UPDATES ON THE DISTRIBUTION AND POPULATION STATUS OF THE PLATTE RIVER CADDISFLY, *IRONOQUIA PLATTENSIS*, AND AN ASSESSMENT OF THREATS TO ITS SURVIVAL

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Masters of Science

University of Nebraska at Kearney

By

Lindsay Anne Vivian

THESIS ACCEPTANCE

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Acceptance for the faculty of the Graduate College, University of Nebraska, in partial fulfillment of the requirements for the degree Master of Science, University of Nebraska at Kearney.

December 2010

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University of Nebraska at Kearney, 2010

Advisor: W. Wyatt Hoback

ABSTRACT

The Platte River caddisfly (PRCF), *Ironoquia plattensis*, was first described from an intermittent slough in south-central Nebraska in 1999 in an area that retains the original topography of the Great Plains. When described, the PRCF attained average aquatic densities of 802 ± 194 larvae per m^2. Because the lifecycle includes a terrestrial aestivation phase, it was noted that the PRCF was likely an important component of energy transfer between the aquatic and terrestrial environments.

Between 1999 and 2004, 49 surveys for additional PRCF populations in central Nebraska were conducted, and only five populations were identified. This study reports recent sampling efforts to identify new populations and visit historic sites to quantify PRCF larval densities and adult activity at each location. Two of the six historic sites were considered extirpated as of 2007, and one of these was the type locality. Between 2009 and 2010, 104 new sites with potential PRCF habitat were visited, and 21 new populations were identified. Six additional sites with either the PRCF or a closely related species were identified on three other Nebraska drainages. Twelve sites along the Platte River and two on other rivers were identified that had larval cases only but no

larvae or adults. Laboratory case degradation studies demonstrate that discarded larval cases left in the environment can persist for a year or more, demonstrating that sites with cases only are not necessarily active. Aquatic and terrestrial sampling found one site to support densities similar to those reported from the type locality. Most other sites represented 30 percent or less of historic numbers.

Because it is endemic and known from few sites in Nebraska, the PRCF has been listed as a Tier I, or at-risk, species. Studies were conducted in the laboratory to understand what may limit the range of the PRCF. The Western mosquitofish, *Gambusia affinis*, is an invasive species to Nebraska and has been implicated in the loss of native fish and amphibians worldwide. However, feeding trials documented little predation by mosquitofish on PRCF larvae. A shade study and behavior trials conducted demonstrate the PRCF is prone to desiccation while it aestivates on land in the summer. This study also documents a previously unidentified behavior by the PRCF. Terrestrial larvae were observed to burrow underground during aestivation possibly to avoid desiccation.

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CHAPTER ONE

INTRODUCTION TO TRICHOPTERA AND THE PLATTE RIVER CADDISFLY, *IRONOQUIA PLATTENSIS*, AND ITS HABITAT

Introduction to Trichoptera

Trichoptera, the caddisflies, are considered to be ecologically important in aquatic systems (McCafferty, 1988; Wiggins, 1977). Caddisflies are abundant and diverse in aquatic habitats, second only to Diptera in diversity in lotic (flowing) habitats (Mackay and Wiggins, 1979; Holzenthal et al., 2007). As an abundant component of the biotic community, caddisflies are important as energy links in aquatic systems (Garono and MacLean, 1988), because of their ability to assimilate smaller food items to become large enough to be utilized by other predators (Ross, 1944). Thus, they are important components of secondary production in aquatic systems (Merritt et al., 1996). Also, surveys of caddisflies in biomonitoring programs can help assess disruptions or anthropogenic disturbances to aquatic ecosystems (Garono and MacLean, 1988), because of their varying degrees of sensitivity to pollution (Houghton et al., 2001).

Taxonomically, caddisflies comprise the order Trichoptera (trichos-hair, pteron-wings; Greek) (Gelhaus and Hamilton, 1981). Trichoptera likely arose from a megalopteran-like ancestor, first appearing in the Triassic during the Mesozoic Era (Ross, 1956; Resh and Solem, 1978). Trichoptera is closely related to Lepidoptera, the butterflies and moths, as both orders spin silk (Holzenthal et al., 2007). Together, Trichoptera and Lepidoptera comprise the superorder Amphiesmenoptera (Holzenthal et al., 2007).

One principal difference separating adult Trichoptera and Lepidoptera is the presence of fine hairs on the wings and bodies of caddisflies, whereas Lepidoptera wings and bodies are covered in scales (Voshell Jr., 2002). Adult caddisflies are often brown, nondescript insects that resemble adult moths (Voshell Jr., 2002). Caddisflies have wings that extend beyond the abdomen (Voshell Jr., 2002) and form a tent shape over the body while at rest (Holzenthal et al., 2007), although some species are wingless (Cummins and Merritt, 1978). Adult caddisflies have long antennae, reduced mouth parts, and a distinct haustellum, which is a short proboscis (Holzenthal et al., 2007); most Lepidoptera adults have a coiled proboscis (Ross, 1944). Adults strictly feed on liquid food, some living up to two months on this diet or longer (Ross, 1944; Svensson, 1972).

Caddisfly larvae are cylindrical and resemble moth and butterfly larvae (Voshell Jr., 2002). The larvae have reduced antennae, no wing pads, and three pairs of legs extending from the thorax (Voshell Jr., 2002). Most larvae have gills on the abdomen (Voshell Jr., 2002). Caddisfly larvae are generally described as hypognathous, or having downward facing mouth parts (Cummins and Merritt, 1978). Caddisflies are holometabolous, having four life stages: egg, larva, pupa, and adult (Gelhaus and Hamilton, 1981). Most Trichoptera have a one year lifecycle (Gelhaus and Hamilton, 1981) and are univoltine (Voshell Jr., 2002).

Trichoptera is found worldwide and in most hydrologic conditions, from lotic (flowing) to lentic (stagnant) bodies of freshwater as well as in shallow marine environments (Anderson et al., 1976; Drysdale, 1999; Holzenthal et al., 2007). Trichoptera represents the seventh largest order of insects, with approximately 13,000 extant species worldwide in 45 families and 600 genera (Drysdale, 1999; Holzenthal et al., 2007). In North America, there are about 1,400 species of caddisflies in 21 families (Voshell et al., 2002), with Limnephilidae being the most diverse caddisfly family

(Mackay and Wiggins, 1979). Locally, caddisflies can be diverse. For example, 148 species of caddisflies have been collected from the Tomah Stream in Maine, a fourth-order tributary of the St. Croix River and its associated wetlands (Huryn and Harris, 2000). The diversity observed in Trichoptera has been attributed to the secretion of silk, as this behavior has allowed caddisflies to exploit a diverse array of habitats (Mackay and Wiggins, 1979; Gelhaus and Hamilton, 1981; Voshell Jr., 2002).

Although an energetically expensive process (Wiggins, 2005), caddisflies fashion silk in many ways, leading to the use of various resources (Mackay and Wiggins, 1979). Silk, spun from glands associated with the labium, is used to construct nets, shelters, or cases (Gelhaus and Hamilton, 1981). There are three suborders of Trichoptera: Annulipalpia, Integripalpia, and Spicipalpia, and these have been classified based on the way caddisflies utilize silk (Holzenthal et al., 2007). Spicipalpia are generally free-living and may construct purse-like cases (Holzenthal et al., 2007). Members of the Annulipalpia construct fixed retreats and nets on the undersides of rocks, while members of the Integripalpia construct portable cases using either organic or mineral material (Holzenthal et al., 2007).

From the suborder Spicipalpia, caddisflies of the family Rhyacophilidae leave a trail of silk as they move and use silk to build a shelter as a fifth instar, or during the pupal phase (Mackay and Wiggins, 1979; Gelhaus and Hamilton, 1981). These free-living caddisflies are generally predaceous (McCafferty, 1988). In contrast, members of Hydropsychidae, from the suborder Annulipalpia, use silk to fashion fixed shelters and nets, to catch drifting and suspended food particles (Williams et al., 1987). Using silk for pupal cocoons or to construct food-capture nets are more primitive behaviors among caddisflies (Ross, 1964; Williams et al., 1987). Members of Limnephilidae and Leptoceridae, from the suborder Integripalpia, use silk to construct cases around the thorax and abdomen and attach either mineral or organic materials (Mackay and Wiggins, 1979). Cases around the abdomen enable caddisflies to forage while offering protection from predators (Williams et al., 1987; Wiggins, 2005).

Case construction in caddisflies is a complex behavior (Holzenthal et al., 2007). Cases are constructed from the front end and trimmed at the posterior end by the larva with its mandibles (Wiggins, 2005). Material is added to the front as the larva grows (Wiggins, 2005). Larvae stripped of their cases are able to rebuild them within a short time period (Fankauser and Reik, 1935). Caddisflies may show preference for either mineral or organic material for case construction (Hanna, 1961; Otto and Svensson, 1980). However, in the absence of the preferred material, caddisflies will stick to the same general case plan (shape, size) and apply available materials (Otto and Svensson, 1980). Some species of caddisflies switch material during case construction, and usually the switch is from organic to mineral material (Otto and Svensson, 1980).

Mineral cases generally require more energy to build than those from plant material, because more silk is needed to fasten the smaller fragments of the case together (Wiggins, 2005). Certain case building behaviors, such as adding heavy ballast stones, rudders made with twigs, or constructing a stream-lined case enable caddisflies to occupy lotic waters with swift currents (Wiggins, 2005). The differences observed in case architectural behavior is thought to have played a role in the diversity of Trichoptera (Wiggins, 2005; Holzenthal et al., 2007).

Aside from using silk in a variety of ways, Trichoptera exhibit resource partitioning, displaying specialized behavior in the way food is obtained (Resh and Solem, 1978; Wiggins, 1978). Different caddisfly species may co-exist in streams, by building silk nets with different mesh sizes (Mackay and Wiggins, 1979) or foraging for food over the entire rock surface while others construct fixed retreats (Wiggins and Mackay, 1978). For example, the exploitation of different food resources has allowed species of the genus *Pycnopsyche* to co-exist (Mackay and Wiggins, 1979). Other caddisfly species may have different lifecycles, with different periods of activity and little temporal overlap (Mackay and Wiggins, 1979). Aside from case building, resource partitioning has likely enabled caddisflies to become a diverse order of aquatic insects (Mackay and Wiggins, 1979).

The caddisfly family Limnephilidae is considered to be the most ecologically diverse family of Trichoptera (Holzenthal et al., 2007) and is the largest caddisfly family in North America with over 900 species in more than 100 genera (Holzenthal et al., 2007). The group is dominant at higher latitudes and elevations, has the widest distribution of any caddisfly family, and comprises one-third of all Nearctic caddisfly species (Wiggins, 1977). Limnephilids are classified as shredders and feed by reducing larger bits of plant material into smaller fragments, and feed on the fungi found on the surface of decaying plant material (Wiggins, 1977). One species of Limnephilidae is apparently endemic to a small range in Nebraska and was described in 1999 by Whiles et al. from a lentic system.

Platte River Caddisfly Described

The Platte River caddisfly (*Ironoquia plattensis* Alexander and Whiles), hereafter PRCF, is a recently described member of the genus *Ironoquia*. The PRCF is found in sloughs along the Platte River in south central Nebraska (Whiles et al., 1999). "Slough" is a regional term used to describe any backwater or pool with a soft muddy substrate and emergent vegetation (dictionary.com). These sloughs are considered ecologically significant because of their high levels of biological productivity and habitat they provide for migratory waterfowl, including the sandhill crane, *Grus canadensis* L., and endangered whooping crane, *Grus americana* L. (Whiles and Goldowitz, 2001). However, because of hydrological and agricultural land changes, slough wetlands are some of the most degraded habitats in Nebraska (Sidle et al., 1989).

