Pond drying cues promote cannibalism in larval *Anax junius* dragonflies

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Abstract: Global climate change is expected to shorten hydroperiods and accelerate drying of ephemeral freshwater habitats, a shift that is likely to increase intraspecific competition and cannibalism in the aquatic animals that rely on those habitats. We experimentally examined the effects of simulated pond drying, tank size (initial larval density), and body size on survival and cannibalism in larvae of the dragonfly *Anax junius*, a species known to show frequent size-structured cannibalism. Thirty tanks of 3 different sizes were each stocked with 8 *A. junius* larvae (6 small, 1 medium, and 1 large) along with *Enallagma* damselfly larvae as prey. *Anax junius* survival and cannibalism were documented daily for 16 d. For tanks in the permanent hydroperiod treatment, we maintained water depth at a constant 14 cm for all 16 d, while we gradually reduced depth in the temporary hydroperiod tanks from 14 to 2 cm to simulate pond drying. We found that cannibalism was strongly size-dependent, as 31, 7, and 0% of small, medium, and large larvae, respectively, were cannibalized. Tank size (initial larval density) and hydroperiod treatment both affected larval survival and cannibalism. However, the effects of simulated pond drying were more pronounced than those of tank size. In addition, hydroperiod treatment was a predictor of daily risk of larval cannibalism, but daily volumetric larval density (number of *A. junius* alive divided by water volume present that day) was not. Our results, therefore, indicate that 1) pond drying can substantially increase cannibalism in larval odonates beyond its simple effect of producing high-density populations as water levels recede and 2) the effect of drying cues on the behavior and life-history characteristics of aquatic invertebrates merit increased attention from freshwater ecologists.
Keywords: cannibalism, Odonata, dragonflies, climate change, hydroperiod, pond drying, density, body size

Shallow and temporary aquatic habitats, particularly those at high latitudes, at high elevations, and in arid and semi-arid environments, are likely to experience dramatic changes in their hydrological regimes as a result of climate change. These changes are expected to include earlier and reduced snowmelt, shorter hydroperiods, increased water temperatures and evaporation, and earlier and more rapid drying (Smol and Douglas 2007, Brooks 2009, Rosset and Oertli 2011, Sim et al. 2013, Tuytens et al. 2014, Lund et al. 2016). Such changes are likely to have profound impacts on the subset of species that are adapted to complete their life cycles in these temporary habitats (Wellborn et al. 1996, Williams 2006, Strachan et al. 2014). Under climate change, species that rely on these habitats are faced with the challenge of adjusting their complex suite of behavioral, ecological, and life-history adaptations to survive and reproduce under novel and rapidly-changing conditions.

One of the behavioral responses likely to increase under these expected climatic and hydrological shifts is cannibalism. Aquatic organisms that live in ephemeral habitats or are otherwise under significant time constraints to complete development as rapidly as possible frequently cannibalize smaller conspecifics, particularly in strongly size-structured populations (Hopper et al. 1996, Johansson and Rowe 1999, De Block and Stoks 2004a, b, de Roos and Persson 2013, Lund et al. 2016). Cannibalism has numerous potential costs, including elevated risk of disease transmission or injury (Elgar and Crespi 1992). However, cannibals also have the opportunity to enhance their fitness under deteriorating environmental conditions by acquiring high-protein supplemental food, accelerating their growth and development, and eliminating competitors for limited resources (Hopper et al. 1996, Johansson and Rowe 1999, De Block and Stoks 2004a, b, Lund et al. 2016).

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Furthermore, recent studies have demonstrated that both increasing water temperatures (Crumrine 2010, Start et al. 2017, Sniegula et al. 2019) and declining water levels (Lund et al. 2016) can provoke intraspecific aggression and cannibalism in aquatic organisms, thereby establishing an explicit link between some of the expected consequences of climate change and cannibalism.

