

# *Combined impacts of warming and salinisation on trophic interactions and mortality of a specialist ephemeral wetland predator*

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## ORIGINAL ARTICLE

# Combined impacts of warming and salinisation on trophic interactions and mortality of a specialist ephemeral wetland predator

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## Abstract

1. Wetlands are of enormous importance for biodiversity globally but are under increasing risk from multiple stressors driven by ongoing anthropogenic environmental change. As the trophic structure and dynamics of ephemeral wetlands are poorly understood, it is difficult to predict how these biodiverse ecosystems will be impacted by global change. In particular, warming and salinisation are projected to have profound impacts on these wetlands in future.
2. The present study examined the combined effects of warming and salinisation on species interaction strengths and mortality rates for two ephemeral wetland species. Using an ephemeral pond specialist copepod, *Lovenula raynerae* Suárez-Morales, Wasserman, & Dalu, (2015) as a model predator species, we applied a functional response approach to derive warming and salinisation effects on trophic interactions with a prey species. Furthermore, the effects of a salinisation gradient on mortality rates of adult copepods were quantified.
3. The predatory copepod exhibited type II functional responses towards larval *Culex pipiens* mosquito prey, owing to high predation rates at low prey densities. Increased temperatures generally resulted in greater predator feeding rates, whilst increased salinities reduced consumption. However, the effects of temperature and salinity interacted: temperature effects on functional responses were suppressed under heightened salinities. Substantial mortality was observed in both male and female adult *L. raynerae* at salinity levels exceeding 10 parts per thousand.
4. Warming and salinisation substantially altered interaction strengths in ephemeral wetland ecosystems, with implications for ecosystem function and stability. Furthermore, we demonstrated salinisation thresholds for mortality in an ephemeral wetland specialist, showing that salinisation may threaten the persistence of endemic species. The ongoing effects of warming and salinisation may therefore

interact to alter trophic dynamics and species composition in ephemeral wetlands. These stressors should be considered synergistically within management practices.

#### KEYWORDS

climate change, multiple stressors, predation, saline waters, temporary pools

## 1 | INTRODUCTION

Wetlands are highly biodiverse and productive ecosystems, yet are under increasing pressure from global environmental change via multiple stressors that often act synergistically (Dalu, Wasserman, & Dalu, 2017; Mabidi, Bird, & Perissinotto, 2018; White, Donohue, Emmerson, & O'Connor, 2018). From a biodiversity perspective, ephemeral wetlands are especially important habitats, characteristically inhabited by a rich array of specialists adapted to transient hydroperiods and that may be unable to persist in permanent aquatic ecosystems (Dalu, Wasserman, & Dalu, 2017; Wasserman, Alexander, Barrios-O'Neill, Weyl, & Dalu, 2016). Ephemeral wetlands often provide aquatic habitats for threatened, rare and/or endemic species which are reliant on transient habitat patches within terrestrial environments (Bird et al., 2018; De Meester et al., 2005). However, despite their significance for regional and global biodiversity, ephemeral wetlands have received relatively little attention due to spatial and temporal heterogeneity, which makes them difficult to detect and conserve during dry periods. The integrity of ephemeral wetlands is thus threatened in multiple ways, such as through land use changes driven by agricultural activity (Brock, Smith, & Jarman, 1999; Marty, 2005), species introductions (Cuthbert, Dalu, Wasserman, Dick, et al., 2018; Dalu, Wasserman, & Dalu, 2017), and climate change (Lu et al., 2016). Warming and salinisation are amongst the most pervasive stressors in ephemeral wetlands associated with ongoing environmental change (Dalu, Wasserman, & Dalu, 2017; Mabidi et al., 2018). Quantifying the ecological implications of these stressors is key to holistically understanding impacts arising from anthropogenic activity on the functioning and stability of these widespread aquatic habitats.

