



Behavioral Ecology

Chronic Road Salt Exposure Across Life Stages and The Interactive Effects of Warming and Salinity in a Semiaquatic Insect

Vicki M. Zhang,^{1,2} Rosemary L. Martin,^{1,2} and Rosalind L. Murray^{1,2,3,◉}

¹Department of Biology, University of Toronto Mississauga, 3359 Mississauga Rd. L5L 1C6, Mississauga, Ontario, Canada, ²Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks St. M5S 3B2, Toronto, Ontario, Canada, and ³Corresponding author, e-mail: rosalind.murray@utoronto.ca

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Abstract

The salinization of freshwater habitats from winter road salt application is a growing concern. Understanding how taxa exposed to road salt run-off respond to this salinity exposure across life history transitions will be important for predicting the impacts of increasing salinity. We show that *Leucorrhinia intacta* Hagen, 1861 (Odonata: Libellulidae) dragonflies are robust to environmentally relevant levels of salt pollution across intrinsically stressful life history transitions (hatching, growth, and metamorphosis). Additionally, we observed no carry-over effects into adult dragonfly morphology. However, in a multiple-stressor setting, we see negative interactive effects of warming and salinity on activity, and we found that chronically warmed dragonfly larvae consumed fewer mosquitoes. Despite showing relatively high tolerance to salinity individually, we expect that decreased dragonfly performance in multiple-stressor environments could limit dragonflies' contribution to ecosystem services such as mosquito pest control in urban freshwater environments.

Key words: salinization, warming, multiple stressor, life history, road salt

The salinization of freshwater ecosystems is a growing global threat to freshwater ecosystems (Findlay and Kelly 2011, Cañedo-Argüelles et al. 2016, Kaushal et al. 2018). In cold climates, deicing road salts applied during the winter months are a major contributor to the salinization of aquatic environments (Hintz and Relyea 2019). The expansion of cities in recent decades has exacerbated salinity damage to freshwater environments (Bester et al. 2006); urbanization is often correlated with increases in impermeable land cover (e.g. paved roads), which is associated with more salt pollutants entering adjacent freshwater systems via stormwater and snowmelt runoff in cold climates (Bartlett et al. 2012, Schuler and Relyea 2018). The amount of salt applied to impermeable surfaces has increased dramatically from 0.2 million metric tons in 1944 to 24.5 million metric tons in 2014 (Harris and Tucker 1947, Bolen 2016). The increased road-associated salts moving into freshwater habitats cause both acute salinity spikes during winter thaw and spring melt events, and chronically elevated salt levels year-round (Todd and Kaltenecker 2012, Cañedo-Argüelles et al. 2013).

Vulnerability to salinity stress can vary across development in freshwater organisms (e.g. Welch et al. 2019) consequently resulting in individual or population level responses, and depending on timing of exposure and timing of vulnerable life history stages. We currently know little about how different life stages of many aquatic invertebrates respond to salinization or how these stage-specific effects could impact freshwater communities. In particular, there has been little research on how important freshwater predators (e.g. many dragonfly species) respond to increased salt stress throughout their life history. For many semi-aquatic animals, transitional periods including hatching, molting, and metamorphosis are intrinsically stressful. These life history transitions may be even more difficult for animals developing within an environment with extrinsic stressors, such as salt pollution, decreasing their individual performance, or leading to increased mortality and population declines (e.g. Metcalfe and Monaghan 2001, McCauley et al. 2011, Welch et al. 2019).

For individuals that successfully transition to adulthood, the initial period following metamorphosis can be a vulnerable time; even under ideal conditions many newly emerged adult insects are

particularly at risk (Lowe et al. 2021). We currently know little about the sub-lethal carry-over effects of juvenile aquatic salinity pollution exposure for terrestrial adult dragonflies, including how salinity affects important indicators of individual and population success such as size dimorphism, body size, and phenology. Age and size at key life history transitions, such as metamorphosis, are a component of fitness (e.g. Werner 1986, Rowe and Ludwig 1991) and extrinsic stressors on these crucial transitions can be detrimental (McCauley et al. 2011), affecting population success and consequently species interactions and community structure.

The salinity stress freshwater organisms currently face occurs simultaneously with other environmental stressors such as climate change. Synergies between stressors, are predicted to exacerbate the effects of climate warming on freshwater taxa (Canedo-Arguelles et al. 2018, Jeppesen et al. 2020). Climate change is predicted to increase precipitation in many temperate regions (IPCC 2014), which will likely necessitate increased use of de-icing road salt during winter months. Additionally, organisms may be exposed to increasing heat stress as a consequence of climate warming during crucial growing periods, affecting life history timing and species interactions (Frances and McCauley 2018). While we know that climate change and salinity pollution occur simultaneously, we do not know how these anthropogenic stressors will interact to affect freshwater communities, particularly in high human density areas where they are most likely to be problematic.