The lifecycle and ecology of the PRCF have been described by Whiles et al. (1999) who characterized the PRCF lifecycle using emergence traps and benthic core samples, taken over the course of a year. The PRCF is a univoltine species (Whiles et al., 1999). Adults are active from late September through mid-October and live for seven to ten days. During this time, adults mate and lay eggs on the surface of the slough. The eggs descend to the slough bottom and incubate for about a month before first instars hatch and begin feeding in mid-November. First instars overwinter in the slough and feed little through late February. Larval growth resumes again in the early spring as waters warm, during which time the PRCF progresses through four more instars.

Beginning in late May and proceeding until early June, fifth instars emigrate from the slough and begin aestivating, using lower metabolism to survive times of environmental stress (Wiggins, 1977). PRCF aestivation coincided with slough drying at the time of the life history study (Whiles et al., 1999). Aestivating larvae were found to feed little, but remained somewhat mobile over the summer months. PRCF larvae aestivate until the beginning of September, when they start pupating. Pupation lasts for about three to four weeks, until adult emergence in late-September. Males emerge before females do, with all adults emerging in a three to four week window (Whiles et al., 1999). Adults live seven to ten days (Whiles et al. 1999). In short-lived species like mayflies and caddisflies, simultaneous emergence of adults helps ensure successful reproduction (Resh and Solem, 1978), and possibly to synchronize asynchronous larval development.

There are six known caddisfly species in the *Ironoquia* genus: *I. plattensis*, *I. punctatissima*, *I. parvula*, *I. dubia*, *I. lyrata*, and *I. kaskaskia*, with *I. plattensis* being the most recently described (BayScience Foundation, 2009). Of the *Ironoquia* species, *I. plattensis* is most similar to *I. parvula* (Alexander and Whiles, 2000). It is thought that these two species diverged from a common ancestor during the Pleistocene glaciation (Alexander and Whiles, 2000).

Ironoquia belongs to the subfamily Dicosmoceniae, which is the only Limnephilidae subfamily known from the South American and Australian regions (Wiggins, 1977). Dicosmoceniae mostly occurs in cool, lotic environments, except for the genus *Ironoquia*, which occurs in temporary pools (Flint, 1958; Flint Jr., 1960; Wiggins, 1977). *Ironoquia* species occur throughout the central and eastern portion of the United States, except for *I. dubia* which is found in Europe (Wiggins, 1977). *Ironoquia* is most often collected from temporary systems, but can also occur in perennial waters (Flint, 1958; Gray and Johnson, 1988) (Table 1.1).

The temporary waters from which *Ironoquia* are collected often undergo large temperature fluctuations and loss of water to drying. *Ironoquia* survive habitat drying by aestivating in the leaf litter surrounding pool margins as waters recede (Flint, 1958; Williams and Williams, 1975; Wiggins, 1977). Aestivation lasts from late spring through early autumn (Wiggins, 1977), except for *I. lyrata*, which emerges in June (Mackay, 1969; Wiggins, 1977). Dry periods which may be characteristic and predictable for one area are not the same as drought, which is an episodic phenomenon (Boulton, 2003; Dodds et al., 2004).

While most caddisflies remain in the aquatic environment through adult emergence, terrestrial behaviors in Trichoptera are not unique to *Ironoquia*. A few species of caddisfly are entirely terrestrial, including *Enoicyla pusilla*, *Philocasca demita*, and *Caloca saneva*; the first two of which are limnephilids (Hayashi et al., 2008). *Nothopsyche ruficollis* and *N. yamagataensis* aestivate on land as prepupae (Hayashi et al., 2008). *N. longicornis* may aestivate on land if water levels decrease enough to make a normally aquatic habitat terrestrial (Hayashi et al., 2008).

Terrestrial aestivation is just one behavior displayed in aquatic taxa that enables some macroinvertebrates to survive habitat drying. Macroinvertebrates in transient aquatic habitats must be able to withstand fluctuating temperatures and decreasing oxygen levels as waters dry, reductions in flow, and habitat loss as waters recede (Miller and Golladay, 1996).

To survive in temporary waters, one species of caddisfly, *Limnephilus externus*, lays its eggs in a gelatinous capsule, under rocks and logs. First instars begin developing in this capsule, in the absence of water (Wissinger et al., 2003). *Polycentropus crassicornis* (Trichoptera) eggs have remained viable after an eight month dry period in Ontario (Wiggins et al., 1980). *I. punctatissima* adults may lay their eggs on moist streambeds, before waters return (Williams and Williams, 1975).

Some caddisfly species avoid desiccation by burrowing into damp substrate (Mackay and Wiggins, 1979; Wiggins et al., 1980). Other macroinvertebrate taxa retreat to favorable habitats until waters return (Delucchi and Peckarsky, 1999). Some crustaceans, oligochaetes, and snails may aestivate or remain in the egg phase until waters return, which could be up to eight months (Harrison, 1966, Williams and Hynes, 1976). Some aquatic taxa survive under leaves and rocks, in crayfish burrows, or as desiccation-resistant stages (Boulton, 2003). Quick larval development and early adult emergence (before habitat drying) have also been observed in macroinvertebrates in temporary systems (Delucchi and Peckarsky, 1989; Wiggins et al., 1980).

Inhabiting temporary pools and wetlands can offer a refuge free from predators and competition (Williams, 1996) and an abundant food source (Zamora-Muñoz and Svensson, 1996). Drying pools can be an important source of dying aquatic species for terrestrial fauna, forming an important link of carbon and energy between aquatic and terrestrial environments (Boulton and Suter, 1986; Williams, 1987; and Boulton, 2003).

Whiles et al. (1999) suggested that because of its abundance and emigration from the slough environment, the PRCF is an important link between the aquatic and terrestrial environments. The PRCF may play an important role as a shredder of coarse particulate organic matter (CPOM), potentially prolonging the lifespan of temporary waters (Whiles et al., 1999). A conservative estimate revealed that PRCF larvae consumed about 13 percent of the standing stock at the type locality, thus facilitating leaf litter break down (Whiles et al., 1999). Gut content analysis corroborated this finding, as PRCF larvae were found with senescent plant tissue in their digestive tracts (Whiles et al., 1999).

Aquatic insects classified as shredders facilitate and hasten leaf decomposition (Simon and Benfield, 2001) and may also be important in generating fine particulate organic matter, or FPOM. FPOM is considered an important food source for collector-gatherer macroinvertebrates (Heard and Richardson, 1995), although the importance of shredders as generators of FPOM in aquatic systems may be overstated (Heard and Richardson, 1995). For example, CPOM shredder importance may depend on the quality of particles generated and whether or not FPOM is limited in a system, because FPOM can come from shredder-independent inputs, like stream run-off or microbial abrasion (Heard and Richardson, 1995). Even if the role of the PRCF as a shredder is marginal in sloughs where it is found, at the time of the life history study, it represented an important processor of energy in its habitat, because of its abundance (Whiles et al., 1999). Finally, shredders are typically rare in seasonal wetlands, and thus, species such as the PRCF may be important (Wissinger, 1999; Whiles and Goldowitz, 2005).

Platte River Caddisfly in Decline

When described by Whiles et al. (1999), the PRCF was considered to be a productive and important component of the south central Platte River floodplain ecosystem, formerly accounting for 40 percent of adult insect emergence production from the type locality. From this slough, an average aquatic density of 802 ± 194 larvae per square meter was observed (Whiles et al., 1999). Also noted during the study was the association of the PRCF to the hydrograph of the type locality. The PRCF emigrated from the water before slough drying, and PRCF adults emerged around the time when the slough refilled in autumn (Whiles et al., 1999). Thus, its lifecycle appeared to be intimately associated to a certain hydroperiod (Whiles et al., 1999).

Despite the proximity of four other wetlands, the PRCF was only documented at the type locality, and this may have been due to differences in hydroperiod (Whiles et al., 1999; Whiles and Goldowitz, 2001). The slough from which the PRCF was described held water for 331 days during 1997-1998, whereas one slough held water for 365 days, and the other three wetlands held water for 296 days or less (Whiles and Goldowitz, 2001). Therefore, wetlands either too ephemeral or permanent are potentially unsuitable for the PRCF (Whiles et al., 1999; Whiles and Goldowitz, 2001).

Because of an interest in the PRCF after its discovery, an effort was made to identify additional populations with the caddisfly (Goldowitz, 2004). Through 49 survey efforts on the Platte and Loup Rivers, six populations, including the type locality, were identified (Goldowitz, 2004). All six populations identified were located along a 45-mile stretch of the Platte River corridor. No populations were identified outside the Platte

River, and no other sites supported densities as large as those of the type locality (Goldowitz, 2004; Goldowitz, personal communication, 2009). Further survey work in 2004 found the PRCF to be extirpated from the type locality, likely because the slough was dry by April (Goldowitz, 2004). Survey work in 2007 also found that another historic site, Brooks (Appendix A), was extirpated, likely due to drought (Riens and Hoback, 2008).

Because of its limited range and apparent decline, the PRCF has been classified as a Tier One species within the Nebraska Natural Legacy Project (Schneider et al., 2005). Tier One species are those that are at-risk of extinction on a global or national scale (Schneider et al., 2005). The reasons for the decline of the PRCF are unclear, although several factors have likely acted in concert to reduce PRCF numbers. Because some genera of Trichoptera seem to be dependent on a particular feature in their environment, certain species in a water body may indicate the presence of pristine or undisturbed conditions (Wiggins, 1977; Wiggins and Mackay 1978). Conversely, the absence of such species from a system could be a sign of environmental change (Wiggins and Mackay, 1978). Because the PRCF seems to have a tight association between its lifecycle and given the hydroperiod of sloughs along the Platte River, losing the PRCF from the Platte River valley may demonstrate larger scale environmental changes.

The Platte River region, where the PRCF occurs, has been highly altered through: changes to hydrology, water depletions, home development, urbanization, recreational vehicle use, invasive species encroachment from the Eastern red cedar, *Juniperus virginiana* L. and common reed, *Phragmites australis* (Cav.) Trin. ex Steud., sandpit development, agriculture, and wetland drainage (Dodds et al., 2004; Schneider et al., 2005). Although the PRCF was described in 1999, declines in the species range likely occurred prior its discovery, because changes to the Platte River ecosystem have spanned the last century (Sidle et al., 1989).

Historic Platte River and Potential Threats to the PRCF

The central Platte River has been described as a braided system, because it has two or more channels that branch out and reconnect (anastomosing) and flow around alluvial islands (Leopold and Wolman, 1957; Johnson, 1994). Historically, the Platte River valley was "…an open, sand-bottomed river channel bordered by marshes, sloughs, and endless prairies, stretching up the Valley slopes and across the tablelands" (Eschner et al., 1981; Johnson and Boettcher, 2000). Despite this view, woodlands were part of the Platte River corridor, with an increase in woody vegetation as one travels east from the present-day Colorado border (Johnson, 1994).

