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Emergence timing and fixation height in *Pachydiplax longipennis* (Odonata: Libellulidae) at varying substrate density and sunlight exposure

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Emergence substrate and sunlight penetration inherently trade off in patchy vegetation. Given the importance of solar radiation at emergence, we expected greater sunlight availability in sparse vegetation to advance emergence timing and reduce the average height of emergence fixation. We used outdoor mesocosms stocked with varying cattail (*Typha*) densities and late-stage *Pachydiplax longipennis* (Odonata: Libellulidae) larvae. As predicted, emergence based on exuviae observations began significantly earlier (5 d) at lower cattail density and greater sunlight exposure, with over 60% of the emergence completed midway into the experiment period, compared to about 50% in the medium and higher density cattail. This finding suggests lag effects under relatively limited light availability in a temperate-centered lentic-breeding heliotherm. Contrary to our prediction, we found significantly greater emergence heights at lower cattail density ($\bar{x} = 18.0$ cm) than at medium ($\bar{x} = 13.0$ cm) and higher ($\bar{x} = 10.0$ cm) densities. We recommend further study of emergence heights using larval choice experiments in natural settings. Variation in emergence timing and fixation height under the substrate–sunlight tradeoff may be driven proximally by larval choices/development and ultimately by adult activity.

Keywords: cattail; dragonfly; exuviae; mesocosms; shading

Introduction

Shading by vegetation has a strong effect on adult Odonata abundance, habitat use, and community assembly (De Marco, Batista, & Cabette, 2015; Remsburg, Olson, & Samways, 2008). Indeed, many studies have highlighted the importance of forest riparian conditions in structuring odonate assemblages (e.g. Dolný, Harabis, Bárta, Lhota, & Drozd, 2012; Modiba, Joseph, Seymour, Fouché, & Foord, 2017; Samways & Sharratt, 2010; Seidu, Danquah, Nsor, Kwarteng, & Lancaster, 2017) and controlling the emergence rates of aquatic insects (e.g. Banks, Li, & Herlihy, 2007; Progar & Moldenke, 2009). Yet, non-forest vegetation can also generate varying degrees of shading at and below the water surface, potentially influencing larval development, microhabitat selection, and emergence. Freshwater marshes, shrub-dominated wetlands, and pond/lake littoral zones can all have patchy dense tall emergent vegetation where light and

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substrate availability inherently trade off (more substrate = less light penetration and vice versa). This substrate–sunlight tradeoff may create opportunities for larval microhabitat preferences tied to emergence and early adulthood.

Larval development and emergence timing, in odonates and other hemimetabolous aquatic insects, is strongly linked with environmental temperature and light availability (Ivković, Miliša, Previšić, Popijač, & Mihaljević, 2013; Lutz, 1974; Suhling, Suhling, & Richter, 2015; Ward & Stanford, 1982). Lowered temperatures in shaded areas could slow larval development, postpone emergence, decelerate final molting, and delay/disrupt maiden flight (Corbet, 1957, 1999; Trottier, 1973). Consequently, and given intraspecific variation in distances traveled to emergence sites (e.g. Boda et al., 2015), it seems plausible that late-stage larvae may seek out areas with increased solar radiation for emergence, or that greater sunlight input in exposed areas could advance the timing of emergence. Alternatively, however, denser vegetation offers emerging nymphs more substrate to reduce overcrowding and more protective cover against predation and extreme weather (Jakob & Suhling, 1999; Worthen, 2010; but see Pitcher & Soluk, 2018), possibly offsetting the potential benefit of sparse canopies to dragonfly emergence.