Dragonflies (Odonata) are an ideal group in which to study the sub-lethal ecological effects of road salt pollutants; their ionic regulatory system allows many odonate species to survive environmentally-relevant levels of salinity (e.g. Nicholls 1983), but they may nonetheless experience negative physiological effects (Mangahas et al. 2019). Additionally, odonates are useful bioindicators of wetland ecosystems (Villalobos-Jimenez et al. 2016), and the effects of salinization on their performance may have profound consequences for both freshwater and terrestrial communities. Dragonflies have a biphasic life cycle and perform essential ecosystem services in both the aquatic (larval) and the terrestrial (adult) habitats (Benke 1976). Larval dragonflies are important predators that can shape aquatic communities (McPeck 1998), and act as keystone predators in some systems (Knight et al. 2005), while terrestrial adult dragonflies are an important prey resource for many birds and fish (Benke 1976). Dragonflies also provide ecosystem services that benefit humans, including being voracious predators of pest/disease-vector species, such as mosquitoes, in both their larval and adult forms (e.g. Quiroz-Martinez and Rodriguez-Castro 2007, Combes et al. 2013). In light of these key ecological functions, dragonflies can be used to indicate the health of both aquatic and terrestrial environments (e.g. Dolny et al. 2012), and therefore their response to the impacts of increasing salinity can be broadly applied to understanding the ecological impacts of road salts. Despite this, few studies have specifically examined the effect of road salt pollutants on the life stages and behaviors of dragonflies.

Additionally, many previous studies have focused on the effect of a single stressor on a single life stage of a species. This approach has yielded valuable insights, but likely underestimates total effects in natural systems where animals encounter multiple stressors simultaneously and often for prolonged periods across multiple life stages. We had two research questions. First, how does salinity pollution affect a dragonfly species (*Leucorrhinia intacta*) across multiple intrinsically stressful life history stages (hatching, larval growth, metamorphosis)? This study allows us to determine whether there are vulnerable stages or whether the life history transitions themselves affect the response to salinization. We predict that hatching,

molting, and metamorphosis are vulnerable life history transitions that may be affected by salinization. Second, how are key behaviors, such as activity and foraging, that have strong effects on individual and population performance, affected in a multiple stressor (salt and temperature) environment? We hypothesize that multiple stressors will interact to reduce these key behaviors more than single stressors (salt or temperature).

Methods

For all experiments, saltwater was prepared by dissolving Windsor Safe-T-Salt deicing salt, primarily composed of NaCl, in de-chlorinated tap water, to create four salinity treatments: control (aged tap water +0 g salt), low (+1 g/L salt; 1.97 mS/cm), medium (+3 g/L salt; 5.558 mS/cm), and high (+4.5 g/L salt; 8.121 mS/cm). These concentrations were selected as they represent the range of deicing salt concentration seen in urban freshwater habitats (Environment Canada and Health Canada 2001). While we acknowledge that these salinity values are high relative to many aquatic environments (Hintz et al. 2022, Lawson and Jackson 2021), for urban areas (including stormwater management ponds) where many dragonfly species reside, these salinities are well within the values we, and others, have observed (Van Meter et al. 2011). Because our experiments all used aged tap water, which contains dissolved ions, we also measured conductivity in our tap water at 0.372 mS/cm (or 0.179 g/L). To replenish water due to evaporation during all assays, RO water was added to each individual cup as needed. All communally kept larval tanks contained window screening to mimic natural habitat structure and aquaria bubblers.

Specimen Collections

Leucorrhinia intacta (Odonata: Libellulidae) dragonflies were collected at two different life stages in two locations for this study. Larval *L. intacta* were collected from the Koffler Scientific Reserve (KSR), Ontario, Canada (44°01'46.1"N 79°31'44.1"W) in May 2019 and August 2020. Collected larvae in 2019 ($n = 165$) were transferred to the lab and fed damselfly larvae, mosquito larvae, and other aquatic arthropods *ad libitum*. These larvae were kept individually in 350ml aged tap water in the lab at KSR and randomly assigned to each of the four salt treatments: control (aged tap water +0 g salt), +1 g/L salt, +3 g/L salt, and +4.5 g/L salt. To study hatching success, adult *L. intacta* females were collected using aerial sweep nets in Palgrave, Canada during June 2019 (43°57'09.5"N 79°51'17.1"W). Females were caught after observing mating in flight to ensure that eggs were fertilized. Captured females had their abdomens dipped in a vial lined with filter paper and filled with local pond water, causing them to release eggs. Following collection, vials were left for 24 h to give eggs time to become sclerotized and adhere to the filter paper. For behavioral assays, the collected larvae in August 2020 were transferred to the lab at the University of Toronto Mississauga. Larvae were kept communally in 10-gallon aquaria in one of three salt treatments (aged tap water +0 g/L salt (control), +1 g/L salt, or +3 g/L salt) until the start of experiments. Larvae used for the foraging assay were starved, while larvae used for the activity assay were fed zooplankton and mosquito larvae *ad libitum* for a week prior.