A group of aquatic insects in which cannibalism occurs frequently is the order Odonata, dragonflies and damselflies. Odonates begin their life cycle as aquatic larvae that often rely on shallow or temporary freshwater habitats. Because shallow ponds at temperate latitudes are vulnerable to winter fish kill and, thus, like temporary ponds, are often fishless, large-bodied dragonfly larvae frequently attain the position of top predator in these habitats, playing a significant role in the structure of food webs and in overall ecosystem function (Wellborn et al. 1996, McPeek 1998, Rudolf and Rasmussen 2013). The complex seasonal breeding and emergence patterns of many dragonfly species often result in overlapping larval cohorts, causing a broad size distribution between the youngest and oldest larvae (Wissinger 1988a, b). Odonate larvae rarely exhibit aggressive behaviors toward similarly-sized individuals under normal conditions, but smaller larvae are commonly subjected to cannibalism by larger conspecifics (Crumrine 2010). Overall, cannibalism can be responsible for up to 95% of the total larval mortality of many species, making it a primary regulating mechanism of odonate population densities (Anholt 1994).

Drying cues might be expected to trigger increased cannibalism in larval odonates because previous research on the taxon has shown that other indicators of deteriorating environmental conditions, such as short day lengths, decreasing food supply, and high larval densities, can provoke a variety of behavioral and life-history responses, including increased cannibalism (van Buskirk 1989, Hopper et al. 1996, Johansson and Rowe 1999, De Block and Stoks 2004a, b), heightened larval activity (Johansson and Rowe 1999), and accelerated development and early maturation (Johansson and Rowe 1999, De Block and Stoks 2004a, b). However, only 1 previous study has experimentally investigated the response of larval odonates to simulated pond drying, and its primary focus was the effect of drying cues on life-history traits, not cannibalism (De Block and Stoks 2005). In addition, that study produced somewhat ambiguous results because the effects of pond drying were partially confounded with the effects of increased density and reduced food supply (De Block and Stoks 2005), a common problem faced by experimental investigations of simulated pond drying (Lund et al. 2016).

The purpose of our study was to experimentally examine the interacting effects of simulated pond drying and larval density on cannibalism in size-structured microcosm populations of larvae of the dragonfly *Anax junius* (Drury 1773), a species known to show frequent size-structured cannibalism (Crumrine 2010). We were particularly interested in testing 2 specific hypotheses: 1) that drying cues would promote cannibalism in *A. junius*, and 2) that the increase in cannibalism in response to simulated drying would exceed what would be predicted from changes in larval density alone. To our knowledge, no previous study has experimentally investigated the relationship between drying cues and cannibalism in odonates or whether any such relationship is mediated by changes in water level per se or by the increases in larval density that are typically associated with pond drying.

METHODS

Microcosm setup and *A. junius* collection

The experimental setup included an array of 30 microcosm aquaria, 10 each of 3 different sizes: large (50 × 25 cm), medium (40 × 20 cm), and small (27 × 17 cm). All aquaria were housed under room temperature and natural photoperiod in the aquatics laboratory at Allegheny College, Crawford County, Pennsylvania, USA. To simulate the 3-dimensional structure of wetlands with emergent vegetation and provide vertical foraging substrate for the *A. junius* larvae, we fabricated vertical perches from wooden dowels (15 × 2 mm) and attached them with aquarium cement to the bottoms of the 30 aquaria. Each large aquarium had 3 rows of 4 evenly-spaced perches (12 perches total, 96/m^2), while the medium and small aquaria, respectively, had 2 rows of 4 perches (8 perches total, 100/m^2) and 2 rows of 2 perches (4 perches total, 87/m^2; Fig. 1A). After attaching the wooden perches, we spread a 1-cm layer of clean sand evenly over the bottom of each aquarium and filled all aquaria with a 1:1 mix of water from our primary collecting pond (see below) and dechlorinated tap water to a depth of 14 cm from the water surface to the top of the sand layer. All aquaria were supplied with an air stone for gentle oxygenation and water circulation.