The effects of climate change on the interactions between species present the greatest uncertainty in global biodiversity impact predictions (Daufresne, Lengfellner, & Sommer, 2009; Gilbert et al., 2014). In the future, patterns of temperature and rainfall will shift on a global scale (Lu et al., 2016), with the potential to significantly alter interaction strengths between species which subsequently cascade through ecosystems (Cuthbert, Dick, Callaghan, & Dickey, 2018; Wasserman, Cuthbert, Alexander, & Dalu, 2018). Whilst the influence of temperature on biotic interactions is well studied (e.g. Englund, Öhlund, Hein, & Diehl, 2011), the role of salinity in mediating species interactions is often ignored, despite a close association with temperature regimes (Atashbar, Agh, Van Stappen, Mertens, & Beladjal, 2014). Salinity is a pervasive factor that affects aquatic biodiversity and species distributions, and in arid regions ionic

compounds often accumulate in association with anthropogenic changes to land cover and land use (Jolly, McEwan, & Holland, 2008; Nielsen, Brock, Petrie, & Crosslé, 2007; Toruan, 2012). Indeed, given that they are internally drained and small, ephemeral wetlands are particularly quick to accrue pollutants that rapidly heighten salinity levels (Nicolet et al., 2004; Williams, 2006). Ephemeral wetlands are therefore increasingly exposed to extended periods of elevated salinity (Finlayson, D'Cruz, & Davidson, 2005), with ecological impacts associated with abiotic regime shifts largely unknown (but see Mabidi et al., 2018). Tolerance to increases in salinity is typically low in freshwater organisms (Nielsen, Brock, Rees, & Baldwin, 2003), and thus we propose that anthropogenic climate change and salinisation may interact to impact ecological communities in ephemeral aquatic ecosystems.

Interactions between consumers and resources can be expressed using functional responses (FRs; see Solomon, 1949; Holling, 1959; Dick et al., 2014; Cuthbert, Dalu, Wasserman, Callaghan, et al., 2018). The FR is particularly useful for examining context-dependencies of interaction strengths between different trophic groups (e.g. Cuthbert, Dick, & Callaghan, 2018). That is, effects of environmental variables on species interactions, such as temperature, can be quantified through examinations of FR form and magnitude (e.g. Cuthbert, Dick, Callaghan, & Dickey, 2018; Wasserman, Cuthbert, Alexander, & Dalu, 2018). Additionally, the emergent interaction effects of multiple environmental factors may be deciphered under a FR approach (e.g. Wasserman, Alexander, Weyl, et al., 2016). In essence, higher FRs (i.e. greater attack rates, shorter handling times and thus higher maximum feeding rates) have the potential for greater ecological impact on resources, which may destabilise trophic groups (Bollache, Dick, Farnsworth, & Montgomery, 2008; Dick et al., 2014). The form of FRs (types I, II or III) can also be a useful indicator of the stability of consumer–resource interactions, whereby type II FRs are typically regarded as destabilising, whilst type III FRs may impart stability to resource populations (Oaten & Murdoch, 1975).

Calanoid copepods are often numerically dominant in ephemeral wetlands during the early and middle stages of the hydroperiod and can occupy top trophic levels (Dalu, Wasserman, Froneman, & Weyl, 2017; Wasserman, Weston, et al., 2018). Such zooplankton groups are internally recruited from dormant eggs within egg banks in the substrate, which hatch following rainfall events which initiate the hydroperiod (Wasserman, Alexander, Barrios-O'Neill, et al., 2016). Whilst the relative contributions of different environmental cues for dormant egg hatchability remain unclear, salinity has been identified as a particularly important determinant

(Brock, Nielsen, & Crossle, 2005; Skinner, Sheldon, & Walker, 2001). Indeed, high salinity levels can completely inhibit hatching (Mabidi et al., 2018; Waterkeyn et al., 2010) and affect juvenile development (Cancela da Fonseca et al., 2008; Hart et al., 1991). Specifically, high salinities may block cues which trigger emergence, or drive mortality through direct toxicity (Nielsen et al., 2007; Vanschoenwinkel, Seaman, & Brendonck, 2010). However, effects of salinity regime on mortality rates of mature zooplankton populations remain unquantified and may pose a substantial threat to the persistence of specialist populations in ephemeral wetlands if reproduction is impacted.