Growth Rate and Emergence Success

Dragonflies at the larval stage were collected in May 2019 and kept individually and placed in front of windows on a lab bench at KSR. Control and saline treatments were prepared in bulk, and individual specimen cups (white Solo® Cups) were filled to approximately

350 mL. The lab was temperature-controlled ($22\pm 1^\circ\text{C}$) and the window allowed for natural light cues to be used rather than artificial light (Supp. Fig. S1). Individual head widths (a good proxy for body size in odonates; Corbet 2002) were measured every three days using digital calipers (accuracy ± 0.1 mm). Each individual container had a small strip of window screening to simulate habitat structure, a 30 cm wooden dowel to facilitate metamorphosis, and was encased in a mesh bag to prevent emerging adults from escaping. Larvae were monitored daily for metamorphosis for a maximum of 16 weeks. Successfully emerged adult dragonflies were measured for body size (head width and mass), and their sex and date of emergence were recorded.

Hatching Success

Eggs collected in June 2019 became sclerotized to filter paper, and filter paper was cut so that five eggs were transferred to 100ml experimental containers (Supp. Fig. S1) with one of four salt treatments: control (aged tap water +0 g salt), +1 g/L salt, +3 g/L salt or +4.5 g/L salt ($N = 40$ trials of five eggs for each treatment, resulting in 800 eggs). Eggs were monitored daily for hatching success for a total of 2 wks. We recorded the number of successful hatches per container (five possible) and date.

Foraging and Activity Assays in Salinity and Warming

Larvae collected from KSR in August 2020 were randomly assigned to one of three salt treatments (control (+0 g/L), 1 g/L, or 3 g/L). Larvae were kept in six 5-gallon tanks for each treatment with bubblers and lengths of window screen added to increase habitat complexity and reduce cannibalism (Van Buskirk 1989). Thirty larvae from each salt treatment were randomly selected and equally divided between heat treatment aquaria with the same salinity as the acclimation tank they had come from. Half of the aquaria (two per salt treatment) were maintained at 22°C (control) and half at 28°C (warmed treatment) using aquarium heaters (Visi-Therm Deluxe) so that there were six treatments total: three salinities by two temperatures. The temperature treatments are based on work that included the effects of warming on *L. intacta* (Frances et al. 2017, Frances and McCauley 2018). Both temperatures are within the natural seasonal range for Ontario-dwelling *L. intacta* larvae, however, 28°C is warm for the time of year that the experiments were conducted. Because there is variation in thermometer accuracy, we performed periodic point measurements of temperature throughout the experiment to ensure the tanks remained within $\pm 2^\circ\text{C}$ of the set temperature. The bubblers allowed sufficient water movement within the tanks so that no point estimate was recorded outside the set temperature $\pm 2^\circ\text{C}$. Larvae from all treatments were starved for one week in preparation for the foraging assays. The same procedure was followed in preparation for the activity assays (thirty larvae per salt treatment separated into control or warming treatments), however, larvae assigned to the activity assay were fed *ad libitum* for the week prior to the assay.

Activity assay—Individual dragonfly larvae were transferred to two-liter experimental aquaria containing the same treatment conditions (salinity and temperature) 2 h before the start of a trial. During observations, we recorded each individual every 5 min for the 105-min duration of the trial. Larval activity was measured by quantifying larval movement distance and movement frequency based on the methods originally described in (Johansson 2000). Briefly, aquaria were divided into 16 cells of equal size—an eight-block grid (four up and four down) was placed on the back of the aquarium and a line

on the side of the aquarium equally partitioned the front and back of the tank (Supp. Fig. S1). We placed the heater in the same position in all trials as well as a piece of mesh for larvae to hide in. We recorded a larva as having moved if it was found in a different cell in subsequent observations. Movement distance was recorded as the minimum number of blocks a larva could have traveled to reach its current position. We also recorded whether larvae were associated with habitat structure (mesh or heater) during each observation and whether they were static or actively moving during each observation.