We used standard aquatic D-nets to collect *A. junius* larvae from semi-permanent ponds in Crawford County in northwestern Pennsylvania in late September 2016. The primary source of *A. junius* larvae was a pond located on State Game Lands 146 (lat 41.7340, long −79.9740). We transported the *A. junius* larvae to the laboratory where we sorted them by total body length (including the head) into 3 size classes: large (35–38 mm), medium (24–27 mm), and small (19–22 mm). To each of the 30 aquaria, we then allocated 1 large, 1 medium, and 6 small *A. junius* larvae, a size ratio that approximated the natural variation in body size and population size structure found in the primary collection pond. To provide live prey for the *A. junius*, we initially added 8 damselfly (*Enallagma* spp.; Charpentier 1840) larvae to each aquarium as well. We collected damselfly larvae primarily from a semi-permanent beaver marsh on Allegheny College's Bousson Environmental Research Reserve (lat 41.5994, long −80.0530).

Experimental design and data collection

Once stocked with *A. junius* and prey, we randomly assigned the 30 aquaria to 1 of 2 hydroperiod treatments

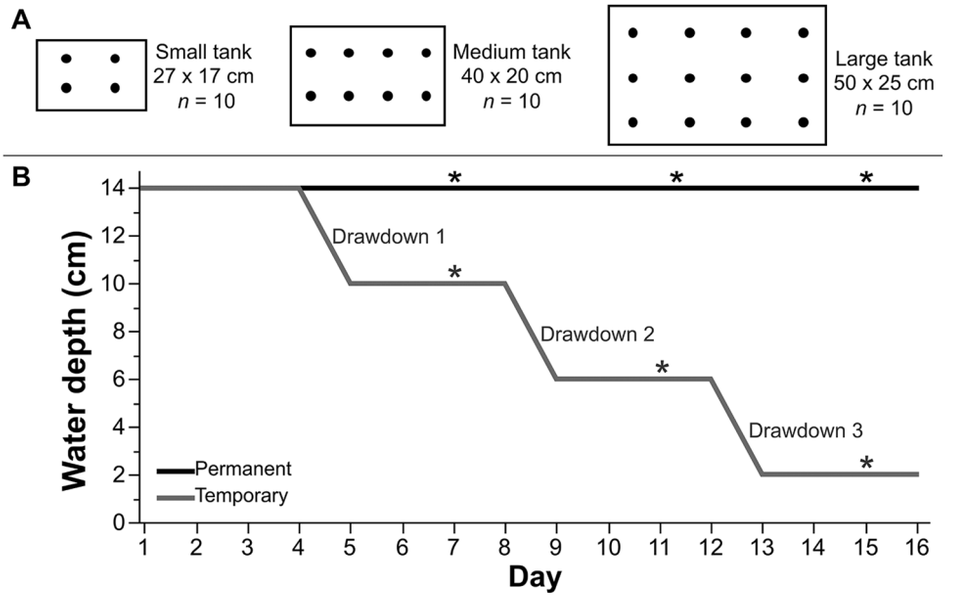

Figure 1. Schematic overhead view of small, medium, and large microcosm tanks. Black circles indicate vertical wooden perches (A). Schedule of water drawdowns and *Anax junius* behavioral observations in the 2 hydroperiod treatments, permanent and temporary (B). Asterisks indicate days when behavioral data were collected.

(permanent or temporary) such that each treatment included 5 large (low-density) tanks, 5 medium-sized (medium-density) tanks, and 5 small (high-density) tanks. For the 15 aquaria in the permanent hydroperiod treatment, we maintained the water level at a constant depth of 14 cm throughout the 16-d duration of the experiment by adding dechlorinated tap water when necessary to account for evaporation. We subjected the 15 aquaria in the temporary hydroperiod treatment to 3 scheduled water drawdowns (d 4, 8, 12; Fig. 1B). We reduced water depth from 14 to 10 cm on d 4, from 10 to 6 cm on d 8, and from 6 to 2 cm on d 12 (Fig. 1B). These scheduled drawdowns were designed to mimic rapid drying in temporary ponds.

