Damselfly (Odonata: Calopterygidae) Population Decline in an Urbanizing Watershed

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Subject Editor: Philippe Usseglio-Polatera

Received 7 March 2019; Editorial decision 27 May 2019

Abstract

Reduction of terrestrial vegetation and degradation of water quality are among the factors driving insect population decline in growing cities. In this study, we investigated the extent of habitat deterioration, behavioral and physiological responses, and fitness of a damselfly (Hetaerina americana [F.]) population in a semitropical region in central Mexico. The study population was located in a riverine area that crosses a small urban area (Tehuixtla city). We related two habitat variables (tree/shrub covered area and numbers of wastewater outlets) to presumable damselfly responses (larval and adult abundance, duration of adults exposed directly to sunlight, lipid content and muscle mass, and egg survival) over the years 2002 and 2016. We detected a reduction in terrestrial vegetation cover, an increase in wastewater outlets, and a decrease in larval and adult abundance. Adults were more exposed to sunlight in 2016 than in 2002 and showed a reduced lipid content and muscle mass in 2016. Egg survival also decreased. Although correlative, these results suggest impairment of damselfly condition (via lipid and muscle reduction) and fitness as urbanization increases.

Key words: Hetaerina americana, vegetation, water quality, abundance, physiology

Impacts of urban development on rivers are generally negative due to poor river management plans and regulations (Sisman and Bolu 2015). The majority of urban development projects at or near rivers clear riparian vegetation and increase the discharge of untreated industrial and domestic wastewater (Monteiro-Júnior et al. 2014, Sisman and Bolu 2015, Tchakonté et al. 2015). Consequently, rivers in urbanized areas usually have reduced biological components and impaired ecological functioning (Tchakonté et al. 2015).

Urban environmental stressors (e.g., resource availability, microclimate, and pollution) are known to negatively affect fitness or fitness-related attributes (Isaksson 2015), such as phenology, population size, reproductive success, and body condition (Meillère et al. 2015, Seress et al. 2018). For example, urbanized environments have selected for large distributions and wing span in birds (Croci et al. 2008) and for small size in salamanders (Murphy et al. 2016). However, for most of these effects, spatial approaches have been used (i.e., assessing two or more geographical sites that differ in environmental stressors) rather than temporal approaches even though the latter are better at avoiding local environmental factors implied by the former (Fattorini 2011, Buczkowski and Richmond 2012). To support the latter, a study of temporal synchrony, e.g., egg clutching, between two events may better reflect how population declines locally over time than across sites (Miller-Rushing et al. 2010, Seress et al. 2018).

Invertebrates have been recognized in maintaining ecosystem services (Sanford et al. 2009) and functions (McKinney 2008) even in urbanized areas. Given their short generation times, invertebrates are better indicators of urbanization rate than many vertebrates (Jones and Leather 2012). One example of such invertebrates are damselflies and dragonflies whose life cycle includes using an aquatic water habitat during the larval stage and a terrestrial habitat during the adult stage (Corbet 1999). Their life histories that straddle aquatic and terrestrial habitats imply that environmental stressors caused by urbanization can multiply their negative effects on damselfly or dragonfly fitness (Villalobos-Jimenez et al. 2016). This indicates that odonates may serve as bioindicators of both aquatic and terrestrial habitats. Observed effects of urbanization on Odonata species richness vary among studies: some works found a clear negative effect (e.g., Lubertazzi and Ginsberg 2010, Craves and O’Brien 2013, Goertzen and Suhling 2013), whereas others found no relationship (e.g., Monteiro-Júnior et al. 2014, Prescott and Eason 2018, Rocha-Ortega et al. 2019). These differences may be a problem of scale (Jeanmougin et al. 2014) such as a lack of direct measurements of individual condition or fitness.

Reduction in riparian vegetation (often quantified as vegetation cover area along watercourses; e.g., Monteiro-Júnior et al. 2014) and water quality degradation (de Jesús-Crespo and Ramírez 2011) are major driving factors of the negative effects of urbanization on
insect populations. Trees can provide both shelter and refuges to adult odonates against natural threats such as high temperatures and predators (Corbet 1999). A reduction in water quality possibly has more drastic effects when 1) development and feeding of odonates takes place in this habitat, 2) odonates spend a longer time in the aquatic stage than in the terrestrial stage, and 3) fitness effects on adults is carried over from the larval stage (Stoks and Córdoba-Aguilar 2012). We do not know if reduced riparian vegetation and water quality can impact odonates at the individual level.

