

Subterranean Behavior and Other Notes for *Ironoquia plattensis* (Trichoptera: Limnephilidae) in Nebraska

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ABSTRACT *Ironoquia plattensis* Alexander & Whiles (Trichoptera: Limnephilidae) was discovered along the Platte River in central Nebraska in the late 1990s, and basic information about its life history is not well understood. Here, we describe previously undocumented life-history traits that demonstrate strategies used by *I. plattensis* for surviving in fluctuating wetland environments in a landscape formally shaped by flooding. In an off-channel aquatic habitat along the Platte River, we observed 1) larvae residing in a slough that did not dry completely, 2) larvae emigrating from aquatic to terrestrial habitats 1 mo earlier than reported previously, 3) larvae moving above ground during the summer aestivation period, 4) larvae residing underground in soil during summer aestivation, and 5) mass emergence and swarming of adults after daybreak in autumn. Underground larval aestivation represents a previously undocumented behavior for this species. It is unclear whether aestivating underground represents an unreported common behavior or an infrequent response to local disturbances. At our site, insects may have been responding to a prescribed burn in April and introduction of cattle in mid-May that yielded the site unsuitable for aboveground aestivation. Additional studies on the life history for *I. plattensis* are warranted to help manage, locate, and protect the few sites where it occurs.

KEY WORDS subterranean behavior, larval movement, life-history strategy, Platte River, caddisfly

Most trichopeteran species have an entirely aquatic larval phase, but larvae of *Ironoquia* species emigrate from aquatic to terrestrial habitats for aestivation and pupation (Flint 1958, Williams and Williams 1975). In 1997, a new *Ironoquia* species, *Ironoquia plattensis* Alexander & Whiles (Trichoptera: Limnephilidae), was described along the Platte River from a single locality along an intermittent slough (linear, wetland depression) on Mormon Island, Hall County, NE (Alexander and Whiles 2000). Subsequent searches have discovered *I. plattensis* in sloughs adjacent to and associated with the Platte River (L.A.V., unpublished data). *I. plattensis* develops in sloughs through five larval instars from November to late May–early June, moves to land to aestivate and pupate above ground during summer, and emerges as adults during a brief period in late September and early October (Whiles et al. 1999). The terrestrial phase of its life cycle coincides with periods when sloughs dry or have intermittent flow (Whiles et al. 1999).

I. plattensis recently was petitioned for listing as federally endangered in the United States due to its limited distribution and susceptibility to habitat alterations (USFWS 2009). However, basic information

about the life history of *I. plattensis* is limited because all publications about the species are from one sampling site (Whiles et al. 1999, Alexander and Whiles 2000). Here, we describe new life-history characteristics for *I. plattensis* observed at another locality that demonstrate additional adaptations to fluctuating wetland environments.

Materials and Methods

Study Site. In 2010, we studied *I. plattensis* in a slough on land managed by the Platte River Whooping Crane Maintenance Trust on Shoemaker Island, Hall County, NE (40° 47.660' N, 98° 26.722' W). Limited numbers of *I. plattensis* were observed here in May 2003 and March and April 2004 (C. K. Meyer, personal communication; Meyer and Whiles 2008). We witnessed swarming of several hundred *I. plattensis* adults from this locality on 29 September 2009. On 25 April 2010, we confirmed aquatic larvae were present in the slough and initiated research. Our site is 5.5 km west of the type locality on the adjacent Mormon Island (Alexander and Whiles 2000).

The slough was surrounded by a tallgrass prairie dominated by big bluestem (*Andropogon gerardii* Vitman), Indiangrass [*Sorghastrum nutans* (L.), Nash], and switchgrass (*Panicum virgatum* L.). Sedges dominated areas immediately adjacent to surface water and formed vegetated island hummocks (≤ 40 cm in diameter) in the channel; see Henszey et al. (2004) and Meyer et al. (2008, 2010) for additional informa-

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tion about plant communities in nearby sloughs. Ground water maintained continuous flow in the majority of our study segment of the slough in 2009 and 2010, and rains supported intermittent flow upstream. Slough banks were 25–50 cm high, the wetted channel perimeter ranged from 65 to 150 cm, and maximum water depth ranged from 6 to 17 cm along the section of slough where *I. plattensis* larvae were present (27 April 2010; six cross-sections measured at 50-m intervals). Water temperature averaged 11°C (± 1 SD), with a pH of 7.8 (± 0.1), conductivity levels of 1,047 $\mu\text{S}/\text{cm}$ (± 33), and dissolved oxygen levels of 6.6 mg/liter (± 1.4) (sampled from six cross-sections on 27 April 2010).