For each of the 16 d following the initial stocking, we recorded the number and size class of all surviving *A. junius* larvae in each microcosm. We counted surviving damselfly larvae in each tank daily and added damselflies when needed to maintain a constant daily 1:1 ratio of *A. junius* to prey, assuring a sufficient food source for the *A. junius* larvae throughout the experiment. During the daily censuses, we also made note of evidence of cannibalism (e.g., partially-eaten *A. junius* bodies at the bottom of the tank).

To assess the behavior of *A. junius* larvae following scheduled water drawdowns, we made focal individual observations 3 d after each drawdown (Fig. 1B). For these direct visual observations, we chose 12 tanks at random (2 from each of the 6 unique treatment combinations of tank size and hydroperiod) and followed 1 randomly-selected individual from each of the 12 tanks for a total of 15 min. We noted the size class (small, medium, or large) and initial location of the focal *A. junius*, along with its behaviors, specifically noting total time active and the number of separate encounters (direct physical contact) with other *A. junius* larvae. During the 15-min focal observation periods, we also watched for evidence of aggressive or cannibalistic behavior between conspecifics, but such events occurred too rarely for statistical analysis.

Statistical analysis

We plotted Kaplan–Meier survival curves using the Survival platform of JMP Pro 12.0 (SAS Institute, Cary, North Carolina). We analyzed survival data using mixed-effects Cox proportional hazards models generated by the *coxme* package (Therneau 2019) of R (version 3.6.2; R Project for Statistical Computing, Vienna, Austria). Cox models, which are frequently used in clinical and epidemiological studies of survival time, are semi-parametric in that they require no a priori assumptions about the form of the baseline hazard function (Moore 2016). In all Cox models, tank number was a random effect. *Anax junius* larval size category (small, medium, and large) and hydroperiod treatment (permanent and temporary) were categorical fixed effects. Because we knew tank size (area of the tank bottom in cm^2; Fig. 1A) precisely for each tank, we included it in models as a continuous fixed effect to preserve degrees of freedom and simplify interpretation of model coefficients. The assumption of proportional hazards was checked by confirming that log cumulative hazard functions for different treatment groups were approximately parallel.

To disentangle the confounded effects of hydroperiod treatment and volumetric density of *A. junius* on the daily

risk of cannibalism, we constructed a generalized linear mixed model (binomial logistic regression with random effects) using the R package *lme4* (version 1.1-21; Bates et al. 2015). The dependent variable in this analysis was whether an individual *A. junius* larvae was cannibalized on a given day (no or yes), while hydroperiod treatment and *A. junius* density that day (number of *A. junius* alive in the tank at the beginning of the day divided by the volume of water present that day) were fixed effects, and tank number was a random effect. To exclude cases of mortality that were not attributable to cannibalism or that occurred before water levels had been experimentally modified, we restricted this analysis to data collected following the 1st scheduled water drawdown on d 4 (Fig. 1B) for small and medium larvae only.

We analyzed behavioral data on the number of physical encounters with conspecifics observed during each 15-min focal observation period via generalized linear models (Poisson distribution, log link function) using the Generalized Linear Model personality of JMP Pro's Fit Model platform. To control for the effects of previous *A. junius* mortality on encounter probabilities, we used the number of surviving individuals present in the focal microcosm tank at the time of data collection as an offset variable in the models. We checked normal quantile plots of the deviance residuals from these models to ensure that the residuals approximated a normal distribution.

RESULTS

Of the 240 *A. junius* larvae that began the experiment, 61 (25%) died by d 16 (Table 1). Most deaths occurred in temporary habitats (48 deaths, 79% of all deaths), and death occurred more frequently in small tanks (28 deaths, 46%) than in either medium-sized (21 deaths, 34%) or large tanks (12 deaths, 20%). Mortality was also strongly associated with *A. junius* size class: 31% (56 of 180) of the small larvae died over the course of the experiment, compared to just 7% (2 of 30) of medium larvae and 10% (3 of 30) of large larvae (Table 1).