Lieutenant Woodbury's 1847 account of the Platte River mentions wooded areas near Fort Kearny and other accounts speak of scanty, shrubby vegetation on river islands (Currier and Davis, 2000). Large islands that may have been heavily wooded (Grand Island, Brady) were beyond the influence of the scouring flows of the Platte River (Currier and Davis, 2000). Side channels and banks contained woody vegetation, primarily cottonwoods, *Populus deltoides* Bartram ex Marsh, American elm, *Ulmus americana* Planch., and willow trees, *Salix* sp. L. (Johnson, 1994).

The Platte River is a dynamic system that historically oscillated between woodland expansion, channel narrowing and channel widening with decreasing woodland cover (Johnson, 1994; Figures 1.1a. through 1.3b.). However, control of prairie fires and regulated water flows has promoted woodland expansion (Currier, 1982; Johnson, 1994). Furthermore, water development has likely altered high flows and ice scours that historically removed vegetation (Johnson, 1994).

By some accounts, the historic Platte River was 1.6 to five kilometers in breadth, with a main channel width of 1.2 kilometers (Willman, 1930; Johnson and Boettcher, 2000) and a history of annual late-spring floods (Jackson and Spence, 1970; Johnson and Boettcher, 2000; Dodds et al., 2004), prairie fires (Johnson and Boettcher, 2000), and drought (Johnson, 1994). More than one hundred years later, the Platte River is only a sliver of its former width (Williams, 1978; Sidle et al., 1989). By comparing historic and current photographs, Sidle et al. (1989) estimated that some areas of the Platte River have incurred an 85 percent reduction in river channel width. Reductions in channel width are visible when comparing historic aerial photographs from 1938 and 2009 (Figures 1.1a. through 1.3b.). The decrease in channel width has been accompanied by a 70 percent reduction in flows since the mid-nineteenth century (Williams, 1978; Sidle et al., 1989). Because water development preceded installation of stream gauges, 70 percent is likely an underestimate (Sidle et al., 1989).

Impoundments, water diversion projects, river channelization, and groundwater withdrawal are major factors in the dewatering and associated hydrologic changes to the Platte River system (Sidle et al., 1989; Johnson, 1994; Dodds et al., 2004). This water development has been accompanied by wetland conversion into agricultural lands (Sidle et al., 1989). Dams and canals along a river can lead to downcutting, or vertical erosion, of a channel downstream of an impoundment by reducing sediment loads, subsequently leading to a lowered water table and loss of backwater areas (Rahel and Thel, 2004). Loss of backwater areas due to water development could potentially have adverse impacts to the PRCF.

Aside from resulting in backwater habitat loss, water development can alter natural fluctuations in flow (Rahel and Thel, 2004). Changes in hydrology could have implications for the PRCF, because slough hydroperiods are influenced by both precipitation and the hydrology of the nearby river (Whiles et al., 1999). Central Nebraska experienced an extensive drought during the early 2000s, and this was implicated in the loss of the PRCF from the type locality (Goldowitz, 2004). Impacts to the species by drought periods, a natural phenomenon, may be exacerbated by water development (Dodds et al., 2004; Rahel and Thel, 2004).

The type locality for the PRCF was an intermittent wetland that supported an array of plant species and a few fish species (Whiles et al., 1999; Whiles and Goldowitz, 2001). Plant species from the site included cattails, *Typha* sp. L., rushes, *Juncus* sp. L., and sedges *Carex* sp. L. Fish species included the common carp, *Cyprinus carpio* L., and the plains topminnow (PTM), *Fundulus sciadicus* Cope (Whiles et al., 1999). The type locality supported the second highest aquatic insect diversity in a series of wetlands investigated by Whiles and Goldowitz (2001). The site that supported the highest macroinvertebrate diversity was also an intermittent slough (Whiles and Goldowitz, 2001).

Wetlands and their communities are being lost rapidly, and this destruction is more widespread in areas with agriculture where irrigation needs compete with wetlands for water (Angeler and García, 2005). Prairie streams and their unique flora and fauna are nearly gone (Samson and Knopf, 1994; Dodds et al., 2004). The Ohio wetlands from which *I. parvula* and *I. punctatissima* have been collected represented approximately one percent of the pre-1912 Ohio landscape, but many have since been lost (Garono and MacLean, 1988). Temporary and isolated wetlands are often neglected or threatened, yet they are used by a high percentage of species on state and federal endangered species lists (Gibbs, 2000).

Several Nebraska non-governmental agencies (NGOs) are actively restoring wetlands in the central Platte region, (Whiles and Goldowitz, 2001); however, restored wetlands often do not parallel natural wetlands in terms of flora and fauna diversity (Galatowitsch and van der Walk; 1996). Restored wetlands may be isolated from natural wetlands, making recolonization difficult, and differences in wetland hydrology between natural and restored wetlands may further limit restoration efforts (Galatowitsch and van der Walk; 1996; Meyer and Whiles, 2008).

A study comparing natural and restored sloughs in central Nebraska found that some aquatic taxa were missing entirely from restored sloughs as compared to natural sloughs (Meyer and Whiles, 2008). Restored wetlands, although beneficial in providing habitat for some species, may not provide the right kind of habitat for specialists like the PRCF. PRCF cases and a low number of individuals have been found in two separate restored sloughs, although the one with live larvae was near the type locality (Vivian, personal observation; C. Meyer, personal communication, 2009). Caddisflies are considered poor fliers, thus it is likely caddisflies mostly disperse by wind as aerial plankton (Bilton et al., 2001; Kelly et al., 2001), making colonization of new available habitat difficult.

Besides wetland hydrology, other factors including cattle grazing, invasive species encroachment and abundance, and transgenic corn crops may threaten PRCF survival. Searches for the PRCF in areas with cattle suggest grazing may have some impact on grassland sloughs that support the PRCF (Vivian, personal observation). Cattle grazing has been shown to reduce odonate abundance and macroinvertebrate diversity through removal of standing vegetation by trampling (Foote and Rice Hornung, 2005). In continuously grazed plots, loss of odonates by trampling was thought to be significant, but this was speculation (Foote and Rice Hornung, 2005). Loss of vegetation along the banks of sloughs could reduce shade cover that may be important for PRCF survival during aestivation.

Invasive species such as reed canarygrass, *Phalaris arundinacea* L., have colonized many North American wetlands. Reed canarygrass has been widely planted for erosion control and forage (Kercher and Zelder, 2004). Extensive stands of reed canarygrass have been observed encroaching on sloughs that support the PRCF (Vivian, personal observation). These stands of reed canarygrass appear to now occupy areas that formerly held water. Common reed is also abundant throughout the main and side channels along the Platte River, and has been observed in side channels that support the

PRCF, but not at the same extent as reed canarygrass (Vivian, personal observation). Thus, invasive species could result in habitat loss for the PRCF.

Transgenic corn expressing *Bt* (*Bacillus thuringiensis*), an endotoxin developed to control the European corn borer, *Ostrinia nubilalis*, (Wraight et al., 2000) has been identified as a potential threat to the PRCF (Riens and Hoback, 2008). However, an experiment designed to test the effects of *Bt* pollen on the black swallowtail butterfly, *Papilio polyxenes*, did not reveal a correlation between exposure and larval mortality (Wraight et al., 2000). Lepidoptera (butterflies and moths) and Trichoptera (caddisflies) larvae have similar life cycles, so effects of *Bt* crops on Lepidoptera may also affect Trichoptera (Rosi-Marshall et al., 2007). Some sites with the PRCF are also in close proximity to agriculture fields.

Thirty-five percent of the corn crop planted in the United States in 2006 was transgenic (Rosi-Marshall et al., 2007). Rosi-Marshall et al. (2007) demonstrated a reduced growth rate in the caddisfly *Lepidostoma liba*, a leaf shredder, when fed *Bt* corn litter in a laboratory setting. However, mortality was not different between caddisflies that ate *Bt* corn and those that did not (Rosi-Marshall et al., 2007). In the field, reduced growth rates could have potential impacts on adult fecundity, because adult insect size and fecundity are positively correlated (Vannote and Sweeney, 1980; Rosi-Marshall et al., 2007). However, other studies have failed to document the impact of *Bt* crops on caddisflies *in situ* (Chambers et al., 2007). Thus, because the lab study from Rosi-Marshall et al. (2007) depicted a worst-case scenario and PRCF larvae do not feed during peak *Bt* corn leaf and pollen input (Whiles et al., 1999), the impact of transgenic crops on the caddisfly is likely minimal.

Some sloughs of south-central Nebraska harbor fish, and the PRCF, when first described, was found in a slough where fish were present (Whiles and Goldowitz, 2001). While the Western mosquitofish, *Gambusia affinis* Baird and Girard, was not among the species reported in this slough (Whiles et al., 1999; Whiles and Goldowitz, 2001), this fish has been identified as a potential threat to the PRCF because of its abundance in backwater sloughs (Riens and Hoback, 2008). The mosquitofish, native to the south coastal United States, has been introduced all over the world for the biological control of mosquitoes (Hurlbert et al., 1972) among diverse aquatic environments (Hubbs, 2000). Declines in native fish and amphibians worldwide have been attributed to exotic fish introductions, particularly by the mosquitofish (Hamer et al., 2002; Kumar and Hwang, 2006). Mosquitofish have also been noted to cause declines of aquatic invertebrates (Kumar and Hwang, 2006).

Mosquitofish were first introduced into Nebraska in 1972, and by 1988 the fish was widely distributed (Haynes, 1993). Mosquitofish may suffer 99 percent mortality in the winter, yet number in the thousands every summer in Nebraska waters (Haynes, 1993). The mosquitofish are adapted for surface feeding (Krumholz, 1948), but their diet includes algae, cladocerans, ostracods and dipterans (Kumar and Hwang, 2006). In a manipulated pool experiment, mosquitofish completely eliminated all insect taxa present, including benthic dwellers, and grazed intensely on zooplankton, subsequently promoting the growth of algae (Hurlbert et al., 1972). Typically, case-building caddisflies that use mineral material for their shelters are better shielded against fish predation than are caddisflies that use organic material (Zamora-Muñoz and Svensson, 1996). However, the potential of mosquitofish to consume caddisflies has not been reported in the literature, and was thus investigated as part of this study.

OBJECTIVES

The objectives of my thesis project were to: 1) identify new populations of the PRCF, 2) quantify larval densities and adult activity at sites with the PRCF, 3) quantify rates of larval case degradation, and 4) investigate and quantify potential threats to PRCF survival by conducting laboratory and field studies.

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FIGURES

Figure 1.1a**.** Historical photograph from 1938 taken just south of Grand Island, NE. The type locality for PRCF (arrow) at the present day Platte River Whooping Crane Maintenance Trust. Few trees dot the landscape.

Figure 1.1b. This photograph was taken in the same area as that of Figure 1.1a. The type locality is shown (arrow). This area has been kept free of trees through habitat management by the Crane Maintenance Trust. Farm Service Agency, 2009.

Figure 1.2a. This picture was taken near Shelton, NE (1938) and shows that some woody vegetation was well established on river islands.

Figure 1.2b. This photograph was taken in the same area in 2009 and shows a decrease in channel width accompanied by an increase in forest cover. Farm Service Agency, 2009.