The Platte River Whooping Crane Maintenance Trust managed the pasture surrounding the slough with rotational burning and cattle grazing. The pasture was burned on 17 March 2010, and grazing by domesticated cattle (*Bos taurus* L.) was initiated on 14 May 2010 (58 cow-calf pairs; 1.7 animal unit months). Cattle obtained water from a river channel along the southern border of the pasture or directly from the slough. The pasture was not grazed or burned the previous 2 yr (2008 and 2009).

Sampling of Aquatic Larvae. In April 2010, we established eight plots (each 12.5 by 12.5 m) along the slough, four grazed and four nongrazed. We sampled aquatic larval caddisflies on 29 April before cattle were introduced and on 28 May after grazing had occurred for 14 d. We first conducted visual surveys by placing a 30- by 30-cm² quadrat on the streambed and counting larvae during a 30-s period. Second, we physically sampled sediments and larvae from an area measuring 30 cm wide by 30 cm long by ≈ 2.5 cm deep via a single sweep of a 30-cm D-loop net in the center of stream flow. We collected three subsamples from each plot on each sampling date. Contents of the net were sieved (3-mm mesh) and sorted. Larval caddisflies were counted and returned to the capture site. The two sampling techniques were compared with Pearson correlation analyses in PASW Statistics version 18 (SPSS Inc. 2010).

Sampling of Terrestrial Larvae. To document emergence of larvae from the slough to the nearby prairie, we counted larvae captured in pitfall traps. Pitfall traps were installed as part of a concurrent herpetological survey and consisted of two 19-liter (5-gal) buckets set flush with the ground surface along a 10-m silt fence that crossed the slough. We examined pitfall traps periodically from 26 April through 23 July. We also searched for larvae above ground on 30 May and 16 July in study plots. We observed few aboveground larvae (see Results) compared with the high aquatic densities, so we searched for larvae buried in the soil. We collected $\approx 720\text{-cm}^3$ soil samples with shovels to a depth of ≈ 15 cm on 30 May, 11 July, and 16 July from vegetated banks < 0.5 m from the slough and counted larvae within the soil.

Description of Adult Emergence. We visually assessed emergence of adults from the slough on 28 and 29 September and 1–3 October 2010. Surveys were conducted at dawn, except 3 October when we sur-

veyed in afternoon and at dusk. To search for adults, we searched for adults flying around and resting upon vegetation surrounding the slough.

Voucher Specimens. We kept several larvae and adult *I. plattensis* as voucher specimens. Identifications were verified by M. R. Whiles (Southern Illinois University, Carbondale, IL) and deposited in natural history collections at Southern Illinois University.

Results and Discussion

Spatial Abundance of Aquatic Larvae. Larvae were observed only in a 250-m segment of the slough on Shoemaker Island (upstream end: 40° 47.629' N, 98° 26.778' W; downstream end: 40° 47.748' N, 98° 26.660' W of slough where larvae were present). Larvae were absent in upper slough segments that were dry September 2009 and from mid-March to early May 2010. Larvae also were absent further downstream, although downstream segments always contained surface water in the slough. It is unclear why larvae were present in the upper 220-m segment of the slough with continuous flow but not present in downstream reaches. The original population of *I. plattensis* was observed in an intermittent slough where larvae migrated onto land when the slough dried during summer (Whiles et al. 1999). Whiles et al. (1999) did not find *I. plattensis* in four other intermittent sloughs with different hydroperiods and one permanent slough near the type locality, and they concluded that the species likely exists within a narrow range of physical habitats. Inhabitation of a slough with continuous flow (except at the uppermost 30 m) at our site demonstrates that *I. plattensis* can exist within wider range of slough habitats than reported previously (Whiles et al. 1999). Subsequent surveys for new populations should focus on both intermittent and perennial sloughs because *I. plattensis* is not restricted to sloughs that dry up in summer.