*Hetaerina americana* (F) is a damselfly common in dry regions of the United States and Mexico (Rivas et al. 2016). Larvae inhabit riverine areas where they complete development in 3–5 mo (A.C.-A., unpublished data). Adults utilize most of their time feeding and building up the physiological traits (e.g., muscle mass and fat) necessary to engage in reproductive activities (i.e., egg production for females and territory ownership for males; Córdoba-Aguilar and González-Tokman 2014). Such a wide distribution may be interpreted as a fairly high ecological tolerance by this species (Rivas et al. 2016). As had been detected in other animals (Lubertazzi and Ginsberg 2010), this wide species-level distribution may mask population vulnerability at the local level and during the breeding season. For example, *Enallagma* damselflies (Coenagrionidae) also have wide distributions and have thus served to uncover habitat requirements and extinction rates (Gibbons et al. 2002, Suohonnen et al. 2010).

In this study, we related the abundance and fitness responses of *H. americana* to urbanization along the Amacuzac River in Tehuixtla City in central Mexico. Our general hypothesis is that the decrease in habitat quality (less vegetation and more wastewater outlets) had a negative impact on damselfly abundance, condition (muscle and lipid mass), and fitness (egg hatching). To test this hypothesis, we first compared a set of variables informing on habitat degradation over 2 yr (2002 and 2016) in the same study site: 1) the numbers of trees and shrubs per area and 2) the total number of wastewaters outlets from urbanized areas to riverine areas. Second, we provided abundance data for both larvae and adults over 2 yr (2002 and 2016). Finally, we compared 1) time under direct sun exposure because of reduced vegetation cover and shade; 2) muscle lipidic content and muscle mass as indicators of insect condition; and 3) egg hatching as a direct indicator of fitness.

### Materials and Methods

#### Study Area

Fieldwork was carried out along a 4,500-m stretch of the Amacuzac River that crosses and surrounds Tehuixtla City, Morelos State, Central Mexico (18°33′34″N, 99°16′15″W; Fig. 1). There was an approximate 100–300 m width of terrestrial vegetation between the riverbank and the residential area. Tehuixtla currently has around 7,000 people, with its main economic activities in agriculture, livestock, and tourism. The river crosses the city and two water parks. Upstream, this perennial river crosses a number of localities where water is also used mainly for agriculture, house discharges, and recreational activities.

Six plots of 600 × 100 m were defined longitudinally along the banks (600 m occurring along the river and 100 m occurring transversally from the river) on both sides of the river (six plots on either side). We did not expand our plot beyond 100 m from the riverbank because our experience suggested that *H. americana* rarely fly beyond 100 m from the river (A.C.-A. and M.R.-O., unpublished data). Plots located on the same riverbank were separated by 100 m from each other. These longitudinal plots facilitated the graphical planning of transects (see below) that took place along the river banks.

#### Tree/Shrub Density and Wastewater Outlets

We modified the transect method of Nowak et al. (1996) to estimate tree/shrub density in urbanized areas. All trees and shrubs in each of our plots (600 × 100 m each) were depicted on an acetate paper. The method described by Nowak et al. (1996) is based on tracing a line longitudinally (from one extreme of the plot to the other extreme) within the plot on the acetate and counting all trees/shrubs that the line ‘touches’. However, given the small size of our plots, we were able to count all the trees/shrubs present in the whole plot area. We did not consider plant diversity as a relevant environmental parameter because adult damselflies (including *H. americana*) do not select this kind of vegetation for perching or resting (Corbet 1999, A.C.-A. and M.R.-O., unpublished data). The minimum height of shrubs counted was 1 m, which is the approximate minimum height preferred by damselflies to rest (Córdoba-Aguilar 1994). Tree/shrub density was recorded four times over a 14-yr period: in 10–18 September 2002, 17–25 September 2003, 25–29 October 2004, and 4–10 November 2016.