In the Eastern Cape province of South Africa, the calanoid copepod *Lovenula raynerae* Suárez-Morales et al. (2015) often dominates ephemeral wetlands and belongs to the Paradiaptominae subfamily that is adapted to arid, temporary habitats. This species is large and predatory (Cuthbert, Dalu, Wasserman, Dick, et al., 2018; Suárez-Morales et al., 2015; Wasserman, Alexander, Barrios-O'Neill, et al., 2016), and occupies high trophic levels in ephemeral wetland ecosystems for much of the hydroperiod (Dalu, Wasserman, Froneman, & Weyl, 2017). Therefore, this species has the potential to have marked ecological impacts on lower trophic levels through biotic processes such as predation (Cuthbert, Dalu, Wasserman, Callaghan, et al., 2018). The present study aimed to examine the emergent implications of warming and salinisation for trophic interactions between species in ephemeral wetlands, before assessing the tolerance threshold for salinity regime shifts, using the copepod *L. raynerae* as a model species. We thus employed FRs to examine the strength of interactions between copepods and a larval prey, which is a species of medically important mosquito (i.e. capable of vectoring pathogens or parasites) that successfully colonises ephemeral wetlands, under shifting temperature and salinity regimes. Salinity levels in excess of 10 parts per thousand (ppt) are known to occur in groundwaters, which, in turn, can heighten wetland salinity regimes in surface waters (Mabidi et al., 2018). We then examined mortality rates of *L. raynerae* across a salinity gradient. This information was used to quantify the potential ecological impacts of warming and salinisation for populations inhabiting such wetlands.

## 2 | MATERIALS AND METHODS

### 2.1 | Animal collection and maintenance

Adult *Lovenula raynerae* (4.5 – 5.0 mm) were collected from an ephemeral pond in Grahamstown (33° 16'47.8"S, 26° 35'39.8"E), Eastern Cape, South Africa by towing a 200-µm zooplankton net across the surface water, and transported in source water to controlled environment rooms at Rhodes University, Grahamstown, South Africa. Larvae of the medically important and widespread mosquito complex *Culex pipiens* were cultured using egg rafts obtained from artificial container-style habitats around the university campus and reared to the desired size (2.0–4.0 mm) on a diet of crushed rabbit food pellets (Agricol, Port Elizabeth, South Africa).

### 2.2 | Functional responses

Adult male copepods were concurrently acclimated and starved under three temperature regimes for 48 h, corresponding to experimental temperatures (13, 18, 28 ± 1°C) under a 12:12 light:dark regime in continuously aerated, filtered source water from the collection site (filter size 20 µm) within 25-L aquaria. Functional response experiments were undertaken under three salinity regimes (0.2 [source water concentration], 4, 8 ± 0.1 ppt) at each of the three experimental temperatures, representative of abiotic undulations across the hydroperiod (Mabidi et al., 2018; Wasserman, Weston, et al., 2018). Seawater was mixed with aerated, filtered source water to obtain the allocated salinity regime under each temperature level, and verified with a salinity probe (Sper Scientific 850036, Scottsdale). Larval mosquito prey were then introduced at five densities (2, 4, 8, 16, 32) across all salinity and temperature treatments (i.e. a 3 [temperature] × 3 [salinity] × 5 [prey density] factorial experimental design) in 80-mL arenas of 5.6 cm diameter and allowed to settle for 2 h prior to the addition of predators. All treatment groups were replicated four times. Once added, copepods were allowed to feed for 6 h, after which they were removed and remaining live prey counted. Controls comprised one replicate of each experimental group in the absence of predators. Experimental treatment groups were run simultaneously to eliminate potential confounding arising from different times.

### 2.3 | Mortality rates

*Lovenula raynerae* were maintained following collection in a controlled environment room at 18 ± 1°C and under a 12:12 light and dark regime. Ten *L. raynerae* (5:5 female:male per experimental replicate) were exposed to a salinity gradient spanning thirteen levels (0.2, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 ± 0.1 ppt;  $n = 5$  per experimental treatment) and mortality recorded after 24 and 96 hours of exposure. Salinity levels ≥10 ppt are known to occur in aquatic environments in arid regions and potentially impact upon communities (Mabidi et al., 2018). Experiments were undertaken in 80 mL glass arenas (5.6 cm diameter) containing the designated treatment. The allocated salinity was, again, obtained by mixing continuously aerated, filtered source water and sea water to reach the desired level.