Light availability could also conceivably influence the height of emergence fixation. Tall vegetation may increase larvae/exuviae densities (Remsburg, 2011; Remsburg & Turner, 2009) and the average emergence height (Zebsa, Khelifa, Kahalerras, Djalal, & Houhamdi, 2014), but studies have not considered emergence heights directly in relation to the substrate–sunlight tradeoff (but see Boda et al., 2015). We hypothesize that emergence heights could decrease with reduced vegetation canopy and greater sunlight penetration because larvae would have a shorter climb to access solar radiation for drying and warming the pharate adult. However, mortality risk factors such as overcrowding and density-dependent predation, or strong intraspecific variation in emergence heights (Cordero, 1995) and fixation occurring mainly in proportion to substrate height (Zebsa et al., 2014), could override any substrate–sunlight effect.

We conducted a mesocosm experiment to test the substrate–sunlight tradeoff on emergence timing and height observed via the exuviae of *Pachydiplax longipennis* (Burmeister). We hypothesized that in relatively limited vegetation canopy larvae would tend to show earlier emergence at lower height due to greater sunlight input.

Materials and methods

We stocked 21 outdoor mesocosms (110-l Sterilite® clear storage containers) with late-stage *P. longipennis* nymphs at varying cattail (*Typha*) densities. Mesocosms were exposed to full sunlight throughout the day and arranged (3 × 7) far enough apart to avoid shading (1.5–2.0 m cattail height) each other. Cattail treatments and environmental conditions were prepared in April 2017, about 2.5 weeks prior to *P. longipennis* inoculation. All experiment material (including dragonflies) came from a lake and pond complex at Greenleaf Lake State Park located about 14 km southeast of the experiment site (35.73°N, −95.25°W, Muskogee Co., OK, USA).

We used 12 culms m^{-2} (116 $\mu mol\ s^{-1}\ m^{-2}$ water surface light intensity, LI-250A, Li-Cor, Inc., Lincoln, NE, USA), 30 culms m^{-2} (76 $\mu mol\ s^{-1}\ m^{-2}$), and 72 culms m^{-2} (42 $\mu mol\ s^{-1}\ m^{-2}$) to represent lower, medium, and higher cattail densities, roughly mimicking the patchy distribution observed at the collection site. We assigned the treatments at random to seven mesocosms each. Tanks were lined with lake sediment (12–15 cm depth) and filled with lake water to about 14 cm above the sediment. We planted stiff (dead) culms in roughly uniform distributions and built a support frame (elevated grid of twine) around each tank to keep the culms erect and in place. To recreate conditions at the collection site and provide habitat structure, each container received about 2 l of *Ceratophyllum demersum*, a submersed macrophyte with dense leaf structure, and a handful of dead oak (*Quercus*) leaves to simulate allochthonous input. All collected sediment and vegetation was carefully examined prior to addition to remove odonate nymphs and potential predators (fish, dytiscids, belostomatids). We also collected prey items (small invertebrates) with standardized tow net (80-μm mesh) sweeps, adding equal volumes of prey to each container one week before the experiment began and every 10–12 d thereafter.

We collected and stocked *P. longipennis* in early May. Collections occurred in areas of varying cattail density, away from the dead material harvested for mesocosms. To mitigate cannibalism and intraspecific competition, and to maximize emergence during the study period, we discarded smaller nymphs and allocated similar size-class distributions across tanks. Based on locally collected exuviae, at least 85% of the stock appeared to be in the final or penultimate stages. We added 24 nymphs to each tank, leaving three (one per treatment, chosen at random) unstocked as controls – *P. longipennis* exuviae did not appear in the controls, suggesting exuviae observed in the density treatments came from stocked nymphs only. The same observer searched for exuviae on the cattail culms every 1–4 d ($\bar{x} = 2.0$ d) starting 9 May and ending 28 June after an extended period of little to no emergence. Emergence heights were measured as distance from the water surface to the tip of the exuviae abdomen (Zebsa et al., 2014). Non-emerging larvae were not all accounted for at the end of the experiment, but larvae and exuviae were never seen on the tank walls or outside the tanks on the ground, suggesting that no escape occurred. Water levels were maintained at 11–17 cm over the sediment and we let water temperatures (Hanna® instrument, HI 769828, Hanna Instruments, Inc., Nusfalau, Romania) fluctuate naturally up to 35°C (below the upper lethal limit for *P. longipennis* and other dragonflies; McCauley et al., 2015; Suhling et al., 2015), cooling the water when necessary using ice bottles. Mesocosms received rainfall over the study period but did not overflow due to small drain holes below the container rim.