We counted the number of wastewater outlets to the riverine area in the survey plots. Wastewater outlet was defined as any liquid flow of at least 15 cm in width from any house into the river. We did not record the volume of these outlets nor the type of water contents.

Fig. 1. A geographical description of the Amacuzac river, which appears in light and dark black, within the Tehuixtla city, which appears in gray. The light black depicts the riverine zone along the urbanized area, where field work was carried out.
However, wastewaters included mainly toilet (detergent), shower (shampoo, soap), and laundry chemicals. We moved along the riverbank and counted outlets simultaneously to tree/shrub density assessment. These outlets were also depicted on the same acetate paper used for tree/shrub density surveys. Each counting was carried out twice, and preliminary paired t-test suggested that the data of tree/shrub density and numbers of wastewater outlets were consistent between the two counts.

Larval and Adult Abundances
Every third day from 23 September to 28 November 2002 and from 18 September to 28 November 2016, larvae were sampled along a 600-m transect from 09:00 to 17:00 h. The sampled transect was one that occupied the middle of the three plots defined for the tree/shrub density assessments along each riverbank. Last-instar damselfly larvae were collected using a square aquatic net (50 cm length × 20 cm height × 40 cm depth, 0.5 mm mesh), approximately every 34 m. We collected only last-instar animals as taxonomic assessment of younger instars can be equivocal. Because *H. americana* reproduces at all times in this site (A.C.-A. and M.R.-O., unpublished data), our sampling of last-instar animals has no temporal bias. The aquatic net was placed downstream onto the river bottom. We gently disturbed the substrate located at the entry of the net, which allowed to dislodge damselfly larvae. The net was moved so that it can capture any larvae. This netting process lasted no more than 1 min per sample. This procedure was done once for each of the 15 sampling points (each point was approximately 1 m along the riverbank) over 23 d per year in 2002 and 2016. Five different points were sampled on each sampling day, so that there was a total sample size of 115 sampling points in 2002 and 2016. All collected animals were placed in a pan, and live *H. americana* larvae were counted then returned to the river. No larvae died in the process of collecting and counting.

Adult and larval abundance assessments were conducted in the same 600-m transect during the same sampling periods in both years. Adult sampling was conducted every 5 d (a total of 16 sampled days in both years) and from 11:00 to 14:00 h, the time when both sexes were at their peak activity (Córdoba-Aguilar et al. 2009). An observer walked the whole transect for 3 h on each sampling day and counted all damselflies that were within the field of vision. *Hetaerina americana* was the only *Hetaerina* species present at the study site.

Time in Sunny Areas
Between 09:00 and 17:00 h on 23 September to 28 November 2002 and 18 September to 3 December 2016, we recorded the duration *H. americana* adults spent under direct sunlight. These two periods correspond to the peak times in adult activity for this species (see, e.g., Córdoba-Aguilar et al. 2009). The observations were conducted in the same transects for the damselfly abundance assessments. For this, we randomly selected focal males and females by finding one individual and flicking a coin to choose whether or not the animal would be observed. For each selected animal, we recorded the duration (in seconds) within a 5-min observation period they spent in sunny areas. In total, 83 and 79 individuals were observed in 2002 and 2016, respectively.

Muscle Mass and Fat Content
A sample of 20 males and 20 females were collected on 29 November 2003 and again on 4 December 2016. These animals were captured from the same transect where damselfly abundance was assessed. All animals were placed in a desiccator for 48 h, and their dry weights were measured to the nearest 0.1 mg. This initial weight included exoskeleton, fat, and muscle. To extract fat, samples were placed in chloroform/methanol for 24 h and salt was added (Barnes and Blackstock 1973) after which they were again desiccated and reweighed. Fat content is the difference between final weight and initial weight (Contreras-Garduño et al. 2008). To obtain muscle weight, samples of the same processed individuals were submerged in 0.8 M potassium hydroxide for 48 h (Contreras-Garduño et al. 2008), and the remaining body was weighed. The difference in weight prior and after the extraction was interpreted as the weight of muscle mass. Both fat and muscle mass were corrected for possible year-related differences in body size, by dividing each fat or muscle value by its corresponding body size. For body length, we measured forewing length (in millimeters), from the base of insertion to the thorax to the wing tip. Each recording of size was taken three times by one of us (A.C.-A.) who was not aware of which year the animal was from. The average of the three measures was used.