Table 1. Summary of the 61 recorded deaths out of 240 total *Anax junius* larvae over the course of the 16-d experiment. The 58 deaths of small and medium larvae were all attributable to cannibalism, but the 3 deaths of large larvae occurred for reasons other than cannibalism.

		Deaths of *Anax junius* of each size			
Tank size	Hydroperiod	Small	Medium	Large	Total
Small	Permanent	5	0	1	6
Small	Temporary	19	2	1	22
Medium	Permanent	5	0	0	5
Medium	Temporary	16	0	0	16
Large	Permanent	2	0	0	2
Large	Temporary	9	0	1	10
Total		56	2	3	61

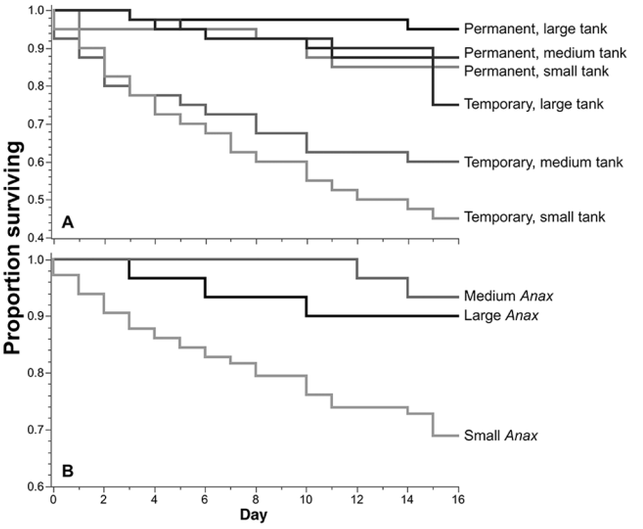

Figure 2. Kaplan–Meier survival curves of *Anax junius* larvae in relation to hydroperiod and tank-size treatments (A) and *A. junius* body-size category (B). Starting sample size was 240 total larvae, including 180 small, 30 medium, and 30 large larvae.

Mixed-effects Cox proportional hazards models showed that larval size category, hydroperiod treatment, and tank size all had substantial effects on *A. junius* survival over the course of the experiment (Fig. 2A, B, Table 2A). Model coefficients indicated that tank size was positively related to survival and that survival was lower in the temporary hydroperiod treatment and in small larvae (Fig. 2A, B). To confirm the effect of hydroperiod treatment, we evaluated additional Cox models in which the 23 larvae that perished prior to the 1st scheduled water drawdown on d 4 (Fig. 1A, B) were excluded, and analysis was limited to survival from d 4 to 16 for the 217 larvae alive at the time of the drawdown on d 4. This analysis (Table 2B) produced results virtually identical to those produced by the complete dataset (Table 2A) and confirmed the role of hydroperiod treatment in *A. junius* survival.

All 58 deaths of small and medium larvae (Fig. 2B, Table 1) were attributable to cannibalism by larger conspecifics. Cannibalism was implicated by visual evidence of larval remains (typically only the heads) or the complete disappearance of larvae that had been present during the previous daily census. The 2 medium larvae cannibalized were both from small temporary tanks (Table 1) and were eaten by the large *A. junius* late in the experiment (Fig. 2B) only after all of the small larvae in those tanks had been cannibalized. In contrast, the cause of death for the 3 large larvae that perished during the experiment (Fig. 2B, Table 1) was clearly not cannibalism. In all 3 instances, the dead body of the large *A. junius* was found floating intact at the

Table 2. Mixed-effects Cox proportional hazards models for survival of *Anax junius* larvae over the course of the 16-d experiment. Larval size (small, medium, large) and hydroperiod treatment (permanent or temporary) were entered as categorical fixed effects, and tank size (area of the tank base for the 3 tank sizes) was entered as a continuous fixed effect. Tank number was a random effect in all models. The 2 parts of the table represent the Cox models for survival of all 240 larvae in the study (A) and survival of the 217 larvae still alive at the time of the 1st scheduled water drawdown on d 4 (B). Likelihood ratio chi-square values for each factor were determined by using a base model of larval size + hydroperiod + tank size as fixed effects, and then either subtracting individual main effects or adding individual interaction terms to compare to the base model. Model coefficients indicated that higher hazard rates were associated with smaller larval size, the temporary hydroperiod treatment, and smaller tank size. df = degrees of freedom.