Emergence heights were averaged for each treatment and compared using two-sample permutation tests with 9999 iterations. To analyze emergence success (overall proportion that emerged) we pooled exuviae observations over time within each mesocosm and fit a binomial generalized linear model to the number of "successes" (# exuviae) out of the total (24) nymphs stocked. Residual deviance did not exceed degrees of freedom, negating the need for a quasibinomial model with overdispersion parameter. We modeled the emergence pattern accounting for temporal nonlinearity and autocorrelation along with random effects from the mesocosms. We used the *mgcv* package (R Core Team, 2017) to fit a generalized additive mixed model:

$$Y_i \sim B(n, \pi_i) \text{ and } E(Y_i) = \pi_i * n \tag{1}$$

$$\text{logit}(\pi_i) = \text{intercept} + s(\text{Days}) * \text{Trt} + \textit{factor}(\text{Trt}) + \text{noise}$$

where Y_i is defined as the cumulative number of exuviae at each observation i, out of $n = 24$ stocked nymphs each assumed to have the same probability π_i of emergence. The interaction term indicates a smoothing function of time, s(Days), to capture the asymptotic trend of cumulative emergence in relation to cattail density (Trt) and account for time-varying effects such as temperature fluctuations. 'Noise' represents both an intercept random effect for the mesocosms and first-order autoregressive correlation structure for observations within mesocosms.

Results

A total of 198 *P. longipennis* nymphs (45% of stocked individuals) emerged from 13 May (5 d post-inoculation) to the last observation on 23 June. During this period, the totals emerging at lower (76 exuviae), medium (62 exuviae), and higher (60 exuviae) cattail culm densities translated to emergence rates (SD) of roughly 3.6 (4.5), 3.0 (3.8), and 2.9 (4.4) exuviae d^{-1},

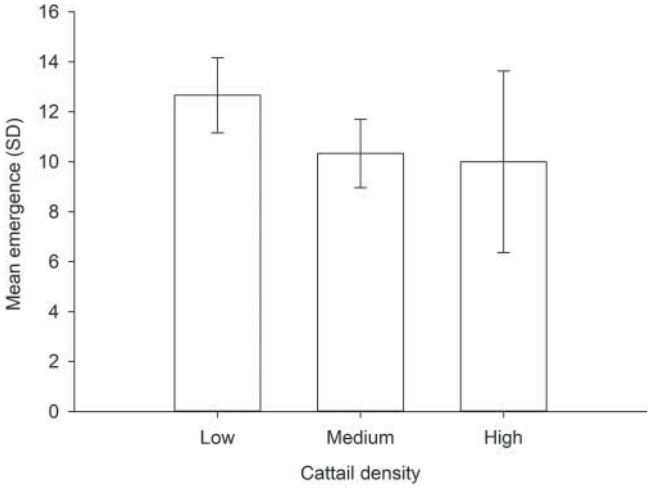

Figure 1. Mean (± 1 SD) number of *Pachydiplax longipennis* exuviae collected from low, medium, and high cattail densities ($n = 6$ per treatment).

respectively. It appeared that more emergence occurred in lower cattail density (greater sunlight penetration) compared to the medium and higher densities (Figure 1), but without statistically significant differences in emergence success among treatments (binomial generalized linear model comparing low vs. medium: $z = 1.65$, $P = 0.099$; low vs. high: $z = 1.89$, $P = 0.060$; medium vs. high: $z = 0.24$, $P = 0.812$), we cannot make a definite conclusion.