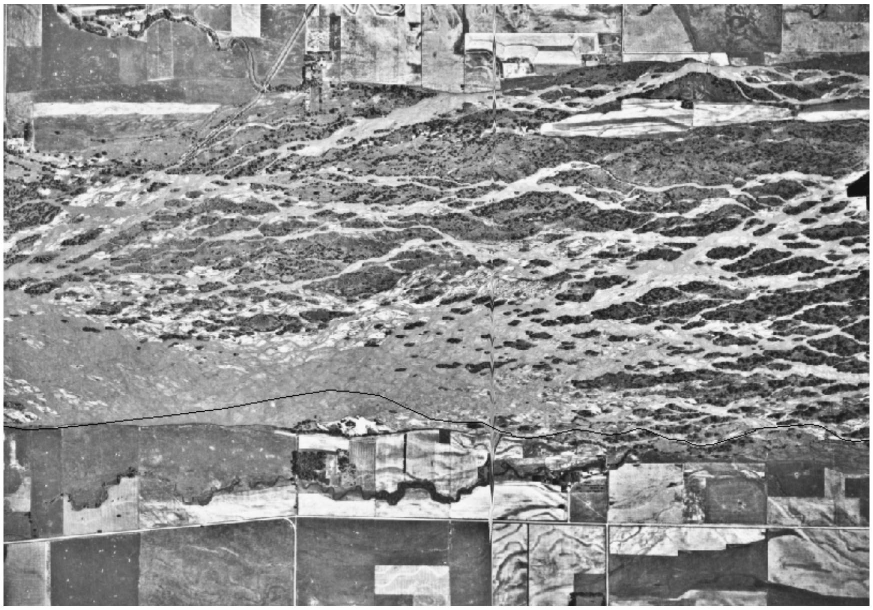

Figure 1.3a. This photograph taken near Overton, NE in 1938 shows a highly braided portion of the Platte River with some woody vegetation.

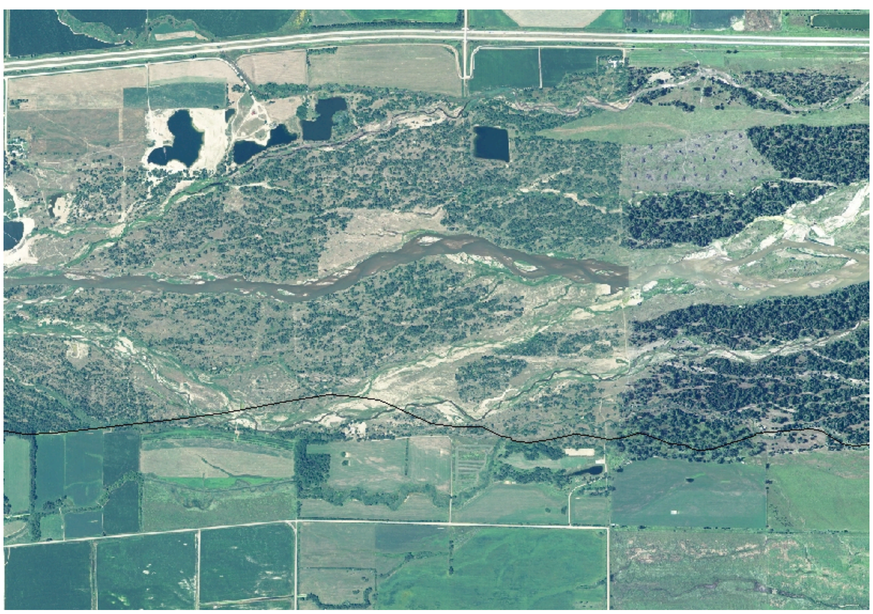

Figure 1.3b. This image from 2009 was taken over the same area shows reduction in channel width and that woody vegetation still dominates the landscape. Farm Service Agency, 2009.

TABLE

Table 1.1. *Ironoquia* sp. and locations where observed and notes on collections.

Species	State, Country, or Province	Habitat and Comments	Reference
I. dubia	Croatia	multiple stream types, ponds with leaves on the benthos	Ćuk and Vučković, 2010
I. lyrata	Quebec	lake/stream system	Roy and Harper, 1981
I. lyrata, I. parvula, I. punctatissima	PA	Linesville Creek and associated tributaries	Swegman et al., 1981
I. parvula	MA, NJ, NY, NH	temporary pool and kettle stream	Flint, 1958
I. plattensis	NE	intermittent and permanent warm water sloughs	Whiles et al., 1999; Goldowitz, 2004
I. punctatissima	IN	intermittent stream, clogs with grasses and rushes when dry	Williams and Williams, 1975
I. punctatissima	Ontario	temporary stream	Clifford, 1966
I. punctatissima	AR	springs and spring runs	Bowles and Mathis, 1989
I. punctatissima	KS	common in headwater springs, rare in intermittent streams	Gray and Johnson, 1988
I. punctatissima, I. lyrata	MN	collections part of comprehensive survey; no habitat affinities noted	Houghton et al., 2001
I. punctatissima, I. parvula	OH	temporary fens and bogs; both species co-occur in glaciated wetlands	MacLean and MacLean, 1984; Usis and MacLean, 1986

CHAPTER TWO

UPDATES ON THE DISTRIBUTION AND POPULATION STATUS OF THE PLATTE RIVER CADDISFLY, *IRONOQUIA PLATTENSIS* AND QUANTIFICATION OF LARVAL CASE DEGRADATION

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ABSTRACT

The Platte River caddisfly (PRCF), *Ironoquia plattensis*, was first described in 1999 and was noted to be abundant at the type locality, with an average larval density of 802 ± 194 larvae per m^2. Surveys for additional PRCF populations occurred from 1999-2004, and these efforts identified five additional populations out of 49 sites surveyed. Surveys in 2004, showed that the PRCF was extirpated at the type locality, potentially because of drought conditions. A search for new populations was conducted in 2009-2010 by identifying potential habitat in aerial photographs, gaining landowner permission, and searching for terrestrial larvae. A total of 104 sites were visited, and 21 new populations along the Platte River were identified. An additional six sites on other drainages in Nebraska were found to support the PRCF or a closely-related species. Fourteen sites had only larval cases with no live individuals observed. Sites with cases only are not considered to be active, because lab trials exposing cases to different environmental simulations reveal larval cases may persist in the environment for a year or more. Survey efforts were also made to quantify larval densities and adult activity across known sites and to compare obtained numbers to historical data from the type locality. At most sites, densities were 30 percent or less of historic numbers. One site supported densities of about 50 percent of historic levels. In 2010, the PRCF was found at historic densities in an area about five km (3.2 mi) upstream of the type locality. Based on surveys, either the PRCF frequently occurs in isolated pockets and low abundances and is locally common in its preferred habitat, or the PRCF has declined in abundance as a result of habitat loss and degradation.

INTRODUCTION

When described, the Platte River caddisfly (PRCF), *Ironoquia plattensis* Alexander and Whiles, once accounted for 40 percent of adult insect emergence production within a warm water slough in south central Nebraska (Whiles et al., 1999). Like other members of the *Ironoquia* genus, *I. plattensis* has a terrestrial aestivation phase, or period of reduced metabolic activity, that lasts about three to four months during the summer (Alexander and Whiles, 2000). Average aquatic PRCF larval density from the type locality was 802 ± 194 larvae per square meter, while a density of 219 individuals per square meter was observed during the aestivation period (Whiles et al., 1999). Thus, the PRCF once represented an important transfer of biomass and energy from the slough to the surrounding upland areas (Whiles et al., 1999).

As a result of its abundance and because it was newly described, surveys were conducted at similar habitats to determine the range and distribution of the PRCF (Goldowitz, 2004). Surveys in 49 locations identified six populations, including the type locality, between Gibbon and Central City, NE (Goldowitz, 2004). Of the 49 surveys, six occurred on the Loup River and 43 were conducted on the Platte. Survey results for the PRCF on the Loup were negative. Later surveys in 2004-2007, did not detect the PRCF at two of the six historic locations, including the type locality (Riens and Hoback, 2008).

Because the PRCF is likely an indicator species of wetland health and function, declining populations could be indicative of wetland loss and changes to hydrology. Currently, the PRCF is listed as a Tier I species in Nebraska (Schneider et al., 2005); however, it is not listed as threatened or endangered. Because of its current rarity and apparent decline, the species is being considered for federal protection under the Endangered Species Act (USFWS, 2009).

One complication for surveys used to establish presence or absence of the PRCF is the potential for discarded cases to persist in the environment. All members of the Trichoptera secrete silk, and some use this material to cement organic or inorganic material together to construct cases around the abdomen (Voshell Jr., 2002). The PRCF forms a mineral case from sand grains. During survey work in 2009, seven sites were identified where only discarded larval cases on land were found; no live individuals were found at these sites. As silk is an environmentally stable protein (Altman et al., 2003), it seems plausible that a case made of inorganic material could persist in nature. Cases of *Neophylax* (Trichoptera: Limnephilidae) and *Brachycentrus* (Trichoptera: Brachycentridae), two common North American genera, can persist in the environment up to three months after pupation (McCabe and Gotelli, 2003). PRCF larval cases have been observed nine months after adult emergence, and the cases showed minimal signs of degradation (Vivian, personal observation). Thus, presence of PRCF cases may not indicate that a site currently supports the PRCF.

The objectives of this study were to: 1) conduct surveys for the PRCF to determine the known range of this species; 2) quantify aquatic or terrestrial larval density and adult activity at all sites with a PRCF population, and to compare these data to historic numbers from the type locality; 3) conduct laboratory experiments with intact and degraded PRCF larval cases to quantify case degradation rates, and 4) conduct a field experiment to quantify the persistence of cases in the environment.

MATERIALS AND METHODS

Identification of New Populations

Unless otherwise noted, potential PRCF habitat was located and identified by first examining satellite imagery in Google Earth® (Google, Inc., Mountain View, CA) (Appendix A). Satellite images of the Platte, Loup, and Elkhorn Rivers were used for identifying potential habitat (Figure 2.1). These drainages comprised the study area for this project. Potential habitat was considered to be any side channel or linear depression with signs of emergent aquatic vegetation that appeared to hold at least some water regardless of the time of year. In Google Earth®, potential habitat appeared light brown or bright green or both. The bright green color was mostly a result of the presence of reed canarygrass (*Phalaris arundinacea*) and duckweed (*Lemna* sp.).

After identification of potential habitat, plat maps were obtained from the Central Platte Natural Resources District (CPNRD) in Grand Island, NE and Nebraska Game and Parks Commission (NGPC) in Lincoln, NE and were used to determine landowner information. Landowner phone numbers were found using Google® PhoneBook. Landowners were contacted during evening hours and called no more than two times. If an answering machine picked up, messages were left, so landowners could call back.

After receiving permission from landowners, potential habitat was subsequently surveyed for the presence of caddisflies. Wildlife Management Areas (WMAs) open to the public and operated by NGPC and land owned by non-governmental agencies (NGO) including Nebraska Public Power District (NPPD), Central Nebraska Public and Power District (CNPPID), Headwaters Corporation, the Nature Conservancy (TNC), and the

Platte River Whooping Crane Maintenance Trust (Trust) were also surveyed for the PRCF. Additional site surveys were conducted as a result of NGO requests or because of the identification of suitable habitat while ground-truthing other sites.

For survey purposes, a site was defined as a backwater area, slough, or side channel that was potentially suitable to the PRCF with at least 100 meters of upland area between it and another such area. One hundred meters was selected, because caddisfly species are generally considered weak fliers (Svensson, 1972), and the PRCF is also thought to be a poor flier (Vivian, personal observation). In cases where sloughs had water that extended further than 100 meters, sampling only occurred within a 100 meter reach of the slough of apparently good habitat. Searches were conducted for 30 minutes, and if no PRCF were found, the site was considered to be unoccupied.