Egg Hatching
On 23 September to 28 November 2002 and 18 September to 3 December 2016, egg survival was recorded under semicontrolled and controlled conditions. Twenty gravid females were located and allowed to lay eggs. These females can be usually distinguished: they came from areas outside the river and attracted the attention of males (Córdoba-Aguilar 2009). We randomly divided the 20 females into two sets of 10 females each by using a random number generator (www.random.org). Aquatic substrates containing the eggs from the first 10 females were collected for the semicontrolled experiment, and each egg mass was placed in a 30 × 15 × 15 cm plastic container (one container per each female’s egg mass) covered with a fine mesh and submerged to a 30-cm depth (firmly attached to the river bottom by using a 50-cm metal nail placed in each container corner). The mesh allowed the river water to enter but impeded both the substrate and larvae from escaping. The containers were placed in a distance of 20 cm from each other. The egg-containing substrates used by the remaining 10 females were collected for the controlled-condition experiment. Each egg mass was placed in a container described above, filled with local tap water, and oxygenated using air pumps in a nearby house. This last set of egg masses was placed on a large table, with containers distant of 20 cm, and exposed to natural day–light changes. Larvae in all the containers were counted every day for 8 d and returned to the river.

Statistical Analyses
The numbers of larvae and adults found across sampling points were compared over the 2-yr period using two independent t-tests. However, because no damselfly was observed in some sampling stations (for larvae) and transect (for adults), we transformed these data with log10 (x + 1), so that data distribution satisfied the assumption of normality. For the consistency of tree/shrub density data, a paired t-test was used where each first counting was compared against its second counting across all 4-yr period. The average of both recordings of each sampling was used to compare the numbers of trees/shrubs for the 4 yr using an analysis of variance (ANOVA) whereby year was entered as a factor and tree/shrub density as a response variable. Tukey tests were used for post hoc comparisons. The same statistical procedure was used for testing consistency and difference across years in the numbers of wastewater outlets. Comparisons of how long adult damselflies spent in sunny areas, as well as fat and muscle masses, for both years, were done using three independent t-tests (note that males and females were pooled in the same group). Time in the sunny area was, however, previously transformed using log10 to fulfill requirements for parametrical
analysis. The numbers of hatched larvae were compared using a two-factor ANOVA where year (2002 and 2016) and treatment (river water and tap water) were entered as factors. We also transformed the data with \( \log_{10}(n + 1) \), given that some egg clutches did not produce larvae. All analyses were carried out with SPSS (IBM Corp 2012).

**Results and Discussion**

Between 2002 and 2016 (the two periods of insect collection), there was a decrease in the abundance of larvae \( (t_{228} = 6.03, P < 0.0001) \) and adults \( (t_{28} = 2.72, P < 0.01; \text{Fig. 2}) \). Tree/shrub density also decreased significantly over the 2002–2016 period \( (F_{3,20} = 83.236, P < 0.0001) \), with the density in 2016 being the lowest (Fig. 3a). Over the same period, wastewater outlet numbers increased from 2002 to 2003, 2004 and 2016 (the four periods of abiotic recordings; \( F_{3,20} = 14.179, P < 0.0001; \text{Fig. 3b}) \).

These results have highlighted the impact of a reduction in vegetation coverage and an increase in wastewater discharge on damselfly abundance, although the general picture of all possible stressors is difficult to depict. A reduction in tree cover as a stressor driving odonate diversity and abundance has been well documented (e.g., Kinvig and Samways 2000, Oppel 2005, Harms et al. 2014). The impacts of wastewater discharge, on the other hand, are less well documented (but see Ferreras-Romero et al. 2009).