### 2.4 | Statistical analyses

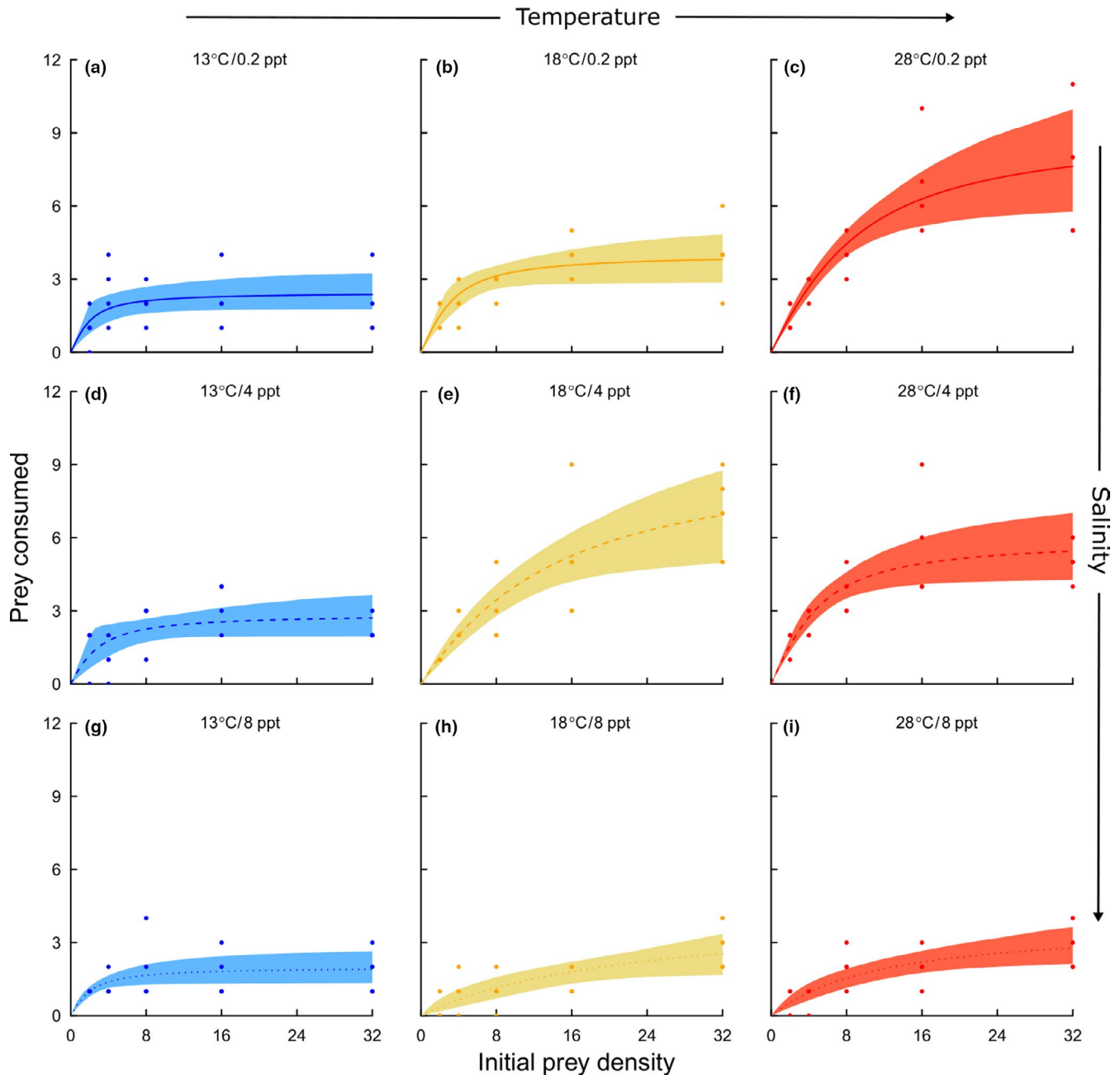
All statistical analyses were undertaken in R v3.4.2 (R Core Team, 2017). Overall consumption from the functional response experiment was analysed using generalised linear models assuming a Poisson error distribution for counts with respect to the *temperature*, *salinity*, and *prey supply* factors, and their interactions. Non-significant terms were removed stepwise from the model to obtain parsimony, following Crawley (2007) via comparisons of model deviance. Analysis of deviance was used to report effect sizes using likelihood ratio tests with type I sums of squares as data were orthogonal. We performed Tukey *post-hoc* comparisons using the *lsmeans* package in R where a factor yielded significance at the 95% confidence level (Lenth, 2016).