Temporal Abundance of Aquatic Larvae. On 29 April 2010, we observed 80 ± 113 larvae per m² (mean \pm SD, 17 samples) in visual searches atop sediment compared with 553 ± 284 larvae per m² (19 samples) in net samples collected to a depth of 2.5 cm. The methods correlated positively ($r = 0.858$, $P < 0.001$; $n = 17$), with ≈ 7 times more larvae residing beneath the sediment compared with the surface ($t = -6.4$, $df = 34$, $P < 0.001$). We cannot directly compare our densities to those of Whiles et al. (1999) because our sampling techniques differed; they sampled to a depth of 10 cm. However, our average numbers (553 larvae per m² on 29 April) were less than their average of 725 larvae per m² (5 April) and 704 larvae per m² (2 May) at the type locality (M. R. Whiles, personal communication; Whiles et al. 1999). This could reflect natural variation between sites and/or that larvae reside deeper than the 2.5-cm depth sampled by us. On 28 May 2010, we could not visually assess larvae atop sediment surfaces due to increased turbidity caused by cattle. From net samples, we observed a 95% decrease in number of larvae from 29 April (553 ± 284 larvae per m²) to 28 May (28 ± 31 larvae per m²; $t =$



Fig. 1. Photographs of *I. plattensis* in a slough on Shoemaker Island, Hall County, NE ($40^{\circ} 47.660' N$, $98^{\circ} 26.722' W$) in 2010. (A) Extremely high larval density in a small pool of water in a drying section of the slough in May. (B) An empty larval case observed embedded vertically in the soil that led us to investigate whether larvae reside underground during their summer aestivation period. (C) Two larvae observed residing underground during the summer aestivation period, which is a new behavior for this species of caddisfly. (D) Cluster of adults in direct contact with one another in early September; caddisflies were mating at the bottom of the group. (Online figure in color.)

9.01, $df = 41$, $P < 0.001$). At the type locality in 1998, the number of aquatic larvae similarly decreased by 84% from 2 May to 1 June, when 117 larvae per m^2 remained in the slough (M. R. Whiles, personal communication). On 4 May 2010, we sampled the type locality on Mormon Island and observed 24 ± 21 larvae per m^2 ($n = 5$), considerably less than densities observed by Whiles et al. (1999). The pasture surrounding the type locality was burned and grazed in 2009, but cows had not yet been stocked when we sampled in 2010. More detailed surveys of intersite and interannual larval emigration are needed to determine which environmental cues (e.g., water temperature, availability of organic matter, disturbance) elicit emigration behaviors for *I. plattensis* from aquatic habitats.

Observations in a Drying Slough. Before onset of rains in mid-May, the uppermost ≈ 30 m of the upstream end of this slough with *I. plattensis* dried and left isolated pools of water where we observed extremely high densities of larvae (Fig. 1A). This observation leads to further questions, such as whether larvae can persist and develop in wet sediments, travel downstream to follow areas with more surface flow, or both. Observing behaviors at such rapidly changing and extreme conditions may be informative regarding selection of habitats across the distribution of *I. plattensis*.

Immigration to Terrestrial Habitats. On 26 April 2010, we observed six larvae in a pitfall trap adjacent to the slough channel, indicating terrestrial activity a month earlier than reported by Whiles et al. (1999).

Larval captures in pitfall traps peaked on 21 May, with 66 larvae. We captured larvae in pitfall traps well into summer (8 June, 36 larvae; 9 June, 28 larvae; and 23 June, 22 larvae), with the last observed on 8 July. Because much of the population emigrated from the slough in late May, we suspect that some larvae fell into pitfall traps while traveling on land after emerging from the water at an earlier date. These patterns demonstrate that terrestrial activity begins earlier and lasts longer than reported previously (Whiles et al. 1999). A better understanding of timing, distances traveled, movements during summer months, and cues that drive such activities is warranted for *I. plattensis*.

Subterranean Behavior. On 30 May, we searched for larvae above ground from the water's edge to ≈ 3 m away for 160 min. Although the prescribed burn in March removed the litter layer and facilitated searching, we observed only seven larvae. Two larvae were 1 m from the water's edge beneath grasses that lacked a litter layer due to the burn; one larva was ≈ 0.5 m from the water's edge in bare understory of grasses; two larvae were < 0.25 m from the water's edge in litter that did not burn; one larva was in the center of a small island hummock at the base of dense sedge stems; and one larva was near the water's edge in bare understory. On 16 July, one investigator searched for 15 min in our four ungrazed plots < 2 m from the water's edge examining an area ≈ 3 – 4 m^2 in each. A total of four larvae were observed; three were found in the bare understory of vegetation, and the other was in litter.