In contrast, temporally explicit analysis indicated a significantly different emergence trend at lower cattail density (generalized additive mixed model comparing low vs. medium: $t = 2.16$, $P = 0.031$; low vs. high: $t = 2.76$, $P = 0.006$); the medium and high trends did not differ ($t = 0.80$, $P = 0.424$). Emergence began 5 d earlier in the lower cattail density and was over 60% completed (out of the total that ultimately emerged) by midway through the observation period, compared to about 50% completed in the medium and higher densities (Figure 2). Emergence did not converge across the canopy levels until almost 30 d into the experiment (Figure 2).

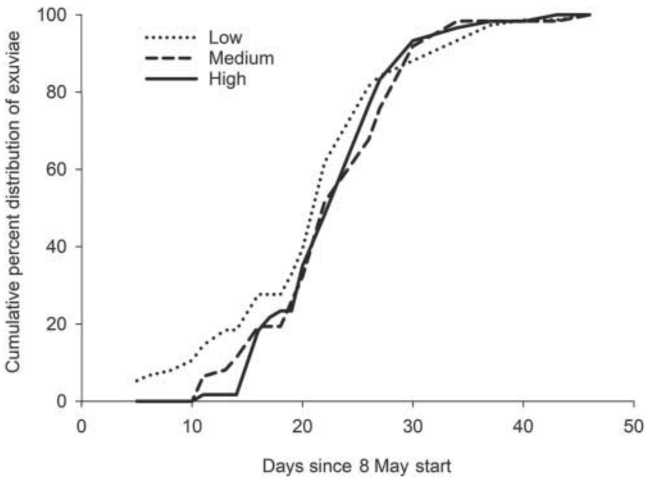

Figure 2. Emergence trends of *Pachydiplax longipennis* at three cattail culm densities. Exuviae numbers were pooled across replicate mesocosms.

We also found significantly (two-sample permutation tests, $P < 0.05$) greater emergence heights at lower cattail density ($\bar{x} \pm \mathrm{SD} = 18.0 \pm 16.1$ cm) than at medium (13.0 ± 11.4 cm) and higher (10.0 ± 5.7 cm) densities, with no significant difference between the medium and higher levels.

Discussion

Given the importance of solar radiation at emergence, we expected greater sunlight availability in sparse vegetation to advance emergence timing and reduce the average height of emergence fixation. Larval populations of *P. longipennis* emerged sooner and climbed higher in outdoor mesocosms with greater light penetration than in denser vegetation, supporting one hypothesis and contradicting the other.

A canopy (cattail) effect on overall emergence success, analyzed as the proportion of stocked larvae that emerged, was difficult to discern. French and McCauley (2018) similarly reported no effects of canopy cover on the growth and survival of dragonfly larvae added to mesocosms. Late instar larvae can have relatively high thermal thresholds for growth and development (Corbet, 1957; Lutz, 1968; Suhling et al., 2015). In addition, fluctuating water temperatures may reduce differences in larval development and emergence among treatments and mask direct effects from sunlight (Ivković et al., 2013; Suhling et al., 2015). Therefore, temperatures should be held relatively constant to allow separation of direct shading effects from shade-mediated temperature effects (McCauley et al., 2015). We let conditions fluctuate naturally below the lethal limit (McCauley et al., 2015; Suhling et al., 2015) yet average water temperatures taken with exuviae observations were similar among treatments (28.2°C in high density, 28.4°C in low density, and 29.0°C in medium density). Although our analysis of pooled observations may not have been confounded by temperature, a more dynamic approach was needed to separate effects against a background of temperature fluctuations, leading us into the temporally explicit analysis.

Emergence started earlier and reached 50% of the total emerged population (the “EM50”) more rapidly in lower cattail density, consistent with *Sympetrum* dragonflies reaching emergence faster in open outdoor mesocosms (French & McCauley, 2018). Warming may significantly advance the timing of *P. longipennis* emergence (McCauley et al., 2015), but as mentioned above, water temperatures were generally similar among treatments. Furthermore, the analysis implicitly accounted for any time-varying effects such as temperature fluctuations. Our finding may thus reflect a direct sunlight effect and not temperature-mediated effect. The apparent effect on phenology may have implications for ecological and evolutionary processes in natural systems. For example, adults emerging earlier from sites or microhabitats with sparse vegetation might gain advantages in establishing territories and breeding sooner than individuals emerging under relatively limited light availability in dense vegetation.