Effect	Chi-square	df	*p*
A. All data, d 0–16 (n = 240 larvae)			
Larval size	29.44	2	<0.0001
Hydroperiod	12.81	1	0.0003
Tank size	5.02	1	0.025
Larval size * hydroperiod	1.62	2	0.44
Larval size * tank size	2.83	2	0.24
Hydroperiod * tank size	0.02	1	0.87
B. Post-drawdown data, d 4–16 (n = 217 larvae)			
Larval size	18.48	2	<0.0001
Hydroperiod	12.22	1	0.0005
Tank size	4.27	1	0.039
Larval size * hydroperiod	2.69	2	0.26
Larval size * tank size	3.45	2	0.18
Hydroperiod * tank size	0.00	1	0.97

surface. The 20 small and medium larvae still alive in these 3 tanks at the time the large *A. junius* died had high subsequent survival: 100% for the 3 medium larvae and 94% for the 17 small larvae.

Because our hydroperiod treatment (experimental reductions of water depth) was confounded with reductions of water volume and corresponding increases in *A. junius* density, we performed a mixed-effects logistic regression on daily risk of cannibalism to statistically disentangle the separate effects of water drawdown and density on cannibalism. In this analysis, which was limited to data collected d 4 through 16 following the initial water drawdowns and included tank number as a random effect, the temporary hydroperiod treatment was associated with an increased daily risk of cannibalism for small and medium larvae ($z = 2.95, p = 0.003$), but *A. junius* density was not ($z = 0.25, p = 0.81$).

Following the initial water drawdown on d 4, *A. junius* larvae from the temporary hydroperiod treatment showed rates of activity and conspecific encounters that were comparable to those of larvae from the permanent tanks (Fig. 3A). However, following the 2nd and 3rd drawdowns on d 8 and 12, when water depth in the temporary treatments was reduced, respectively, to 6 and 2 cm (Fig. 1A, B), activity and encounter rates increased considerably in the temporary treatment tanks (Fig. 3A). A generalized linear model of the number of encounters with conspecifics showed an interaction between drawdown number and hydroperiod treatment (Table 3), suggesting that behavioral encounters increased in frequency in temporary tanks only when water depth dropped to 6 cm or lower. Surprisingly, tank size showed no clear relationship with either overall activity levels or conspecific encounter rates (Fig. 3B), and the effects of tank size and larval size on encounter rates were negligible (Table 3).

DISCUSSION

The primary goal of our study was to examine the interacting effects of simulated pond drying and larval density (microcosm tank size) on cannibalism in size-structured populations of *A. junius*. Four conclusions can be drawn from our results. First, experimental reductions of both water depth and tank size increased cannibalism of small *A. junius* larvae (Fig. 2A, B, Table 2). Second, the water depth reductions we used to simulate pond drying in the temporary hydroperiod treatment had more pronounced effects on cannibalism than did experimental reductions in tank size (Fig. 2A, B, Table 2). Third, the increase in cannibalism associated with simulated pond drying was statistically attributable to the hydroperiod treatment, not to the confounded associated increase in *A. junius* density. Fourth, the increase in cannibalism associated with simulated pond drying appears to have been at least partially related to increased rates of overall activity and conspecific encounters when water depth dropped to 6 cm or lower (Fig. 3A, Table 3). In contrast, tank size had no comparable effect on *A. junius* activity or encounter rate (Fig. 3B). Collectively, our results suggest that pond drying and reductions in water depth are likely to promote cannibalism in *A. junius* populations, a result that could have significant implications for the abundance and population size structure of this important predator in temporary and fishless wetlands (McPeek 1998, Rudolf and Rasmussen 2013).