Foraging assay—Fifteen fourth instar *Culicidae sp.* mosquito larvae were collected the day of the foraging experiment and placed in the two-liter salinity-temperature experimental treatments for 1 h prior to the addition of a starved dragonfly larva. Individual dragonfly larvae were added to mosquito-filled experimental two liter tanks with the same temperature and salinity conditions to which they had acclimated in the previous 2 wks. Individual dragonflies were left to consume mosquitoes for 90 min, and we recorded the number of remaining mosquitoes at the end of the assay.

Statistical Analysis

All analyses were conducted in R (R Core Development Team 2014) and models were fit using the lme4 package (Bates et al. 2015) unless otherwise specified. For each model when a Gaussian (default) distribution was used, we first tested our assumption of normality using the 'shapiro.test' function.

Hatching

To analyze differences in hatching success in response to salinity, we fit a generalized linear mixed-effects models (GLMER) with a Poisson error distribution. We used hatching count (i.e. a count of the successfully hatched larvae per vial) as the response variable, salinity treatment as a fixed effect and clutch as a random effect (where clutch refers to the collection event from a single female dragonfly).

Growth Rate

Dragonfly juvenile growth occurs in a step-wise progression through a series of molting events rather than continuous growth throughout the larval period. Because of this larval growth pattern, we cannot fit classic growth rate curves (e.g. Gompertz, logistic) to the step-wise growth data for individual or grouped *L. intacta* larvae. Therefore, to estimate differences in growth rates due to road salt exposure, we calculated the maximum growth rate in each salinity treatment as the largest mean change between two time points. Additionally, we fit a repeated measures ANOVA to measure an effect of salinity treatment on individual growth rate.

Body Size

We fit a linear model for each metric of body size measured to test for an effect of salinity treatment on adult body size. The model was fit with a Gaussian error distribution, body size (adult head width or the cubed root of adult mass) as a response, and salinity treatment and sex as predictor variables.

Emergence Success

To test for an effect of salinity exposure on emergence success, we fit a binomial generalized linear model (GLM) with emergence success/failure as the binomial response variable, and salinity treatment as a fixed effect. To test for an effect of salinity on phenology, we fit a linear model with the emergence date as a response, salinity treatment as a fixed effect, and a Gaussian error distribution. Next, we performed chi-square tests of the sex ratio of successfully

emerged adults to test for sex differences in the effect of salinity on emergence success. We also tested for an effect of salinity treatment on sexual dimorphism in emergence time (phenology); we fit a model with emergence date as a response and sex and treatment as fixed effects.

Activity

To test for an effect of salinity and temperature on the total movement (distance traveled during observation trial) of larval *L. intacta*, we used the 'pscl' package (Zeileis et al. 2008) in R to fit a zero-inflated Poisson model (to account for overdispersion associated with individuals that had a movement distance of zero). We tested for zero inflation in our data using the 'performance' package in R with a tolerance of 0.05 (Lüdecke et al. 2021). Our model included the total movement distance as the response, salt, temperature, and their interaction, and individual head width as the fixed effects. Similarly, to test for an effect of microhabitat use (time spent associating with structure), we fit a GLM with a binomial error distribution that included salt, temperature, and their interaction as fixed effects.

Foraging

To test for an effect of salinity and temperature on the foraging of larval *L. intacta*, we fit a general linear model with a Poisson error distribution that included the total movement distance as

the response, and salt, temperature, and individual head width (known to correlate with foraging effort; Wissinger 1992) as the fixed effects.

Results

Hatching Success

All salt treatments had high hatching success (proportion successfully hatched ranged between 82% and 88%; Table 1). We found no effect of salinity treatment on hatching success (results from Poisson GLMER with clutch as a random effect: $P > 0.5$ for all treatments; Supp. Table S1).

