others (e.g. *C. glabra* and *C. glauerti* compared with *C.* n. sp. 2).

In the experiments of Williams & Mellor (1991), individuals of *C. striata* from a single population in South Australia showed a broad salinity tolerance (lower IC50 = 3.8 mS/cm, upper IC50 = 125.3 mS/cm). Williams & Mellor (1991) also found that if *C. striata* individuals were directly transferred to tanks of higher or lower salinity without being allowed to gradually acclimatise their salinity tolerances lessened (lower IC50 = 10.4 mS/cm, upper IC50 = 111.7 mS/cm). These estimates suggest that the upper and lower limits of salinity tolerance in *C. striata* are broadly comparable to those obtained for the six *Coxiella* species in this study. However, we acknowledge that the *C. striata* results are not directly comparable with those obtained in the present study because of differences in experimental method. For example, Williams & Mellor (1991) raised and lowered salinities in their 'gradual' experiment every six days as opposed to every 24 h as done here.

Field vs experimental data

For each *Coxiella* species, the experimentally determined salinity tolerances were wider than the salinity range observed in field records. This suggests that the experimental conditions were conducive to activity over a broad range of salinities. The reason why individuals of *Coxiella* are active over a narrower range of salinities in the field could be because their field distributions are influenced by other variable/s in addition to salinity. In experiments testing the salinity tolerance of freshwater and halotolerant macroinvertebrates, artificially manufactured saline water was found to be less toxic to the macroinvertebrates than water from salt lakes at the same conductivity

(Kefford, 2000). This suggests that electrical conductivity is not the only factor responsible for these species response to 'salinity' and that the increased toxicity of salt lake water may be due to the presence of elevated nutrients or other unknown pollutants (Kefford, 1998). It is also worth noting that the present study only tested salinity responses over a 24-h period and so the results do not necessarily suggest that *Coxiella* individuals could remain active at the same salinities for sustained time periods or could complete their life cycle at these salinities.

The discrepancy between experimentally determined and field records may also be explained if the current field records do not adequately cover the range of habitats/salinities occupied by the tested species. Since *C. striatula*, *C. glabra*, *C. pyrrhostoma* and to a lesser extent *C. exposita* have been sampled over their entire known distribution and from a range of different lakes (Lawrie et al., 2023), it is likely that field data for these species are a fair reflection of their salinity distributions. *C. glauerti* and *C.* n. sp. 2 are at the other extreme, having been encountered in relatively few lakes, which may explain the lack of field observations of both species at low salinities, despite the experimental data indicating that they can tolerate such conditions. Regardless, the field data included herein are the most comprehensive available for the studied species of *Coxiella*. It is worth noting that the identity of the species present at each site has been confirmed with genetic data (Lawrie et al., 2023). In contrast, Based on the results of Lawrie et al. (2023), the species-specific salinity data for *Coxiella* contained in some publications (e.g. Pinder et al. (2002), Pinder et al. (2005) and Timms (2009a)) are questionable due to uncertainties about the species identifications, although records for *C. glabra* and *C. striatula* in Halse (1981) and Edward (1983), respectively, are accurate.

Coxiella ecology

Despite overlap in their geographical distributions and salinity ranges, multiple *Coxiella* species rarely co-occur in the same lake. The potential influence of salinity on *Coxiella* species distributions is not clear. The experimental results and field records suggest that *C. exposita* and *C. glabra* have significantly less capacity to tolerate salinities as high as *C. glauerti* and *C.* n. sp. 2 and therefore these species could

be excluded from some salt lakes occupied by *C. glauerti* and *C.* n. sp. 2 on the basis of salinity alone. However, the known geographic distributions of *C. glabra* and *C. exposita* do not overlap with those of *C. glauerti* and *C.* n. sp. 2 (Lawrie et al., 2023), making this situation unlikely. Their geographic distributions do overlap with those of *C. pyrrhostoma* and *C. striatula* and there is considerable overlap in their lower and upper IC50 tolerances but co-occurrence among any combination of these species is rare (Lawrie et al., 2023). It is suggested that, although the upper and lower limits of the salinity tolerance are broadly important in determining whether a *Coxiella* species can occur in a particular lake, within these limits other factors including food availability, hydroperiod, sediment characteristics, biological interactions and stochasticity are potentially at least as important (Williams et al., 1990; Kefford et al., 2004b; Timms, 2009b). More research is needed to understand the influence of biotic and abiotic processes on species occurrence in *Coxiella* and other invertebrate taxa in Australian salt lakes (Lawrie et al., 2021).

Two studied lakes, E22 and E23, are separated by a sandspit 30 m wide and yet support *C. glauerti* and *C.* n. sp. 2, respectively. These two lakes differ in their salinities with the more saline lake supporting the more saline tolerant species *C.* n. sp. 2 while *C. glauerti* occurs in E22. Each species is abundant in their respective lake but absent from the other despite the close proximity. The relatively low tolerance of *C. glauerti* to high salinities may explain why *C. glauerti* does not occur in E23 but not vice versa as the salinity of E22 is well within the tolerable salinity range of *C.* n. sp. 2. It is possible that *Coxiella* has colonised one or both lakes relatively recently and, given more time, the two species will disperse between them. However, this seems unlikely given that both E22 and E23 were previously sampled by Timms (2009a) (site numbers 33 and 34; note that Timms misidentified *C.* n. sp. 2 as *C. glauerti*), who also identified differences in the crustacean fauna of these two lakes suggesting that whatever factors are driving these differences they are applicable to a range of taxa.