The Platte River was the primary focus for searching for new PRCF populations, because the PRCF was previously thought to occupy only a narrow stretch of the Platte River (Goldowitz, 2004). However, with the incidental identification of a potential sister species/or new PRCF population along the Elkhorn River in Holt County, NE (S. Butler, personal communication, 2009) search efforts were expanded to included the Loup and Elkhorn Rivers. For the other drainages, only areas on public lands were used except for three private sites, which were searched at the request of NGPC staff. Public lands consisted of WMAs and rights-of-way along roadsides. On the other drainages, only presence/absence data were collected.

In 2009-2010, 87 new sites were sampled along the Platte River, 12 sites were sampled on the Loup River drainage, four sites were sampled along the Elkhorn River drainage, and one site was sampled on Wallace Creek, south of Sutherland, NE (not shown in Figure 2.1). For purposes of establishing PRCF presence, only sites where larvae were observed, were recorded as occupied. A Garmin® (Olathe, KS) GPSMAP® 60CSx was used to record the coordinates of each location sampled in Universal Transverse Mercator (UTM, Zone 14T), and a geographic information system (GIS) map displaying each location sampled was made using ArcView 9.2x (ESRI®, San Diego, CA) (Figure 2.2, Figure 2.3).

Aquatic Sampling

Aquatic sampling in May 2009 and May 2010 was conducted at five historic sites and 13 new sites with a PRCF population (Appendix A) to determine the population status of the PRCF in relation to historic numbers. In 2010, five sites were sampled twice. Aquatic sampling for larvae coincided with the aquatic phase of the PRCF lifecycle (Whiles et al., 1999). In each slough, a minimum of four, one-meter passes was made using a 30 cm, D-frame net. Every effort was made to stay within the top three centimeters of substrate. Four passes were made at all sloughs sampled except at the Havens site (Appendix A) in 2009 and the Trust Grassland site (Appendix A) in 2010 (Geluso et al., unpublished data). At the Havens site, 38 samples were taken, because of the high number of isolated pools that were originally considered separate populations. Larval counts were multiplied by 3.33 and divided by four to obtain number of larvae per square meter. The highest average larval density observed at each site (because some populations were sampled more than once) was then divided by 410.67 and expressed as a percentage of the May 1998 densities reported by Whiles et al. (1999).

Terrestrial Sampling

A terrestrial sampling protocol was used to survey all but two populations in 2009 and all new populations identified in 2010. A thirty-minute search protocol was used to determine if the PRCF was present or absent at a given location and to quantify larval density on slough banks during the aestivation phase of the PRCF lifecycle. If no individuals were observed within 30 minutes, the PRCF was considered absent. Larvae are distinguished from old, discarded cases by the presence of the head capsule, visible at the open end of the larval case.

At the beginning of each site visit, a timer was started. If larvae were observed, the timer was stopped, and a 0.25 m^2 quadrat was placed on the ground with the detected larva in the center. All aestivating larvae inside the quadrat were counted. This was done four times, if larvae were found at four separate locations during the thirty-minute search. If larvae were only detected in one instance, then the remaining samples were recorded as zeroes. Total number of larvae observed was multiplied by four and averaged to obtain number of larvae per square meter. Counts were expressed as a percentage of historic numbers by dividing the highest average larval density obtained at each site by 219, because at the type locality, 219 aestivating larvae per m^2 was observed in 1997-1998 (Whiles et al., 1999).

Adult Sampling

Sampling for adults consisted of both nighttime and daytime sampling. A nighttime protocol was implemented first, because most species of Trichoptera are active only at night (Huryn and Harris, 2000). For night sampling, a 175-Watt mercury vapor light was operated for one hour at nine sites. The number of adults that landed on a white sheet (167 cm x 259 cm) hung from a tree branch was counted. The sheet was hung as close to the water line as possible.

Because of several nights below 10°C, rain events, and a lack of insect activity during these sample periods, a daytime protocol was implemented and night survey efforts were stopped. Surveying for adults during daylight hours consisted of walking, crawling along the ground, and intermittently disturbing vegetation for 30 minutes to quantify adult activity. The number of adults observed while day sampling was multiplied by two to obtain number observed per hour. Numbers of adults from sites visited more than once were averaged. During adult sampling, four sites were visited where no larvae had been observed during terrestrial sampling efforts. Two historic sites that are presumed to be extirpated were also visited during adult sampling.

Case Degradation Lab Experiments

Over 300 PRCF larval cases were collected from the Bombeck site (Appendix A) near Gibbon, NE in October 2009. Uniform and moist, intact cases and dry, degraded cases were collected and taken to a laboratory at the University of Nebraska at Kearney. Cases were kept on moist soil in a sealed container until lab trials commenced. Prior to lab trials, soil particles and leaf material were dislodged from cases using a small paintbrush, and cases were allowed to air dry for 24 hours. Cases were then weighed individually to the nearest ten-thousandth of a gram on an Ohaus® SC4010 electronic scale to obtain the initial weight (W_0) for each case.

Twenty degraded and 20 intact cases were used in each lab simulation. Degraded cases were those that had a tear or hole or were non-uniform in appearance. Intact cases were uniform in appearance and had no visible damage. Cases were exposed to one of four conditions: 1) wet for the experiment's duration (wet-only), 2) subjected to a weekly wetting/drying cycle, 3) subjected to a weekly freeze/thaw cycle, or 4) controls kept in individual airtight vials. Standard-sized ice cube trays were used to keep cases separate in each of the simulations.

For the wet-only, wet/dry, and freeze/thaw simulations, water was placed into each ice cube tray compartment around each case. Freeze/thaw cases were immersed in nanopure water, placed in a freezer (-20°C) for one week, then allowed to thaw and dry for 12 hours, weighed, and then covered with water and refrozen. The remaining cases were kept in a closed, dark Percival® environmental chamber (Percival Scientific, Inc., Perry, IA). Once weekly, water was removed from individual cases in the wet/dry and freeze/thaw simulations using an eyedropper. Cases were left to dry for 12 hours and subsequently weighed then wetted or frozen again. Treatments were repeated seven times. Cases in the wet-only simulation and control cases were weighed at the end of the study. Case weights at the end of the study were recorded as W_1.

UV Case Experiment

In a separate lab experiment, 20 degraded and 20 intact cases were exposed to constant UV light (Germicidal UVC T5, 14-Watt G14T5L/4P, cureUV.com, Delray Beach, Florida) for a six week period. This light emitted UV light at a wavelength of 253.7 nanometers (cureUV.com), and was selected based on its short frequency, for a maximum exposure or worse-case scenario (Chen et al., 2010). The UV bulb was placed 30 cm above the ice cube trays. Cases in the UV simulation were stored in a Percival environmental chamber with only the UV light on. Cases in the UV light trial were weighed once every seven to ten days for a total of six times. Control cases were stored individually in sealed vials and placed in a dark Percival® chamber for the entire study.

Case Degradation Field Experiment

Twenty mesh frame boxes (10 cm x 10 cm x 1 cm) constructed of 10 mesh woven stainless steel (0.0098 cm wire diameter, TWP, inc., Berkeley, CA) were randomly placed on slough banks of two separate sloughs at the Crane Trust near Alda, NE. Ten boxes ($n = 10$) were placed on the banks of a slough in a grassland setting (Trust Grassland, Appendix A), and ten boxes ($n = 10$) were placed on the banks of a forested slough (Trust Forested, Appendix A). Both sloughs currently support PRCF populations. Five cases, previously weighed together (W_0) in the same fashion as cases weighed during lab trials, were placed in each frame and left exposed in the field from 13 July 2010 to 30 September 2010.

Only five mesh boxes from each site were subsequently recovered. Five at the grassland slough were not recovered because of apparent trampling by cattle. Five were not recovered at the forested slough, either because the boxes were lost, or the boxes no longer contained five cases. Cases were weighed in the laboratory to obtain a final weight (W_1).

Statistical Analysis

Mean W_0 and mean W_1 for degraded or intact cases in each condition were compared using a student's T-test ($p = 0.05$). For the field tests, the average percent of initial weight lost through the experiment was compared between cases placed in the Trust Forested site and cases placed in the Trust Grassland site using a Mann-Whitney U test ($p = 0.05$).

RESULTS

Survey Results

Over five hundred locations were identified as potentially suitable habitat in Google Earth®, along the Platte, Loup, and Elkhorn drainages in Nebraska. Using plat maps 225 private landowners were identified. From a list of private landowners, 195 phone numbers were entered into Google® Phonebook, and 125 were retrieved. Some landowners were not contacted, because their listed phone number was from outside central Nebraska. Out of 125 landowners, 67 were contacted either through direct communication, or leaving a message. Of 67 landowners contacted: one number was out of service, 22 landowners either never responded or did not have answering machines, 30 landowners granted permission, and 13 did not grant permission. A total of 22 private properties were visited. Some private and NGO lands contained more than one slough that was sampled. Two surveys were not conducted because of the lack of suitable habitat on their property.

Between 2009 and 2010, 104 surveys for the PRCF were conducted, and six historic sites were revisited to survey for the PRCF. One population at the Crane Trust near Alda, NE, was identified by Geluso et al. (unpublished data, 2010). Of 110 sites visited in 2009-2010, 43 occurred on public lands or rights-of-way, 34 were on NGO land, and 33 occurred on private lands (Appendix A).

Out of 92 areas surveyed along the Platte River, 21 new PRCF populations were identified, or approximately 23 percent (Figure 2.2). Including historic populations, 26 extant PRCF populations occur along the Platte River (Figure 2.2). An additional 13 percent (12 sites) were found to have only PRCF cases without live larvae or adults (Figure 2.2). Approximately 60 percent (54 sites) did not contain cases or living PRCF (Figure 2.21). Of these 54 locations: seven had evidence of cattle grazing (compacted soils, reduced vegetation cover), 10 were sloughs that had been restored within the last ten years, and 10 were sites with vegetation and water characteristics that visually differs from sites with extant populations. Twenty-seven areas contained apparently suitable habitat but did not contain PRCF (Appendix A).

Out of 18 sites surveyed outside the Platte River, six new populations of either the PRCF or a closely-related species were identified, two sites were identified that contained cases only, and 10 sites did not contain cases or living PRCF (Figure 2.3). Among these sites, two sloughs were previously restored, one had evidence of cattle grazing, and two had marsh habitat which appeared too deep to support the PRCF (Appendix A).

Through all survey efforts, the known distribution of the PRCF now stretches approximately 375 kilometers (210 miles) along the Platte River, approximately five times the historical distribution (Figure 2.2).

PRCF Population Status

Aquatic and Terrestrial Sampling

Based on 2009 and 2010 aquatic sampling, the Bombeck site most often contained the highest densities of the PRCF (Table 2.1). However, densities from the Bombeck site represented less than 15 percent of the densities observed at the type locality (Whiles et al., 1999). During 2010 aquatic sampling, an average larval density of 125.7 ± 95.47 larvae per m^2 was observed at the Patrick site, or about 31 percent of historic numbers. Also during 2010 aquatic sampling, an average larval density of 553 ± 284 larvae per m^2 (n = 19) was observed at the Trust Grassland site (Geluso et al., 2010, unpublished data) (Table 2.1). The average density observed at the Trust Grassland site represented 135 percent of historic numbers and represented the largest known PRCF population observed during 2009-2010 (Table 2.1). Out of 16 sites sampled for larvae, 14 represented less than 10 percent of numbers from the type locality in 1997-1998 (Whiles et al., 1999; Table 2.1).