Functional response analyses were undertaken using the *frair* package in R (Pritchard, Paterson, Bovy, & Barrios-O'Neill, 2017). We followed a non-parametric bootstrap procedure ( $n = 2,000$ ) to generate 95% bias-corrected and accelerated confidence intervals around FRs, which enables their visual comparison at the population-level (see Barrios-O'Neill et al., 2014). The Rogers' random predator equation (type II) was applied as prey were not replaced as they were consumed (Juliano, 2001; Rogers, 1972; Trexler, McCulloch, & Travis, 1988):

$$N_e = N_0(1 - \exp(-a(N_e h - T))) \quad (1)$$

where  $N_e$  is the number of prey eaten,  $N_0$  is the initial density of prey,  $a$  is the attack constant,  $h$  is the handling time and  $T$  is the total experimental period. The Lambert W function was integrated to enable model fitting with the data (Bolker, 2008). Maximum feeding rates ( $1/h$ ) were then calculated using initial handling time maximum likelihood estimations. Where support for a categorical FR type was equivocal, flexible and type III FR models were also fit to the data, with Akaike's information criterion (AIC) used to select models that minimised information loss (see Pritchard et al., 2017).

In the mortality experiment, proportional mortality was assessed with respect to the *salinity*, *sex*, and *time* factors, and their



**FIGURE 1** Matrix of functional responses of *Lovenula raynerae* towards culicid prey at temperatures of 13°C (a, d, g), 18°C (b, e, h), and 28°C (c, f, i) and salinities of 0.2 ppt (a, b, c), 4 ppt (d, e, f), and 8 ppt (g, h, i) with bootstrapped ( $n = 2,000$ ) 95% confidence intervals. Points are raw data

**TABLE 1** First order terms derived from logistic regression of proportional prey consumption across prey supplies, alongside estimates of attack rates, handling times and maximum feeding rates across all salinity and temperature treatments

Salinity (ppt)	Temperature (°C)	1st order term, <i>p</i>	Attack rate ( <i>a</i> ), <i>p</i>	Handling time ( <i>h</i> ), <i>p</i>	Maximum feeding rate (1/h)
0.2	13	-0.09, <0.001	2.17, 0.13	0.41, <0.001	2.46
4	13	-0.08, <0.001	1.53, 0.16	0.35, <0.001	2.87
8	13	-0.08, <0.001	1.39, 0.25	0.50, <0.001	1.99
0.2	18	-0.07, <0.001	2.20, 0.09	0.25, <0.001	4.04
4	18	-0.04, 0.001	0.88, 0.002	0.10, <0.001	9.58
8	18	-0.03, 0.17	0.21, 0.06	0.24, 0.14	4.15
0.2	28	-0.06, <0.001	1.57, <0.001	0.11, <0.001	9.23
4	28	-0.08, <0.001	2.14, 0.01	0.17, <0.001	5.97
8	28	-0.04, 0.02	0.37, 0.04	0.27, 0.02	3.69

interactions using a generalised linear mixed model assuming a binomial error distribution (Bates, Mächler, Bolker, & Walker, 2015). A random effects structure was used to account for repeated measures over time of male and female copepods nested within each arena, with observation time included as a within-subject variable. Non-significant terms were again removed stepwise from the model to obtain the minimum adequate model complexity (Crawley, 2007). Effect sizes were then inferred through likelihood ratio tests via analysis of deviance.

### 3 | RESULTS

#### 3.1 | Functional responses

All control prey survived across all temperatures, salinities, and prey supplies and, ergo, prey deaths were attributed to predation by the calanoid copepods, which was also observed visually. Consumption levels increased concurrently with increasing prey supplied overall ( $\chi^2 = 79.88$ ,  $df = 4$ ,  $p < 0.001$ ). There was a significant temperature  $\times$  salinity interaction term ( $\chi^2 = 10.32$ ,  $df = 4$ ,  $p = 0.04$ ), reflecting interactive complexity between these factors (Figure 1). At the baseline salinity (0.2 ppt), overall consumption was significantly greater at the highest temperature (28°C; Figure 1c) compared to the low (13°C; Figure 1a) and intermediate treatments (18°C; Figure 1b; both  $p < 0.05$ ). At the intermediate salinity (4 ppt), consumption at the highest temperature (28°C; Figure 1f) was, again, significantly greater than the lowest (13°C; Figure 1d) temperature treatment ( $p = 0.007$ ), but was similar to the intermediate temperature (18°C; Figure 1e;  $p > 0.05$ ). However, at the highest salinity (8 ppt), the effect of temperature broke down, with no significant pairwise consumptive differences driven by temperature (all  $p > 0.05$ ; Figure 1g–i). Thus, feeding magnitudes peaked at the highest temperature under lowest salinities and under intermediate–high temperatures at intermediate salinities, whilst consumption was similar at the highest salinity across all temperature treatments.