Cannibalism in larval odonates generally increases in high-density situations (Van Buskirk 1989, Hopper et al. 1996, De Block and Stoks 2004b), and it is often suggested that high density or high activity levels may increase cannibalism simply by bringing potential victims into more frequent contact with potential cannibals (Van Buskirk 1989, Johansson and Rowe 1999, Start and Gilbert 2017, Start et al. 2017). In our study, we similarly found a relationship between density and cannibalism, as cannibalism-linked mortality increased as tank size decreased (Fig. 2A, B, Table 1). However, our results also suggest that pond drying and reductions in water depth per se may be more

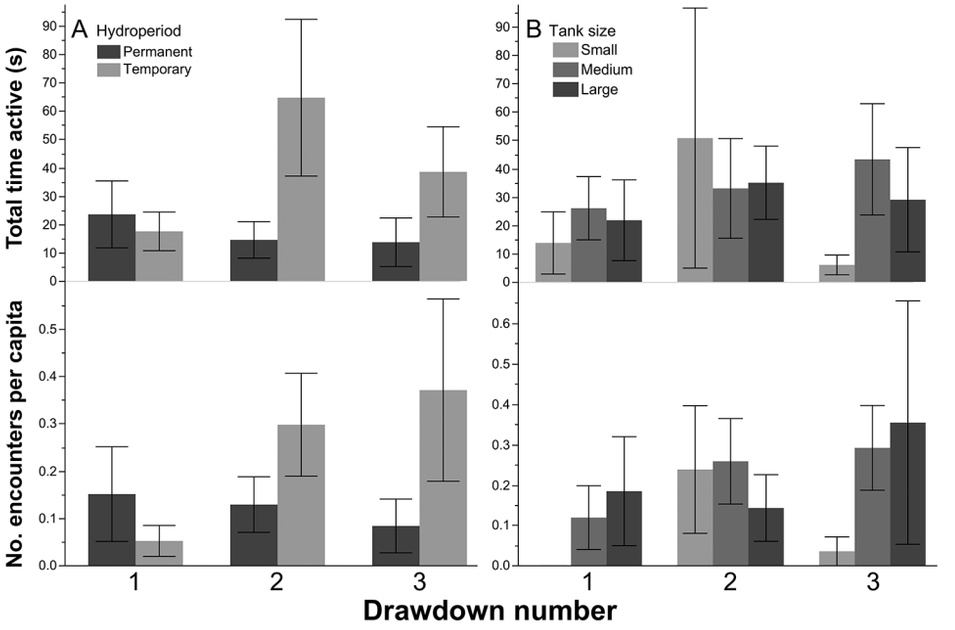

Figure 3. Effects of hydroperiod treatment (A) and microcosm tank size (B) on focal *Anax junius* activity time (upper panels) and number of physical encounters/capita (lower panels) following scheduled water drawdowns in temporary tanks. Drawdowns were performed only in temporary tanks, but behavioral data were collected from both permanent and temporary tanks for comparison 2 d after scheduled drawdowns (see Fig. 1B). Encounters/capita was calculated as the number of encounters observed during the 15-min focal behavioral observations divided by the number of conspecifics present in the microcosm tank at the time of data collection. Error bars represent the standard error of the mean.

important determinants of cannibalism than increases in density. Cannibalism was much more prevalent under conditions of simulated pond drying, and this result could not be statistically attributed to higher density. Instead, our results provide evidence that dragonfly larvae can perceive drying cues (e.g., reduction of water depth) and respond to them accordingly by cannibalizing smaller conspecifics to increase growth and eliminate competitors under deteriorating environmental conditions, as has been reported in other studies of larval odonates (Hopper et al. 1996, Johansson and Rowe 1999, De Block and Stoks 2004a, b). This increase in cannibalism under pond drying could in turn have significant consequences for community structure as larger conspecifics eliminate smaller larvae from younger cohorts (Hopper et al. 1996, Crumrine 2010, Rudolf and Rasmussen 2013, Sniegula et al. 2019).