Growth Rate and Emergence Success

There was no difference in larval growth rate across salinity treatments as measured by a repeated measures ANOVA ($F_{3,159} = 0.148$, $P = 0.931$) or maximum growth rate estimates (Table 1; Supp. Fig. S2). We also found no effect of salt treatment on dragonfly emergence success (Table 1; Supp. Table S2), nor did we see an effect of salt pollution on the proportion of males and females emerging as adults (chi-square results for sex ratios of emerging adults $p > 0.6$ for all salinity treatments). There was no effect of salinity or sex on emergence phenology (Table 1; Supp. Table S3). We found no effect of salinity treatment on the size of adult dragonflies (Supp. Table S4), but we did find that despite no sexual dimorphism in adult mass at

Table 1. Summary statistics of the effect of salinity pollution on *Leucorrhinia intacta* dragonflies across multiple life history stages

Salinity treatment	max growth rate (mean mm/day +/- SE)	emergence success (mean proportion success and 95% binomial CI)	hatching success (mean proportion successful and 95% binomial CI)	sexual dimorphism in emergence phenology (mean date of emergence +/- SE)
0 g/L	0.040 ± 0.014	0.79 (0.65–0.89)	0.84 (0.78–0.88)	F: 159.00 ± 3.35 M: 162.95 ± 3.65
1 g/L	0.037 ± 0.017	0.92 (0.79–0.97)	0.82 (0.76–0.87)	F: 164.63 ± 2.68 M: 164.00 ± 3.57
3 g/L	0.040 ± 0.009	0.92 (0.79–0.97)	0.83 (0.77–0.88)	F: 167.47 ± 3.65 M: 165.07 ± 3.16
4.5 g/L	0.057 ± 0.027	0.68 (0.43–0.86)	0.88 (0.82–0.92)	F: 158.62 ± 2.96 M: 166.60 ± 4.55

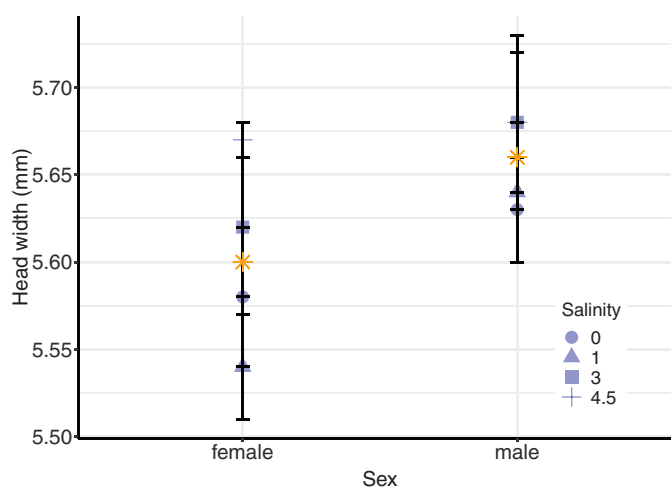


Fig. 1. The effect of juvenile road salt pollution on adult body size in *Leucorrhinia intacta* dragonflies. Salinity is measured as g/L road salt added to aged tap water; error bars represent standard error. The stars represent the mean male and female sizes at emergence; error bars for overall means have been excluded for clarity. We found that males have significantly larger head widths than females at emergence (Supp. Table S4). Note: mean head widths for males from the 3 g/L and 4.5 g/L salinity treatments are both 5.68mm and exactly overlapping.

emergence (Supp. Table S4), males had larger head widths than females ($P = 0.021$; Supp. Table S4; Fig. 1).

Larval Behavior in Response to Salinity and Temperature Stressors

The results of our zero-inflated Poisson model showed that while there was no significant effect of salinity or temperature warming independently on activity, we do see a negative interactive effect of the two stressors; increased salinity caused a decrease in larval movement with a steeper decline in low temperature individuals compared to high temperature individuals (Table 2; Fig. 2A). We found no difference in microhabitat use across warming and salt treatments ($P > 0.1$ for all treatments).

We found a significant effect of temperature on foraging; larvae that had been held in warmed conditions ate significantly fewer mosquito larvae after controlling for body size. We found no effect of salinity or the interaction of salinity and warming on *L. intacta* foraging success (Fig. 2B; Table 3).

Discussion

We investigated the effect of road salt pollution across multiple intrinsically stressful life history stages and measured the interactive effects of warming and salinity on behavioral traits critical to individual and population success (and which affect dragonfly predation on pest species). We found that salinity and increased temperatures interact to decrease activity in larval dragonflies and that warming alone depressed dragonfly predation on mosquitoes. Below, we discuss our results in the context of extrinsic stressors, such as climate warming and road salt pollution, in urban habitats and how differential responses to varying levels of stress at specific trophic levels can influence population health, community assemblages, and ecosystem services.