Type II FRs were observed across all experimental treatments (Table 1; Figure 1) as first order terms were significantly negative at the 95% significance level. The 18°C treatment at a salinity of 8 ppt

was an exception to this; however, when compared to both flexible and categorical type III models, AIC values indicated a categorical type II model to provide the best fit (AIC: type II, 49.76; flexible, 51.63; type III, 51.76; see Pritchard et al., 2017). At baseline salinities (0.2 ppt), *L. raynerae* exhibited the greatest magnitude FR under the highest temperature (Figure 1c), owing to lower handling times and thus higher maximum feeding rates (curve asymptotes) in comparison to the intermediate and lowest temperatures at this level of salinity. However, this effect broke down at intermediate salinities (4 ppt), with FR magnitudes here peaking at the intermediate and high temperatures, given lower handling times and greater maximum feeding rates here (Table 1; Figure 1e). The effect of temperature was nullified at the highest salinity, wherein FR magnitudes were similar across temperature groups given the overlapping of 95% confidence intervals at intermediate–high prey supplies. Inversely, FR magnitudes were also not different between all three salinity levels at the lowest temperature. Attack rates (initial curve slopes) trended towards being reduced by the highest salinity levels overall, particularly under the higher temperature treatment groups (Table 1). Thus, it can be deduced that the effects of salinity on FRs are most pronounced under higher temperatures, as indicated by the greater divergence in 95% confidence intervals here across prey supplies (Figure 1).

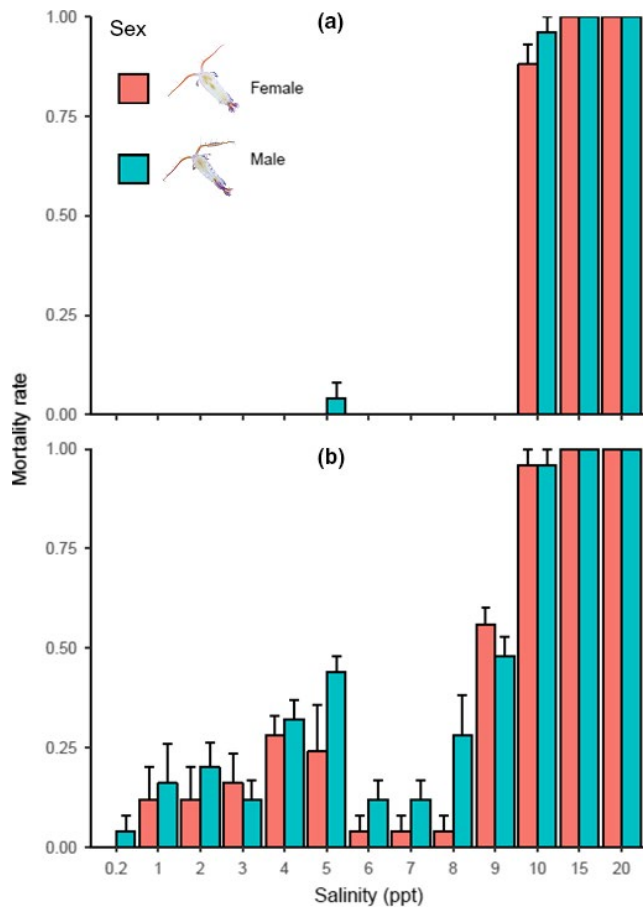
#### 3.2 | Mortality rates

At salinities above 10 ppt, all adult male and female *L. raynerae* died (Figure 2). Overall, mortality rates were thus significantly affected by the salinity gradient ( $\chi^2 = 142.36$ ,  $df = 12$ ,  $p < 0.001$ ). Mortality rates of males were significantly higher than females overall ( $\chi^2 = 5.23$ ,  $df = 1$ ,  $p = 0.02$ ) and were significantly higher following 96 hours as compared to 24 hours exposure ( $\chi^2 = 43.07$ ,  $df = 1$ ,  $p < 0.001$ ; Figure 2).