Through all terrestrial sampling efforts, the Bombeck site contained the highest densities of aestivating larvae (Table 2.2). One quadrat sampled at the Bombeck site contained 280 larvae per m^2, which is more than the average number of aestivating larvae per m^2 observed at the type locality in 1997-1998. However, despite terrestrial sampling efforts at 13 sites with extant populations, overall, only 12 percent of 1997-1998 densities were observed. The Bombeck site had 53 percent of historic numbers of aestivating larvae (Table 2.2). No larvae were collected at one historic site, Brooks (Appendix A), and it is considered to be extirpated (Figure 2.2).

Adult Sampling

Night sampling for adults seemed to be highly influenced by several evenings with temperatures below 10°C and a general lack of insect activity below this temperature (Vivian, personal observation). The highest number of adults observed during night trapping was 5.4 individuals per hour at the Bombeck Site (Table 2.3). Four adults per hour were observed at the Trust Forested site (Table 2.3). Out of nine sites sampled, zero adults were observed at four locations (Table 2.3). One of these sites was where larvae were not observed during aquatic or terrestrial sampling in 2009, and two of these locations were historic sites that were presumed extirpated, including the type locality. One adult was observed at two of nine sites.

During daytime sampling, the highest number of adults observed was at the Bombeck and Hord Lake sites, where an average of 8 ± 3.06 adults and 8 ± 2 adults was observed per hour, respectively (Table 2.4). Havens had the third highest number of adults observed per hour, with 6 ± 6 adults. Five sites visited were those where larvae had not been observed previously and one was the type locality, and no adults were found at these sites (Table 2.4).

Case Degradation Lab and Field Experiments

The initial weight (W_0) of intact cases in laboratory studies ranged from 19.6 to 22.5 mg. After seven weeks, the average percent (%) of weight loss in the intact cases used in the wet/dry, freeze/thaw, and wet-only simulations was 8.46% ± 0.94, 7.10% ± 1.12, and 9.08% ± 1.36, respectively. Control cases gained 1.80% ± 0.20 of W_0. Intact cases in the wet/dry condition lost about zero to 2.00% of case weight weekly and those in the freeze/thaw treatment lost between 0.04 and 3.62% (Figure 2.4).

The starting weight (W_0) of degraded cases used in the lab simulations ranged from 16.6 to 18.7 mg. The average percent of weight lost by the degraded cases used in the wet/dry, freeze/thaw, and wet-only simulations was 11.66% ± 1.72, 15.52% ± 2.56, and 9.52% ± 1.48, respectively. Control degraded cases gained 1.04% ± 0.64 of W_0. Degraded cases in the wet/dry treatment lost between zero and 3.07% of case weight weekly (Figure 2.5). For the freeze/thaw treatment, degraded cases lost 1.11 to 3.72% case weight weekly (Figure 2.5). Using a student's T-test for treatments, the only statistical difference detected between W_0 and W_1 was with the degraded cases in the freeze/thaw treatment ($p = 0.013$, $n = 19$) (Figure 2.7). Differences in W_0 and W_1 in all other simulations were not significant ($p > 0.05$, $n = 20$) (Figure 2.6, Figure 2.7).

Neither intact nor degraded cases in the UV light treatment lost any weight, but instead gained between 1.48 and 3.37% of their initial W_0.

Cases in open grassland sites had an average weight loss of 5.08% ± 2.86 compared to the cases placed under shade, which lost 2.18% ± 2.60. However, the differences were not statistically significant (Mann-Whitney U-test, $p = 0.421$, $n = 5$).

DISCUSSION

An Increase in the Known Range of the PRCF

With an identification of 21 new PRCF populations in 2009 and 2010, the known range of the PRCF is now approximately five times larger than the range previously reported for the species (Goldowitz, 2004). Survey efforts also identified six additional populations of a caddisfly species which may be either the PRCF or a closely related species on the Loup and Elkhorn drainages. These observations suggest that the PRCF could be distributed among the Platte, Loup, and Elkhorn Rivers in the eastern third of Nebraska. The South, Middle, and North Loup Rivers all converge near St. Paul, NE, which is about 40 km (25 mi) north of the Platte River. Also, one population on the Loup River at Prairie Wolf WMA, occurs near Genoa, NE, and lies about 32 km (20 mi) west of the convergence of the Loup and Platte Rivers (Figure 2.1). The habitat of sites from the Platte, Loup, and Elkhorn drainages appear similar in vegetation structure and community and hydroperiod (Appendix A).

Although new PRCF populations have led to an increase in the known range of this species, the distribution of the PRCF appears to be highly disjunct (Figure 2.2). Currently, a gap in PRCF populations of approximately 155 km (97 mi) occurs between Elm Creek, NE and Hershey, NE and along the Platte River. Twenty-four potential sites were visited and searched for the PRCF. At two sites, only cases were found, but PRCF are considered absent because no larvae or adults were observed (Appendix A). The failure to detect the PRCF within this area of the Platte could be a result of flooding that occurred in 2010, cattle grazing, lack of suitable habitat, or time of year that the surveys were conducted.

Within the 155 km gap, several canals divert water for irrigation, and this water diversion has accompanied wetland loss in the Platte River region (Sidle et al., 1989). During surveys at Dogwood WMA (Appendix A), several depressions were observed, and these areas were dry but showed signs of past beaver (*Castor canadensis* Kuhl) activity, indicating that changes in water levels had occurred. Whether these changes in water level were a result of irrigation withdrawal or drought, the habitat no longer appears suitable for the PRCF.

The methodology used to detect new PRCF populations in 2009-2010 was to search the ground for PRCF cases and aestivating larvae. In May 2010, aestivating larvae were observed to burrow into the ground, potentially to avoid desiccation (Wiggins et al., 1980). Larvae were found five to ten centimeters below the ground surface and against plant roots. This behavior was first observed at the Trust Grassland site (Appendix A) near Alda, NE (Vivian, personal observation; Geluso et al., unpublished data). Burial by PRCF larvae could result in false negatives.

Because of the discovery of burial behavior by the PRCF, a shovel was used to supplement terrestrial sampling at seven sites during 2010 survey efforts. Two sites with cases only were identified in this manner, and surveys for adults showed caddisfly (unknown if PRCF) presence at Prairie Wolf WMA (Appendix A). However, no sites were identified as having living PRCF only through digging, and all sites with extant PRCF were observed to have cases on the soil surface.

In 2010, several rain events occurred in May and June and resulted in extensive flooding along the Platte River. However the flooding did not appear to impact aestivating larvae, because larvae were observed in late June 2010, after flood waters had receded at the Bombeck site (Vivian, personal observation). Flooding may wash away discarded cases from past emergence events and distribute them to new areas. Dispersal of discarded cases by flooding events is another reason to be cautious in interpreting PRCF presence at a site based only on discarded cases.

Between Elm Creek and Hershey, NE, cattle grazing and ranches are common along the Platte River. It is unknown whether cattle grazing negatively affects the PRCF. However, overgrazing can reduce macroinvertebrate diversity and abundance through loss of standing vegetation (Foote and Rice Hornung, 2005). Moreover, increased water temperatures in grazed areas, have been noted to impact other aquatic insect populations (Johnson et al., 1977; Kauffman and Krueger, 1984).

During surveys, nine sites were visited where grazing appears to have resulted in reduced vegetative cover and the presence of compacted soils. The PRCF was found at one slough where cattle graze, but the area had been idle for two years prior to identification of this population (M. Harner, personal communication, 2010). Research is needed to determine potential impacts of grazing on the PRCF.

In 2009 and 2010, most surveys for PRCF populations occurred after mid-July. By this time of year, mortality may occur to aestivating PRCF larvae, possibly because of desiccation (Vivian, personal observation). Mortality has been reported in aestivating *I. parvula* larvae (Flint, 1958). Overall, more aestivating larvae appear to be present in

May-June, which may also be a result of the burial behavior as discussed above. Surveys for aestivating larvae should be conducted in early summer.

Population Status

Larval sampling during the aquatic phase of the lifecycle appears to be the best method for assessing PRCF population status. During terrestrial and adult sampling, sampling for aestivating larvae may have been affected by burial behavior and differences in emergence times from the slough, while sampling for adults was likely complicated by weather conditions and narrow activity periods.

Aquatic sampling efforts in 2010 detected a population with maximum larval densities that exceed those observed at the type locality, with 135 percent of historic numbers observed (Whiles et al., 1999; Geluso et al., unpublished data). The Trust Grassland site is approximately five km upstream of the type locality and is similar to the type locality in plant structure and geomorphology (Vivian, personal observation). Slough hydroperiod in 2009-2010 was also similar to what was observed at the type locality (Whiles et al., 1999). Near Chambers, NE on the Elkhorn drainage, caddisfly densities also approach numbers observed at the type locality. Over two hundred larvae have been collected in a minimum of five D-frame net grab samples.

Except for the Trust Grassland site, all sites sampled along the Platte River supported larval densities of 30 percent or less of historic numbers. Several sites were found to have densities of less than one individual per square meter. Given the abundance of PRCF larvae at the type locality in 1997-1998 and at the Trust Grassland site, it appears that the PRCF achieves higher population abundances in certain locations than others. *Ironoquia punctatissima* has been observed with similar population characteristics and has been described as locally abundant in certain systems (Gray and Johnson, 1988).

Low densities compared to those of the type locality may indicate that sloughs where most PRCF populations occur are either marginal habitat or degraded. One factor affecting habitat quality could be hydrology. Hydrology may influence PRCF presence and abundance, as areas with water depth greater than one meter deep or sites where water appeared to be permanent had fewer PRCF (i.e. McCormick 1, Table 2.2), perhaps because permanent waters may be more favorable to amphibians which can prey on caddisfly larvae (Wissinger et al., 2003; Tarr and Babbitt 2007).

During both day and night sampling for adults, the greatest number of adults observed per hour was at the Bombeck site (Table 2.2). At most sites, less than one individual was observed per hour. Collecting few adults by light-trapping and day sampling was potentially hampered by cold weather. PRCF adults also seem to be more active during morning and evening hours with reduced activity during the day, further affecting survey results.

When comparing observed PRCF densities from sites sampled in 2009-2010 to those observed at the type locality and at the Trust Grassland site, it seems that the PRCF has declined. However, between 1999 and 2004, PRCF densities at the other five historic sites were similar to those observed in 2009-2010 (B. Goldowitz, personal communication, 2009). Because insect species can be locally common but rare in other areas (Gray and Johnson, 1988), it is difficult to establish by comparing larval densities whether or not the PRCF declined either prior to 1999 or since that time. More survey work and population monitoring at known sites is needed to assess changes to PRCF population densities.

Case Experiments

Lab experiments indicate that PRCF cases could persist in the environment for a year or more even if they are repeatedly wetted and dried or if they are frozen and thawed. Significant differences were only detected between initial and final weights for degraded cases after seven freeze/thaw simulations, and these cases lost less than four percent of their initial mass.

Cases in the laboratory were allowed to dry completely or were completely covered in water or encased in ice. Cases in the field are unlikely to experience the conditions from the laboratory experiments, and thus cases may persist for years.