Road Salt Pollution Does Not Affect *L. intacta* Development or Life History

We did not see an effect of road salt pollution across intrinsically stressful life history stages in dragonfly ontogeny. Using environmentally-relevant levels of salinity pollution, we found no effect of salt on hatching success, larval growth rate, metamorphic success or adult

Table 2. The interactive effect of salinity and warming on *Leucorrhinia intacta* larval movement (measured as the summed total distance travelled during a 105 min trial)

	Estimate	Std error	z	P
<i>Count model (Poisson)</i>				
intercept	2.27	0.56	4.06	<0.001
salt	-0.09	0.06	-1.55	0.28
temperature	0.37	0.12	3.02	0.19
salt*temperature	-0.36	0.09	-4.09	0.03
head width	-0.05	0.12	-0.43	0.73
<i>Zero-inflation model (binomial)</i>				
intercept	-1.92	3.76	-0.51	0.61
salt	0.09	0.32	0.31	0.75
temperature	-1.37	1.03	-1.33	0.19
salt*temperature	0.80	0.50	1.62	0.10
head width	0.16	0.81	0.20	0.84

Results are from a Zero-inflated Poisson model with total moves as a response, salt, temperature and their interaction, and individual head width as fixed effects. Significant result in bold.

body size (Table 1; Supp. Tables S1–S4). Similarly, we did not find an effect of salinity stress on *L. intacta* emergence phenology (Table 1). Our findings suggest that while these (and even lower) levels of salinity are known to cause catastrophic impacts on some freshwater fauna (e.g. amphibians (Sanzo and Hecnar 2006), zooplankton (Arnott et al. 2020), and some macroinvertebrates (Delaune et al. 2021)), at least one dragonfly species appears insensitive to environmentally relevant levels of salinity exposure during their larval period. In contrast, other studies have found evidence of sublethal effects of high levels of salinity during the larval stage, including a decrease in larval growth rate (Herbst et al. 2013), and a decline in odonate emergence success at high salinity (Lambret et al. 2021). While the dragonflies in our study suffered no detectable changes in any of the important developmental parameters measured, our study was conducted in a controlled laboratory setting; abiotic conditions were stable and by keeping dragonflies individually we eliminated the potential stress of competition and cannibalism (Murray et al. 2020). Several other stressors that have been shown to cause changes to ontogeny and developmental success in dragonfly larvae include predation (McCauley et al. 2011) and warming (Guo et al. 2009). It will be important to test for interactive effects of salinity and other common stressors (e.g. Castano-Sanchez et al. 2020, Orr and Buchwalter 2020) going forward to better understand how sublethal effects might change salt-affected freshwater habitats. Additionally, comparative studies will be vital as not all dragonflies are likely to respond in the same ways, creating potential for ‘winners and losers’ in urban contexts, similar to what has been observed in response to warming. The species used in this study, *L. intacta*, is a habitat generalist making it likely to have broad environmental tolerances (Frances and McCauley 2018).

Adult Body Size Was not Influenced by Larval Road Salt Exposure

We did not see a carry-over effect of larval salt pollution on the size of successfully emerging adult dragonflies. Male head widths were larger at emergence than females, but the sexes did not differ in their wet mass (Supp. Table S4). Adult body size in odonates is positively related to fitness for both sexes (e.g. Sokolovska et al. 2000). Head size is a fixed (sclerotized) trait at the time of emergence, while mass changes as adults become fully mature (Anholt et al. 1991). *L. intacta* adults are sexually size dimorphic (SSD) as measured by mass once they are fully matured, but teneral (young adult) dragonflies are not typically SSD (Anholt et al. 1991). In *L. intacta*, females are eventually heavier than males; abdominal mass in females increases by 400% during the teneral phase (attributed to ovarian mass gain), while males increase abdominal mass by ~50% (both sexes show similar mass gains for the thorax; see Table 2 from Anholt et al. 1991). By assessing SSD in early adulthood, we may be missing some of the differential effects of salinity pollution on males and females. In particular, sublethal carryover effects due to salinity pollution might disproportionately affect females if teneral foraging ability is affected and emerging females are proportionally smaller than males.

Salinity and Warming can Interact to Affect *L. intacta* Behavior

Activity

We did not see individual effects of either warming or salinity, however, we did find a significant interaction; increasing salinity showed a trend of decreased activity and a stronger negative slope in the low temperature treatment compared to the higher