### 4 | DISCUSSION

The results of the present laboratory study demonstrate emergent complexities in the direct and indirect biotic impacts of warming and





**FIGURE 2** Mortality rates of adult female and male *Lovenula raynerae* following (a) 24-hr and (b) 96-hr exposures to different salinity levels. Means are  $\pm$  SE ( $n = 5$  per treatment group)

salinisation on ephemeral wetland specialists. Focusing on the regionally widespread and abundant model copepod species *L. raynerae*, temperature and salinity regimes interacted to significantly influence the interaction strengths upon a major prey species in these wetlands. Furthermore, the salinity tolerance of *L. raynerae* was shown to be limited to mesohaline conditions not exceeding 10 ppt.

Predation is a fundamental process which alters the functioning, structure and stability of ecosystems (Paine, 1980; Sih, Crowley, McPeck, Petranka, & Strohmeier, 1985). Although *L. raynerae* consumed *C. pipiens* mosquito prey across all temperature and salinity treatments below lethal upper limits, there were substantial variations in interaction strengths in this predator–prey system mediated by environmental context. The categorical FR form displayed by *L. raynerae* did not, however, change across temperature and salinity regimes. Type II FRs were exhibited across all treatment groups, characterised by high rates of proportional consumption at low prey densities. This contrasts to other studies (e.g. South, Welsh, Anton, Sigwart, & Dick, 2017) where variables such as temperature caused fundamental changes to FRs, for instance by inducing a shift towards a more stabilising type III form.

Functional response parameters (attack rate, handling time) did, however, differ substantially with variations in temperature and

salinity regime. Within all temperature groups, attack rates were lowest at the highest salinity level. Given attack rates correspond with predatory impact at low prey densities, higher salinity levels in ephemeral ecosystems may reduce ecological impacts of predators when prey are present at low densities. In turn, this may be more stabilising for prey populations through low density predation alleviation and, in our study system, may increase vector mosquito proliferations under certain conditions. Indeed, many larval mosquitoes are tolerant to high salinity (Patrick, Gonzalez, & Bradley, 2001). Conversely, increased temperature will be likely to exacerbate ecological impacts on prey such as mosquitoes in these systems, especially where salinity levels remain low. Such increased predation pressure could also intensify trophic cascades in ephemeral wetland ecosystems driven by predators towards other prey types, potentially reducing the longevity and population viability of lower trophic groups. In larger freshwater study systems, however, greater predation pressure has been exhibited in more brackish waters (Jeppesen et al., 1994), and increasing salinities have been shown to alter zooplankton community composition through, for instance, transitions from copepod to rotifer dominance (Jeppesen et al., 2007). However, given that ephemeral wetlands function differently to permanent ecosystems, the effects of salinisation on broader community compositions requires further investigation over the hydroperiod, as interactions cannot be reliably inferred from more permanent waterbodies. Furthermore, although paired predator–prey experiments have been shown to correspond with in-field ecological impacts (Dick et al., 2017), the context-dependency of ephemeral wetland trophic interactions requires further investigation. In particular, multiple predator effects may alter interaction strengths through antagonistic or synergistic interactions (Soluk, 1993; Wasserman, Alexander, Dalu, et al., 2016), and the presence of habitat complexities, such as vegetation, may alter predatory impact through refuge effects (Wasserman, Alexander, Weyl, et al., 2016). Nevertheless, whilst incorporations of such context-dependencies can further predictions of interaction strengths, simple laboratory experiments that do not necessarily mimic natural conditions can still yield informative and predictive comparative functional responses.