Cases left in the grassland and forested sloughs for a period of two months lost an average of 2.2 and 5.1 percent of their initial weight, respectively. However, several cases from the grassland slough and some from the forested slough were dry and fragile to the touch, breaking apart during weighing. This may have been because some cases in the experiment were exposed to direct sunlight and at times were exposed to high temperatures (maximum recorded temperature was 52°C). In the laboratory, cases dried at 40^{o} C for a period of 12 hours disintegrated upon handling (L. Vivian personal observation). Thus, the silk may degrade rapidly at warmer temperatures, and this may explain the absence of cases from open areas and the burial behavior observed at grassland sites.

During the 2009-2010 surveys, twelve sites were found to only contain cases. Most of these cases were collected from moist and shaded environments. These areas are shielded from direct sunlight, high temperatures, direct rain events, and desiccation. The majority of sites where PRCF are currently found contrast with the type locality. Most sites with the PRCF support shade canopies, whereas the type locality was treeless (Whiles et al., 1999). Because it seems likely that PRCF cases could persist for several years in moist, shaded conditions, locating PRCF cases at a site does not indicate PRCF presence at that location.

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FIGURES

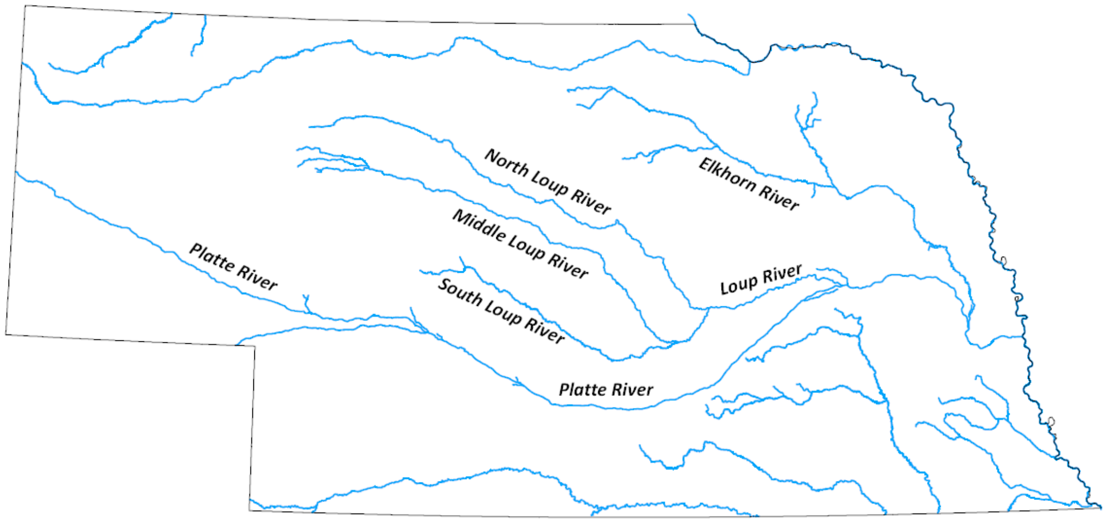

Figure 2.1. Searches for the caddisfly occurred at sites on the Platte, Loup, and Elkhorn Rivers.

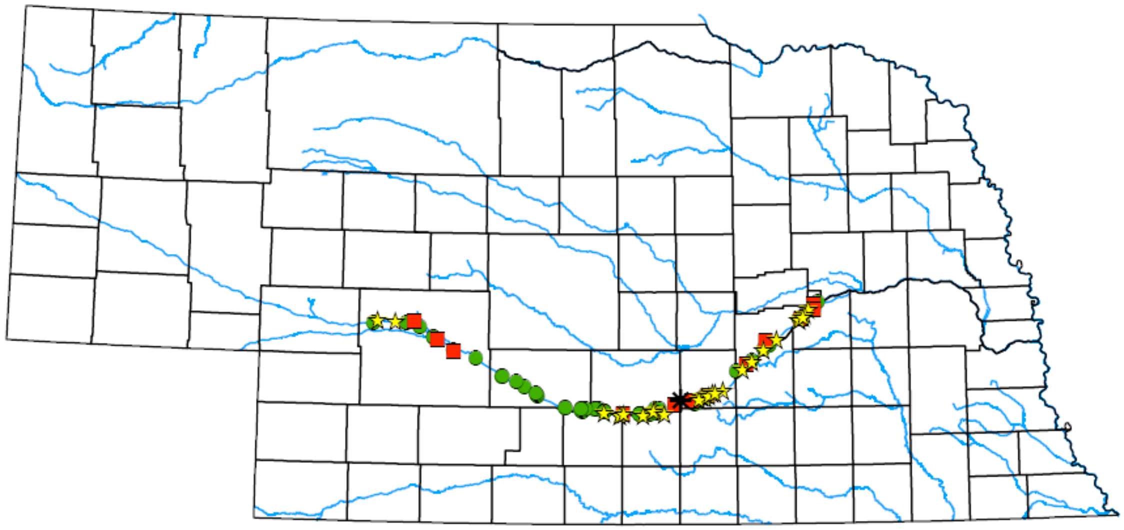

Figure 2.2 Legend

PRCF Present
PRCF Absent
PRCF Larval Cases Only
PRCF Extirpated

Figure 2.2. Map shows all surveys conducted in search of the PRCF on the Platte River.

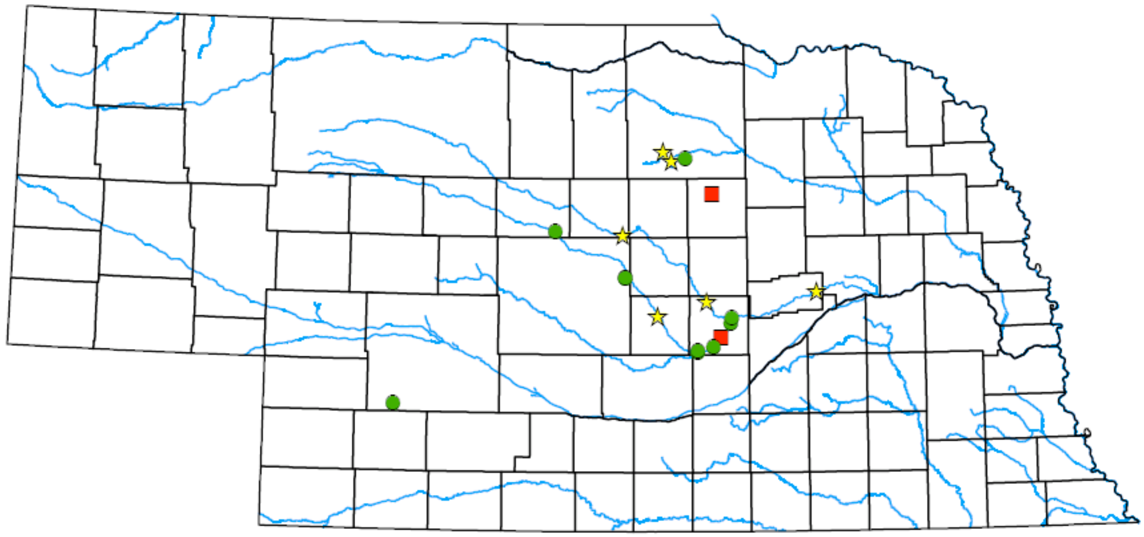

Figure 2.3 Legend

☆ Caddisfly Present
○ Caddisfly Absent
□ Caddisfly Larval Cases Only

Figure 2.3. Survey locations for the PRCF or a closely related species on drainages outside the Platte River.

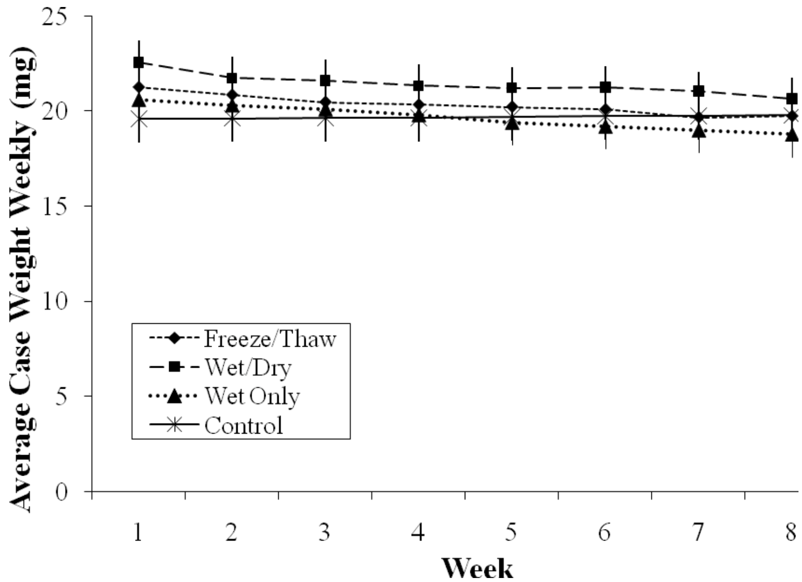

Figure 2.4. Mean weight (mg) (± 1 S.E.) of intact cases as measured weekly after removing the cases from experimental conditions and air drying them for 12 hours at room temperature.

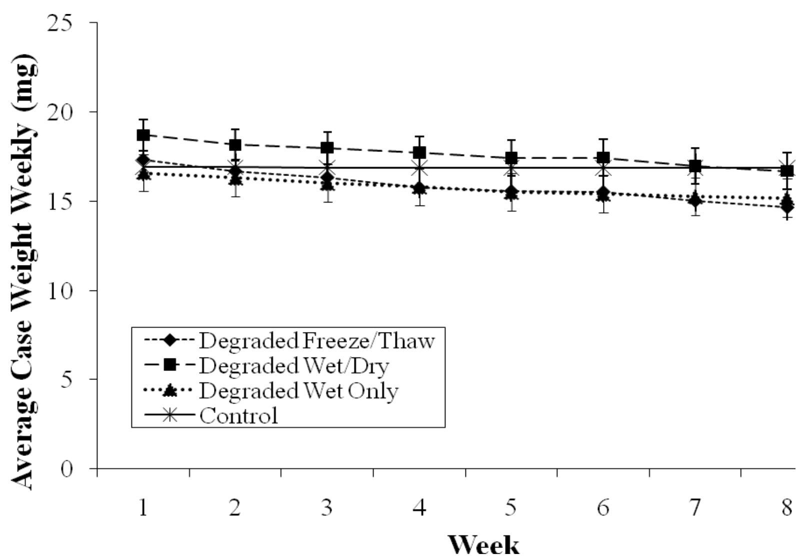

Figure 2.5. Mean weight (mg) (± 1 S.E.) of degraded cases as measured weekly after removing the cases from experimental conditions and air drying them for 12 hours at room temperature.