A reduction of tree/shrub vegetation may have several consequences for adult activities and functions. One function of tree cover is to provide shade that allows thermoregulation in hot areas or times (Kortello and Ham 2010, Carvalho et al. 2013), an assumption that, to our knowledge, has not been tested properly. In this study, the time damselflies spent in sunny areas increased significantly in 2016 when compared with that in 2002 \( (t_{118} = -7.39, P < 0.001; \text{Fig. 4}) \). Unlike other odonate species, adult *Hetaerina* spp. use shrubs that are located along the river after sexual activities in the afternoon and at night for resting. Apart from thermoregulation, by aggregating in large numbers, *Hetaerina* spp. reduce individual probability of predation (Grether and Switzer 2000, Switzer and Grether 2000). Thus, in the absence of trees and shrubs, we hypothesize a cost in terms of thermoregulation during the day and predation at night.

The weights of both fat \( (t_{78} = 3.72, P = 0.0001) \) and muscle mass \( (t_{78} = 2.29, P = 0.012) \) decreased in 2016 compared with those in 2002 (Fig. 5). Perhaps, animals had to either move more to find a place to thermoregulate or become energetically exhausted when exposed for longer times to sunlight (as detected in other insects; Blanckenhorn 2018). An impairment of lipidic and muscle mass can also be explained by other reasons: 1) a reduction in food intake (due to fewer prey) prior to sexual maturation (Jiménez-Cortés et al. 2012) and 2) energy expenditure after costly activities such as egg production (Córdoba-Aguilar 2009) and territory defense (Córdoba-Aguilar and González-Tokman 2014). Unfortunately, we had no data to separate these two possibilities from the consequences of a poor-quality habitat.

Our exploration of potential effects of water sources on egg hatchability in 2002 and 2016 indicated that the numbers of hatching larvae were different between years \( (F_{1,10} = 68.04, P < 0.0001) \), but not different between water sources \( (F_{1,19} = 1.60, P = 0.21) \), with lower numbers of larvae hatched from eggs collected in 2016 compared with 2002 (Fig. 6). In general, a reduction in diversity and abundance of larval odonates have been documented when riverine habitats have been exposed to wastewater outlets (e.g., Watson et al. 1982, Catling 2005). However, odonate responses to wastewater outlets may be species and community dependent. Our findings suggest that egg survival was affected in recent years. This lower egg survival and the lower numbers of larvae and adults in 2016 suggest that urbanization pressure,
seen as a decrease in vegetation cover and increase in wastewater outlets, may have affected damselfly populations.

As indicated before, recent studies on the effects of urbanization on odonate diversity and abundance have produced inconsistent results, but have not discussed the causes of these inconsistencies. For example, Odonata diversity may show a negative relationship (e.g., Willigalla and Fartmann 2012, Monteiro-Júnior et al. 2014) or no relationship with urbanization (e.g., Lubertazzi and Ginsberg 2010, Craves & O'Brien 2013, Goertz and Suhling 2013). We believe that an explanation for detecting—or not—the negative effects of urbanization on odonates may be a problem of scale (Jennougin et al. 2014). In this regard, note that the persistence of the study species in the study site over the 14-yr time window may be taken as ‘no effects’ if only the species presence is taken into account. Note also that a few odonate species can be resilient to changes in, for example, land use which may be the case of the study species (Rocha-Ortega et al. 2019). However, when using more complex and realistic measures such as abundance (as in our case), this picture can change. This implies that assessment of effects at the individual/population level is badly needed as detrimental effects are more likely to be found at these levels. In our case, we also detected changes in condition and hatchability, which may explain why abundance decreased. Whether this is the case for other species remains to be investigated.

Acknowledgments

A number of field assistants kindly provided technical assistance. Catalina Suárez Tovar provided enriching comments of damselfly ecology. The authors thank Raul I. Martínez-Becerril for logistic support. This project was supported by a PAPIIT Grant IN206618.

References Cited

Barnes, H., and J. Blackstock. 1973. Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanillin method for ‘total’ lipids. J. Exp. March Biol. Ecol. 12: 103–118.

Blanckenhorn, W. U. 2018. Behavioral, plastic and evolutionary responses to a changing world, pp. 292–308. In A. Córdoba-Aguilar, D. González-Tokman, and I. González-Santoyo (eds.), Insect behavior: from mechanisms to ecological and evolutionary consequences. Oxford University Press, Oxford, United Kingdom.