Importantly, in the present study we observed emergent complexities in the effects of temperature and salinity regime shifts on the feeding rates of *L. raynerae* towards larval mosquito prey. Whilst at the lowest salinity the highest feeding rates were found at the peak temperature regime, corresponding with the ectothermic nature of copepods observed in other aquatic ecosystems (e.g. Cuthbert, Dick, Callaghan, & Dickey, 2018), at intermediate temperatures FR magnitudes were highest at intermediate–high salinities. Contrastingly, at the highest salinity, the effects of temperature on interaction strengths were nullified, with similar maximum feeding rates across the board. These emergent effects probably emanate from differential behavioural responses across environmental change between predators and prey (Broitman, Szathmary, Mislán, Blanchette, & Helmuth, 2009; Englund et al., 2011). Thus, interaction strengths in ephemeral wetlands may be in a state of flux given the highly dynamic nature of these ecosystems with respect to abiotic context



(O'Neill & Thorp, 2014; Wasserman, Alexander, Weyl, et al., 2016), and biotic interactions will be likely to change in line with anthropogenic impacts associated with salinisation and warming. Whilst the effects of temperature on trophic interactions are often considered in isolation, we demonstrate here that salinisation may further mediate significant changes to interaction strengths that would not otherwise be constrained if temperature was considered alone. Moreover, in other study systems, the effects of salinisation have synergised with additional factors, such as higher order predation, with implications for the persistence of lower trophic groups (Hintz & Relyea, 2017; Hintz et al., 2017). Given that higher-order predation in ephemeral wetlands is often imported *via* aerial colonisation (Wasserman, Weston, et al., 2018), the effects of salinity increases on predatory impacts from external colonists such as notonectids requires assessment. Indeed, if these predatory colonists are more tolerant to salinisation than resident zooplankters, predatory impacts and cascade effects on prey groups could be intensified.

Our laboratory results show that mortality rates of adult *L. raynerae* increased sharply at salinities of 10 ppt or above. Previous research has demonstrated that hatching success of ephemeral pond specialists, and particularly branchiopods, is considerably reduced at salinity levels above 2.5 ppt (Mabidi et al., 2018) as a result of hatching cue inhibition or direct toxicity to salt in temporary wetlands (Nielsen et al., 2007; Vanschoenwinkel et al., 2010). Indeed, whilst some crustaceans continued to emerge at a salinity of 5 ppt, Mabidi et al. (2018) demonstrated negligible hatching rates for copepod and branchiopod crustaceans at a salinity of 10 ppt. Thus, our results align with those of Mabidi et al. (2018), wherein exposure to 10 ppt salinity drives near total mortality in mature zooplankton populations, irrespective of sex. However, mortality rates tended to be higher in males than females in the present study. Rapid salinity regime shifts could, accordingly, have drastic impacts on wetland biota, even inhibiting the viability of resting eggs or dormant life history stages. Indeed, loss of invertebrate diversity under salinisation has been reported in many studies (Atashbar et al., 2014; Nielsen et al., 2007; Toruan, 2012), and the loss of mature zooplankton due to the breaching of salinity thresholds may have profound implications for the reproductive success and persistence of populations under environmental change. Furthermore, in addition to direct lethal effects, salinity increases may have indirect effects that impact wetland biota across different life history stages (Hintz & Relyea, 2017), and this necessitates further examination, alongside other environmental context-dependencies.

In conclusion, using a laboratory approach, we demonstrated that the effects of warming and salinisation driven by global environmental change interact to affect invertebrate interactions in vulnerable temporary wetland ecosystems. Here, interaction strengths between species are profoundly influenced by both temperature and salinity, with high salinisation generally reducing the strength of interactions between species and offsetting temperature effects. As predation is a critical biotic process that influences the overall structure and functioning of ecosystems (e.g. Wasserman, Noyon, Avery, & Froneman, 2013), our results

suggest that the implications of environmental change for ephemeral wetland ecosystems will be marked and warming alone could intensify trophic cascade effects which characterise these wetlands. Further to implications for predator–prey dynamics, beyond a specific threshold, salinisation could directly cause mortality in specialist and endemic species in freshwater environments, with implications for species persistence within these systems. Indeed, as many ephemeral pond specialist species are internally recruited *via* dormant eggs deposited in egg banks, these species are under particular threat from ongoing climatic or land use changes. Hence, failure to properly manage multiple environmental stressors may have substantial future impacts on the conservation and integrity of ephemeral wetlands. Further research should decipher the influence of additional abiotic stressors associated with global environmental change on wetland ecosystem interactions, and link stressors across trophic groups.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA ACCESSIBILITY

Underlying raw data are available as supplementary material (Table S1).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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