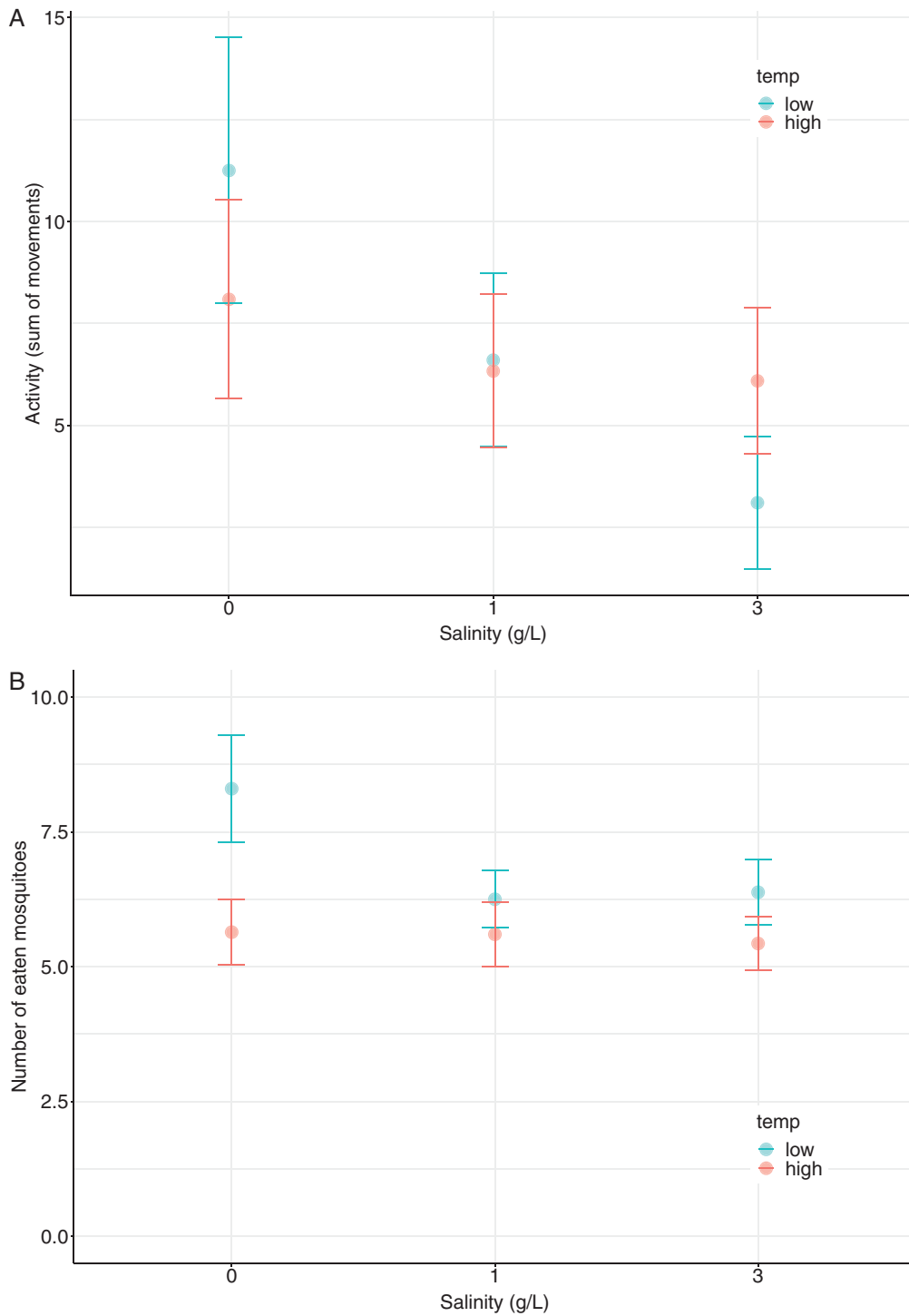


Fig. 2. Larval *Leucorrhinia intacta* activity (measured as mean total distance moved during a trial; A) and mosquito foraging success (B) in response to salt and warming treatments. Larval activity decreased with the interactive effects of warming and salinity (see Table 2), while larval foraging decreased in response to warming, but salinity had no significant effect (see Table 3). ‘Low’ temperature is 22°C; ‘high’ temperature is 28°C. Error bars represent standard error.

temperature treatment (Fig. 2A). Invertebrate salinity tolerances vary considerably but we know relatively little about how salinity tolerance changes during exposure to other, simultaneous stressors. Previous studies in other insect species found that the interactive effects of salinity and temperature can exacerbate the effects of these individual stressors (e.g. Jackson and Funk 2019). Seasonal variation in temperatures, as well as freeze-thaw events, can affect levels of toxicity experienced by freshwater taxa (see

Fig. 4 from Jackson and Funk 2019) with lower temperatures in the late winter/early spring generally correlating with decreased vulnerability to salt toxicity. Our findings differ from the broad patterns observed in Jackson and Funk (2019); we found the greatest suppression of activity in the combined low temperature (22°C), high salinity treatment (Fig. 2A), however, Jackson and Funk (2019) tested insects during freeze-thaw temperatures near freezing, which might account for observed differences.