Buczowski, G., and D. S. Richmond. 2012. The effect of urbanization on ant abundance and diversity: a temporal examination of factors affecting biodiversity. PLoS One 7: e41729.

Carvalho, F. G. D., N. S. Pinto, J. M. B. D. Oliveira Júnior, and L. Juen. 2013. Effects of marginal vegetation removal on Odonata communities. Acta Limnol. Bras. 25: 10–18.

Caffing, P. M. 2005. A potential for the use of dragonfly (Odonata) diversity as a bioindicator of the efficiency of sewage lagoons. Can. Field-Nat. 119: 233–236.

Contreras-Garduño, J., B. A. Buzatto, M. A. Serrano-Meneses, K. Nájera-Cordero, and A. Córdoba-Aguilar. 2008. The size of the red wing spot of the American rubyspot as a heightened condition-dependent ornament. Behav. Ecol. 19: 724–732.

Corbet, P. S. 1999. Dragonflies: behaviour and ecology of Odonata. Comstock Publishing Associates, Ithaca, NY.

Córdoba-Aguilar, A. 1994. Male substrate use in relation to age and size in Hetaerina cruenta (Rambur) (Zygoptera: Calopterygidae). Odonatologica 23: 399–403.

Córdoba-Aguilar, A. 2009. A female evolutionary response when survival is at risk: male harassment mediates early reallocation of resources to increase egg number and size. Behav. Ecol. Sociobiol. 63: 751–763.

Córdoba-Aguilar, A., and D. M. González-Tokman. 2014. The behavioral and physiological ecology of adult rubyspot damselflies (Hetaerina, Calopterygidae, Odonata). Adv. Study Behav. 46: 311–341.

Córdoba-Aguilar, A., G. Rainahi, M. A. Serrano-Meneses, and J. Contreras-Garduño. 2009. The lek mating system of Hetaerina damselflies (Insecta: Calopterygidae). Behaviour 146: 189–207.

Craves, J. A., and D. S. O’Brien. 2013. The Odonata of Wayne county, MI: inspiration for renewed monitoring of urban areas. Northeast. Nat. 20: 341–362.

Croci, S., A. Butet, and P. Clergeau. 2008. Does urbanization filter birds on the basis of their biological traits? Condor 110: 223–240.

de Jesús-Crespo, R., and A. Ramirez. 2011. Effects of urbanization on stream physicochemistry and macroinvertebrate assemblages in a tropical urban watershed in Puerto Rico. J. North Am. Benth. Soc. 30: 739–750.

Fattorini, S. 2011. Insect rarity, extinction and conservation in urban Rome (Italy): a 120-year-long study of tenebrionid beetles. Insect Conserv. Divers. 4: 307–315.

Ferreras-Romero, M., J. Marquez-Rodriguez, and A. Ruiz-Garcia. 2009. Implications of anthropogenic disturbance factors on the Odonata assemblage in a Mediterranean fluvial system. Int. J. Odonatol. 12: 413–428.

Gibbons, L. K., J. M. Reed, and F. S. Chew. 2002. Habitat requirements and local persistence of three damselfly species (Odonata: Coenagrionidae). J. Insect Cons. 6: 47–55.

Goertz and Suhling 2013. Promoting dragonfly diversity in cities: major determinants and implications for urban pond design. J. Insect Conserv. 17: 399–409.

Grother, G. E., and P. V. Switzer. 2000. Mechanisms for the formation and maintenance of traditional night roost aggregations in a territorial damselfly. Animm. Behav. 60: 569–579.

Harms, T. M., K. E. Kinkade, and S. J. Dinsmore. 2014. Evaluating the effects of landscape configuration on site occupancy and movement dynamics of odonates in Iowa. J. Insect Conserv. 18: 307–315.

IBM Corp. 2012. IBM SPSS statistics for Windows, version 21. IBM Corp, Armonk, NY.

Isaksson, C. 2015. Urbanization, oxidative stress and inflammation: a question of evolving, acclimatizing or coping with urban environmental stress. Funct. Ecol. 29: 913–923.