Cannibalism in larval odonates and other aquatic organisms is strongly related to population size structure and variance in body size. Large larvae regularly cannibalize larvae appreciably smaller than themselves but seldom cannibalize individuals of comparable size (Wissinger 1988a, Hopper et al. 1996, Crumrine 2010, Dugas et al. 2016, Okano and Okuda 2018, Sniegula et al. 2019). In many cases, this pattern arises because the costs and risks of being a cannibal increase when cannibals and potential victims are evenly matched (Elgar and Crespi 1992). Our results illustrate the importance of population size structure in facilitating cannibalism, although we did not experimentally manipulate size structure. In our study, none of the 30 large *A. junius* and only 2 (7%) of the 30 medium larvae were cannibalized. Medium larvae were cannibalized only after all the small larvae in their tanks had been consumed. In contrast, 56 (31%) of 180 small larvae were cannibalized.

Among the expected consequences of global climate change for shallow and ephemeral wetlands are shortened hydroperiods, more rapid drying, and warmer water temperatures, particularly in arid regions and at high latitudes and altitudes (Brooks 2009, Sim et al. 2013, Lund et al.

Table 3. Generalized linear model (Poisson distribution, log link function) of the effects of drawdown number and hydroperiod treatment (permanent or temporary) on the number of physical encounters observed between *Anax junius* larvae during 36 15-min focal observation periods. The number of surviving larvae present in the tank was used as an offset variable. Effects of tank size ($p = 0.08$) and larval size ($p = 0.35$) were negligible and excluded from the final model. SE = standard error, df = degrees of freedom.

Effect	Coefficient	SE	Chi-square	df	p
Drawdown number	0.108	0.056	3.66	1	0.056
Hydroperiod (permanent)	−0.410	0.181	5.21	1	0.023
Drawdown number * hydroperiod	−0.147	0.056	7.14	1	0.0075

2016). All these anticipated changes are likely to increase intraspecific competition and cannibalism in the aquatic organisms that rely on these wetlands. While recent studies have begun to investigate the impact of rising water temperatures on competition and cannibalism (Start et al. 2017, Sniegula et al. 2019), our results suggest that the effects of altered hydroperiods and more rapid drying also merit experimental attention (De Block and Stoks 2005, Lund et al. 2016).

Our findings raise 2 additional questions for further experimental investigation. First, is the increase in cannibalism in response to drying cues universal across all *A. junius* populations, or is it restricted to populations living under particular drying-prone hydrologic regimes? Local adaptation may be unlikely in *A. junius* and other odonates that are migratory and have high levels of gene flow (Freeland et al. 2003, De Block et al. 2005, Pfeiler and Markow 2017), although local genetic adaptation to variable hydroperiods is known from some aquatic organisms (e.g., Weitere et al. 2004). Second, is the increase in cannibalism in response to drying cues attributable to decreasing water depth (i.e., the change in depth per se) or to the low water levels that were ultimately reached? Our results suggest the possibility that both may be important. For example, the approximately-linear survival curves of larvae in temporary tanks (Fig. 2A) suggest that change in depth, not the low water levels achieved toward the end of the experiment, is what stimulated higher rates of cannibalism under simulated pond drying. On the other hand, we did not observe changes in larval activity or encounter rates until after the 2nd water drawdown (Fig. 3A, B), suggesting that low water depth per se, not the change in depth, was responsible for the behavioral shifts we observed. Carefully-controlled laboratory studies will be required to address the relative importance of change in water levels vs low water levels in altering behavioral and life-history traits of odonate larvae.

In conclusion, our findings indicate that pond drying is likely to have important consequences for cannibalism in larval odonates beyond its simple effect of increasing density. Because pond drying and reductions of water depth are usually confounded with increases in larval density (De Block and Stoks 2005, Lund et al. 2016), future studies that experimentally manipulate the rate of simulated pond drying while simultaneously controlling for volumetric density and water depth would be particularly valuable in disentangling the separate effects of drying, water depth, and density on cannibalism and other behavioral and life-history traits.

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