Table 3. Results from a mixed effects Poisson model of the effect of warming and salinity on foraging success in *Leucorrhinia intacta* larval dragonflies consuming *Culicidae* sp. mosquito larvae

	Estimate	Std error	z	P
intercept	1.73	0.10	17.5	<0.001
salt	-0.01	0.05	-0.25	0.80
temperature	0.32	0.14	2.28	0.02
salt*temperature	-0.06	0.07	-0.855	0.39

Dragonfly larval head width was fit as a random effect. Significant result in bold.

Activity level can be an important indicator of prey encounter rates (Werner and Anholt 1993) and decreased activity in multiple-stressor environments could limit foraging success for *L. intacta* larvae. Our results are consistent with previous studies investigating how climate warming affects activity in *L. intacta*; Frances and McCauley (2018) showed that despite predictions for increased activity in warmer temperatures, there is no increase in activity for the temperatures measured here. At higher temperatures (32°C) however, *L. intacta* dragonfly larvae do show increased activity, which is consistent with predictions and patterns observed in other taxa (e.g. Gannon et al. 2014). Dragonflies seem relatively robust to many environmental stressors (Rosset and Oertli 2011), but our results suggest there may be sublethal effects that emerge in a more realistic environment.

Foraging

We did not see an effect of salinity on foraging behavior in larval dragonflies, nor did we see the same significant interactive effect as in activity. This contrasts with other studies investigating prey consumption by odonates, which found that feeding rate decreased at high salinities (Herbst et al. 2013), although these differing results may be due to the differences in methodology (feeding rate does not correspond exactly with foraging behavior). We did find, however, that warming contributes to decreased foraging success (Fig. 2B). Previous studies have shown that warming generally increases insect feeding rates on multiple prey types (e.g. Vucic-Pestic et al. 2011, Frances and McCauley 2018), however, *L. intacta* have been shown to maintain their foraging rate in response to a temperature increase from 24°C to 28°C (Fig. 1a from Frances and McCauley 2018). Our experimental set up differs from Frances and McCauley (2018) in how long the larvae were exposed to the warming treatment (16 h acclimation (Frances and McCauley 2018) compared with 2 wks in this study) leading up to the foraging experiment. It's possible that longer periods of increased temperatures (e.g. heat waves) might pose a larger threat (e.g. Colinet et al. 2015), and future work investigating chronically warmed taxa might be important for understanding and predicting the consequences of warming climates in littoral communities.

We did not measure larval mosquito behavior in this study, however previous work has shown that mosquitoes can be affected by some salts and ice melt additives (Schuler and Relyea 2018). Additionally, some species of mosquito larvae have been shown to change their behavior in response to predator presence (Kesavaraju et al. 2007) and predation by mosquitofish (*Gambusia holbrooki*) has been shown to increase with temperature (Grigaltchik et al. 2016). We found the opposite response to temperature—fewer mosquito larvae were consumed in the high temperature treatment at low salinity (Fig. 2). While it is difficult to compare across

experiments, this discrepancy is likely due to differences in predator behavior between dragonflies and mosquitofish. Finally, evidence of mosquito larvae behavior in response to salinity is lacking, however, freshwater mosquitoes are generally robust to salinity levels well beyond the concentrations used in this experiment (e.g. Clark et al. 2004). Temperature and salinity influences on mosquito larval activity would be excellent future research directions.

While we found different significant effects for our two behavioral assays, the trends of increased stress via increased salinity and temperature resulting in the decrease of certain aspects of behavior are consistent. In natural settings (i.e. with more and varied stressors), small effect changes to foraging efficiency can translate into larger scale changes to habitat use and community structure (e.g. Werner and Hall 1979). Future work should consider the effects of salinity and temperature stress in a more natural setting (e.g. a mesocosm study) that could incorporate and measure community level changes.

Concluding Ideas

Individual responses to environmental stressors are expected to be variable among taxa within a community, and, in this respect, freshwater habitats affected by salinity pollution are no different. Here, we have shown that at least one species of dragonfly is tolerant to environmentally relevant levels of salinity across multiple juvenile life history stages. However, we know that many of the dragonflies' predator, competitor, and prey species are more sensitive to salinity and that there is likely to be variation in sensitivity between dragonfly species. Because dragonfly larvae can be top predators and shape aquatic communities, in future studies it will be important to measure their road salt tolerance in a more realistic field setting to understand how tolerant dragonfly species, as 'winners' of road salt pollution, contribute to ongoing ecosystem services offered by freshwater environments.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

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Data Availability

Data will be made available in the Dryad repository.

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