Experimental design

The experiment used in this study measured salinity tolerance while holding constant some key abiotic variables, such as temperature and water pH/ionic

composition. This is because we prioritised testing multiple populations and species over different combinations of abiotic variables. However, we recognise that salinity tolerance in other halophiles is influenced by the interactions between salinity and the ionic pH/composition of water (Bayly, 1969, 1972) and especially between salinity and temperature (Browne & Wanigasekera, 2000; Ismail et al., 2010). Nevertheless, although the exact tolerance estimates may have been different if different environment conditions had been used, the broader finding that *Coxiella* species have wide-ranging and high salinity tolerances is likely to be robust.

The experiment used *Coxiella* individuals that were recently collected from field sites with different environmental conditions, including salinity (see Table 2). Thus, there is the potential that the recent environmental history of the individuals has influenced the experimental results. This is a common problem in experimental studies (O'Dwyer & Murphy, 2021). For our experiment, there was no other option than to use individuals collected from the field as it is difficult and time-consuming to breed *Coxiella* individuals in the laboratory. To mitigate against this problem, snails were acclimatised to a constant set of environmental conditions in holding tanks in the laboratory for at least two days prior to being used in the experiment, noting that 24–28 h should be sufficient for body tissues to become isosmotic (Williams & Mellor, 1991). Also, individuals were only included in one experimental run and never reused. In addition, no relationship was found between the estimated salinity tolerance for a population and salinity of habitat from which the experimental individuals were collected except in the upper tolerance of *C. exposita* populations. Other factors that could not be controlled in the experiment were the age and size of snails, however, as much as possible the largest individuals available in each population were selected across runs to reduce the impact of these factors.

Conservation implications

This study has demonstrated that *Coxiella* species have very broad salinity tolerances. In this sense, relative to halotolerant taxa, *Coxiella* may be resilient to the drying/salinizing effects of climate change (Atkinson et al., 2021). Nevertheless, the experimental data only focussed on the tolerances of adults and

it is unclear how increasing salinity might influence reproduction and recruitment as adults are generally more tolerant of osmotic stress than juveniles in other aquatic invertebrates (Kefford et al., 2004a). Also, although *Coxiella* species can tolerate high salinities, the experimental data demonstrated that there is an upper limit to these tolerances. This upper limit was higher for some species than others suggesting an uneven risk of extirpation within *Coxiella*. Field data suggest that the upper salinity limits of *Coxiella* species are noticeably less than those of some of the other halophilic invertebrates, such as *Parartemia* (Timms, 2014), *Australocypris*, *Diacypris* and *Reticypris* (Lawrie et al., 2021; Rahman et al., 2022), from Australian salt lakes. This is important to recognise as it suggests that the fate of salt lake invertebrates in response to salinisation should not be generalised at an ecosystem level as potential outcomes judged by the response of the most resistant halophilic fauna could grossly underestimate the implications for more sensitive taxa (Timms, 2005). Although *Coxiella* species may be able to aestivate during unfavourable periods, the maximum duration of aestivation is unknown and may also vary amongst species. Given that the regulatory mechanism for salinity tolerance could have arisen as an exaptation to drought conditions (Gomez-Mestre & Tejedo, 2005; Arribas et al., 2014), it would be interesting to know if salinity tolerance in *Coxiella* species is positively correlated with desiccation resistance.

Anecdotal evidence suggests that extirpations of *Coxiella* populations are happening in some locations, particularly in the wheatbelt region of Western Australia. For example, no live individuals were found in Lake Stubbs (Newdegate, Western Australia) in 2021 despite Williams & Mellor (1991) having previously collected *Coxiella* from this lake. Also, many salt lakes in the wheatbelt region have swathes *Coxiella* shells that appear to have formed recently (<150 years), rather than being subfossils, but do not appear to contain any live individuals either in the water or aestivating (A. Lawrie unpublished data). These observations coincide with the progressive salinisation of lakes in this region and elsewhere since the onset of European land clearing, with many fresh or low salinity lakes now hypersaline (George et al., 2008). It is known that changes in hydrology of a water body can lead to the loss of a resident *Coxiella* population, i.e. *C. striata* from

Lake Corangamite, Victoria (Williams, 1995) and a range of lakes on the Eyre Peninsula, South Australia (Timms, 2009b). Furthermore, extirpations in other aquatic invertebrates in the wheatbelt region have been linked to increasing salinisation of water bodies (Timms et al., 2009) and increasing aridity of climate (Atkinson et al., 2021). It seems reasonable to hypothesize that these two factors are causing the loss of *Coxiella* populations from salt lakes.

Conclusion

This study has experimentally demonstrated that six *Coxiella* species possess broad salinity tolerances that are amongst the highest recorded for gastropods from any environment. The experimental results revealed relatively little inter-population variation in upper salinity tolerance in most species but not all. Overall, there was little intra or inter-specific variation in lower salinity tolerance. The experimental results were consistent with field data in that the populations of species that are found in higher salinities in the field tended to show higher upper salinity tolerances in the experiments. However, for all but one species, the upper salinity tolerances observed in the experiment were higher than those observed in the field. This study demonstrates that, although *Coxiella* maybe more resilient to the effects of climate change relative to other halotolerant/freshwater taxa, some species will likely be at greater risk than others and extirpations have already occurred in south-western Australia. Future research needs to focus on how desiccation duration/tolerance varies between species and determining the sub-lethal effects of salinity on *Coxiella*.

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