Denser vegetation implies that emergence supports are more readily available, reducing travel distances to emergence sites and potentially leading to greater emergence densities (Remsburg, 2011; Remsburg & Turner, 2009). In addition, faster emergence among dense supports might occur if there was high mortality risk from predation, overcrowding, or other sources (e.g. extreme winds), but these risks may account for only a small fraction of mortality at emergence (Corbet, 1957; Bennett & Mill, 1993; Jakob & Suhling, 1999; Zebsa et al., 2014), and in a recent experiment distances among substrate patches showed greater effect on emergence than the density of each patch (Pitcher & Soluk, 2018). Additionally, dragonfly emergence may decrease in denser vegetation due to incomplete ecdysis spurred by reduced temperatures or physical injury (Corbet, 1957, 1999; Jakob & Suhling, 1999). Given how water temperatures were similar among treatments (at least during exuviae observations) and that populations within tanks experienced a single substrate density and light regime, the most plausible explanation seems to be earlier emergence triggered by greater sunlight penetration in sparse canopy.

Contrary to our prediction, average heights of emergence fixation were greater in the lower cattail density. We expected sparse canopy to provide more immediate access to solar radiation, thereby reducing the distance to emergence fixation. Additionally, dragonflies are vulnerable at emergence (Corbet, 1999), and sparser canopy could leave emerging larvae more exposed to weather hazards (heavy precipitation, extreme winds) and predators (ants, wasps, spiders, birds), which might further reduce emergence heights. The height climbed in combination with substrate/location choice may reflect adaptation to reducing mortality risks and competition (Boda et al., 2015; Cordero, 1995; Worthen, 2010; Zebsa et al, 2014), analogous to adult perch heights and locations potentially mitigating reproductive interference (Switzer & Walters, 1999; Worthen, 2017). Therefore, greater emergence heights may offer a lower risk of predation from below (by fish, amphibians, other dragonflies) or less competition for emergence space, and perhaps a better chance of avoiding spider webs during maiden flight. But this does not explain why heights would vary with substrate density.

We suggest running experiments allowing larvae a choice of light regimes, including different substrate densities and canopy heights, and using species with differing shade tolerance as adults. We further recommend incorporating multiple types of vegetation growth forms, as plant morphology and habitat complexity can influence horizontal and vertical travel distances and emergence site selection (Boda et al., 2015; Grof-Tisza, LoPresti, Heath, & Karban, 2017; Worthen, 2010). Late-stage larvae could be added to open-topped enclosures placed along natural shorelines with contrasting emergent vegetation structure. Detailed field observations across varying microhabitats would help to complement the natural experiments (e.g. Boda et al., 2015).

The potential for strong intraspecific variation in emergence heights (Boda et al., 2015; Cordero, 1995) suggests a labile behavioral trait, and it seems that dragonflies, especially heliothermic species like *P. longipennis*, would seek to maximize sunlight exposure (i.e. higher fixation points in sparse canopy) for the final ecdysis and early adulthood. Indeed, trait-based behavioral syndromes (e.g. size-based dispersal) may carry over from larvae to adults (Stoks & Córdoba-Aguilar, 2012). Carry-overs also work in the opposite direction, with adult habitat choices and postcolonization processes (reproduction, oviposition) influencing larval densities and performance (Knorp & Dorn, 2016; Remsburg, 2011) and ultimately determining larval distributions (De Marco, Batista, & Cabette, 2015). As such, variation in emergence timing and fixation height under the substrate-sunlight tradeoff may be driven proximally by larval choices/development and ultimately by adult activity.

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