During searches we observed a number of old, empty larval cases embedded vertically in the soil

(Fig. 1B). This suggested that larvae reside or move underground to aestivate, pupate, or possibly escape harsh conditions, such as high temperatures or low relative humidity. On 30 May, we observed 14 larvae embedded in a soil sample (Fig. 1C). In another sample, we failed to detect larvae in the soil with roots but discovered five larvae in the sandy soil below roots. On 16 July, we observed 10 larvae in dense roots in a sample, and 16 *I. plattensis* larvae in another soil sample. In addition, we observed a larger larva underground, along the water's edge with its case consisting of plant material, probably *Ironoquia punctatissima* (Walker).

Such subterranean behavior has not been noted previously for *I. plattensis*, and it is unclear why larvae inhabited soil at this site in 2010. Larvae retained mobility once removed from the soil, and Whiles et al. (1999) also noted that larvae retained some mobility in the litter layer during aboveground aestivation. Such mobility suggests that larvae can respond to changing abiotic conditions. Burying below ground probably reflects an adaptation to historical disturbances by wildfires, grazing ungulates, and flooding in this landscape. The ability to move below ground may protect larvae from heat, desiccation, trampling, or floods. At our site, the prescribed burn in March removed most aboveground vegetation, and subsequent cattle grazing kept cover low along parts of the slough. Loss of cover likely changed the temperature and relative humidity profiles at the site, possibly yielding it unsuitable for long-term (≈ 4 -mo) aboveground aestivation. Hence, burning, grazing, or both may initiate burrowing behaviors of larval *I. plattensis*, but further research is needed to investigate such effects. This behavior also has important implications for conducting surveys for *I. plattensis* during the terrestrial phase of larval development. Aboveground and belowground searches for larval cases seem to be required before concluding larvae are not present in an area during the summer aestivation period.

Adult Emergence. We discovered this population of *I. plattensis* on 29 September 2009 when we witnessed a mass emergence of hundreds of adults above the vegetation surrounding the slough in the morning. In autumn 2010, we did not observe such a mass emergence and may have missed the time when adults were most abundant. On 30 September 2010, we observed clusters of 10–35 adults crawling on vegetation and flying within plant cover ≈ 15 min after sunrise, whereas none were observed just before sunrise on the same morning. After sunrise adults were observed only in vegetation hanging over or immediately adjacent (≤ 1 m) to the water. We observed small, dense groups of individuals in direct contact with one another, with some mating (Fig. 1D). On 28 September and 1–3 October 2010, we observed only a few adults during the morning. Our anecdotal observations suggest that diurnal reproductive behavior for adults occurs primarily in the morning. We suspect there may be only one synchronous emergence as with other aquatic insects that have short-lived adults or small populations (McCafferty 1983), but this remains to be

determined with more detailed observations for *I. plattensis*.

Whiles et al. (1999) reported that adult emergence in 1997 corresponded with onset of autumn rains and return of water to the slough. In 1997, rains occurred in the region 5 and 6 d prior (Wunderground.com) to the largest adult emergences reported by Whiles et al. (1999). In 2009 and 2010, no precipitation was recorded in the region for the 3 d before our largest observations of adult emergence. For both years, rain fell 4–7 d before adult emergence (Grand Island and Hastings, NE; Wunderground.com). Because the majority of the slough at our site had continuous flow, water was always present. Determining cues that elicit adult emergence could help focus future sampling efforts for adults.

In conclusion, much remains to be learned about the life history of *I. plattensis* along the Platte River in central Nebraska. Our study provides new observations on the types of aquatic habitats occupied by larvae, temporal and spatial variation in larval density in aquatic and terrestrial habitats, variation in timing of emigration from aquatic to terrestrial habitats, subterranean behaviors not described previously for this species, timing of adult emergence and mating behaviors near dawn, and possible cues that elicit adult emergence in autumn. We expect further research on *I. plattensis* will continue to uncover additional life-history attributes that enable it to survive in fluctuating wetland environments, because this paper only represents the second publication that describes facets of its life history. Our study exemplifies the importance and need to understand such life-history traits that can then be incorporated in experimental research projects, which are needed to understand potential threats to this species.

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