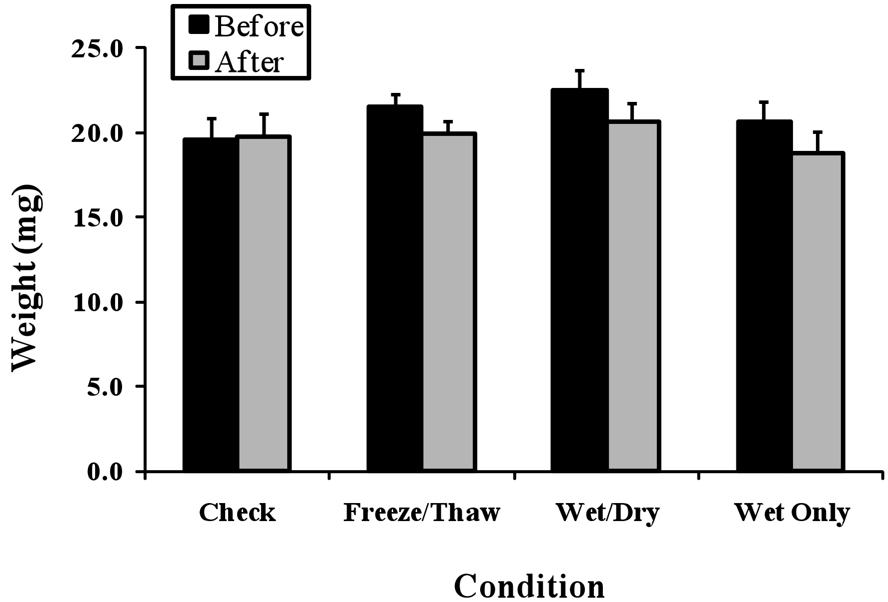

Figure 2.6. Mean W_0 and W_1 (± 1 S.E.) of intact cases from all conditions used in the case degradation experiment.

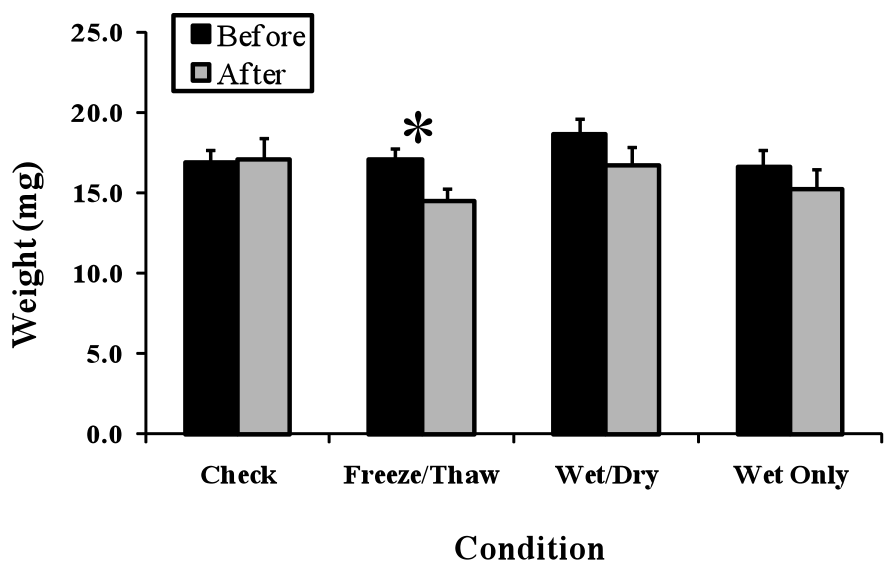

Figure 2.7. Mean W_0 and W_1 (± 1. S.E.) of degraded cases from all conditions used in the case degradation experiment. Significant differences ($p < 0.05$) marked with an asterisk (*).

TABLES

Table 2.1. Average number (± 1 S.E.) of larvae collected at each site through D-frame sampling. One asterisk (*) denotes sites where live individuals have been observed, but where no larvae were detected during aquatic sampling. Site marked with two asterisks (**) was not sampled by L. Vivian (Geluso et al., unpublished data). Second column 2010b reports densities from sites sampled a second time. All densities are expressed as a percentage of 410.67, because 410.67 larvae per m^2 was the average density observed at the type locality in May 1998.

Site Name	**Larvae per m^2 2009**	**Larvae per m^2 2010a**	**Larvae per m^2 2010b**	**Percent of 1997 (410.67)**
Bader	0.83 ± 0.83	0.83 ± 0.83	--	0.2
Bassway	--	1.7 ± 1.67	--	0.41
Binfield 1	1.7 ± 0.96	0 ± 0	--	0.41
Binfield 2	--	1.7 ± 0.97	--	0.2
Bombeck	54.0 ± 14.14	45.8 ± 8.95	98.2 ± 43.53	23.96
Dearking	--	0.83 ± 0.83	--	0.1
East Odessa*	--	0 ± 0	--	0
Glasser	--	5.8 ± 2.91	--	1.41
Havens	23.2 ± 4.98	29.1 ± 10.65	15.0 ± 2.15	7.11
Hoback	--	12.5 ± 4.78	--	3.05
Hord Lake	4.2 ± 3.15	75.8 ± 47.55	43.3 ± 22.21	18.5
McCormick 1	--	7.5 ± 6.43	0 ± 0	1.84
Newark	--	1.7 ± 0.96	--	0.41
Patrick	--	125.7 ± 95.47	--	30.68
Sock	--	30.8 ± 22.02	--	7.52
TNC Wood River*	--	0 ± 0	--	0
Trust Forested	--	10 ± 5.61	0.83 ± 0.83	2.45
Trust Grassland**	--	553 ± 284.0	--	134.67

Table 2.2. Average PRCF larval densities (± 1 S.E.) observed through terrestrial sampling using the quadrat protocol. All densities are expressed as a percentage of 219, because 219 larvae per m^2 was the average density observed at the type locality in June 1998 during the aestivation period of the PRCF lifecycle.

Site Name	**Larvae per m^2 2009**	**Larvae per m^2 2010**	**Percent of 1997 (219 larvae/m^2)**
Bassway	1 ± 1.00	--	0.46
Binfield 1	0 ± 0	--	0
Binfield 3	5 ± 1	--	2.28
Bombeck	9 ± 3	116 ± 54.72	52.97
Dearking	3 ±1.91	--	1.37
Glasser	3 ± 1	--	1.37
Havens	N/A	25 ± 3.42	11.42
Hoback	9 ± 5.74	--	3.65
Hord Lake	5 ± 2.52	14 ± 4.16	6.39
McCormick 1	--	11 ± 4.43	5.02
North River	--	1 ± 1	0.46
Patrick	2 ±1.15	--	0.91
Sock	3 ± 3	--	1.37
Trust Forested	1 ± 1	26 ± 5.29	11.87

Table 2.3. Number of adults observed per hour using a 175-Watt mercury vapor light and white sheet (167 cm x 259 cm) during 2009 nighttime sampling. Sites with an asterisk (*) indicate sites surveyed despite finding no PRCF during larval sampling.

Site	**Adults/hour**
Binfield 1	1
Binfield 3	0
Blue Hole	0
Bombeck	5.4 ± 2.96
Brooks*	0
Hoback	1.33 ± 2.33
McCormick 1	1
Trust Forested	4
Type Locality*	0

Table 2.4. Number of adults observed per hour during day sampling. Sites with an asterisk (*) indicate sites surveyed despite finding no PRCF during larval sampling.

Site	Adults/hour
6th and C*	0
Bader Park	0
Bell	0
Blue Hole*	0
Bombeck	8 ± 3.06
Havens	6 ± 6
Hoback	2
Hord Lake	8 ± 2
Patrick	4
Sock	0
TNC Wood River*	0
Trust Forested	0
Type Locality*	0
Willhoft*	0

CHAPTER THREE

AN ASSESSMENT OF THREATS TO PLATTE RIVER CADDISFLY, *IRONOQUIA PLATTENSIS*, SURVIVAL

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ABSTRACT

The Platte River caddisfly (PRCF), *Ironoquia plattensis*, was first described from a slough on the central Platte River in 1999. At the type locality, the PRCF was an abundant component of the invertebrate community, attaining larval densities of 1,000 individuals per m^2. Surveys for the species conducted between 1999 and 2004 revealed a limited range for the PRCF. Recent sampling has found 26 locations to support the PRCF, but only one population is as dense as was reported in 1999. Because of its apparent decline in abundance, there is a need to understand the biotic and abiotic factors limiting the distribution and abundance of this species. Water quality parameters including dissolved oxygen, conductivity, turbidity, total dissolved solids, and pH were measured at sites with PRCF (occupied) and similar (unoccupied) sites and compared. No water quality parameter measured differed. During a field study, PRCF larvae were observed to prefer moist, shaded microclimates during the aestivation period. In the laboratory, the effects of fish predation, desiccation, and immersion were examined. Low numbers of cased-caddisfly larvae were consumed by native plains topminnow, *Fundulus sciadicus*, and less than 2% of larvae were consumed by the exotic Western mosquitofish, *Gambusia affinis*. Laboratory behavioral trials conducted with terrestrial cased-caddisfly larvae showed movement away from heat and light. In response to heat, 20 of 30 larvae selected moist soil with leaves over soil only, sand only, and no substrate within 30 minutes. Immersion for 72 hours during simulated flooding resulted in low (7%) mortality of aestivating larvae.

INTRODUCTION

The *Ironoquia* genus consists of six species distributed mostly in the Midwest and Northeastern United States and into Canada (Flint, 1958; Williams and Williams, 1975; Williams and Hynes, 1976; Swegman et al., 1981; Garono and MacLean, 1988). One species, *Ironoquia dubia* Stephens, is known only from Europe (Svensson and Tjeder, 1975; Johansson and Nilsson, 1994). Although *Ironoquia* has been collected from a variety of habitats, *Ironoquia* are predominately collected from intermittent streams and pools (Flint, 1958; Williams and Hynes, 1976; MacLean and MacLean, 1984; Garono and MacLean, 1988, Urbanič, 2006). Recently, a new species of *Ironoquia* was described from an intermittent slough in central Nebraska (Alexander and Whiles, 2000).

All *Ironoquia* species are known to aestivate on land during the summer months prior to pupating (Flint, 1958; Svensson and Tjeder, 1975; Wiggins, 1977; Johansson and Nilsson, 1994; Whiles et al., 1999). This behavior likely enables *Ironoquia* to withstand summer drying of temporary streams and pools (Wiggins et al., 1980; Johansson and Nilsson, 1994). Aestivation of terrestrial caddisfly larvae and pupae usually occurs in a moist environment adjacent to the water's edge (Flint, 1958), perhaps as a means to avoid desiccation (Wiggins et al., 1980), which can cause mortality during terrestrial life stages (Wisseman and Anderson, 1984). The pupal characteristics of *Ironoquia* are similar to those of the entirely terrestrial caddisfly, *Enoicyla pusilla* (Flint, 1958). Other completely terrestrial caddisfly species are known, including *Philocasca demita*, *Nothopsyche montivaga* and *Caloca saneva* (Rathjen, 1939; Anderson, 1967; Neboiss, 1979; Nozaki, 1999; Hayashi et al., 2008). But these terrestrial species are restricted to moist environments (Wiggins and Mackay, 1978).

As a semi-terrestrial caddisfly, the Platte River caddisfly (PRCF), *Ironoquia plattensis* Alexander and Whiles, faces a range of mortality factors depending on its life stage. The PRCF has a one-year lifecycle, is aquatic from November through May, and aestivates on land from late May through late September (Whiles et al., 1999). Species that inhabit temporary waters face many challenges including reductions in surface area and volume of their habitat (Boulton, 1989; Williams, 1996; Wissinger et al., 2004). In addition, these species must survive dramatic changes in water chemistry, increases in temperature fluctuations, and low dissolved oxygen levels (Williams and Hynes, 1976; Williams, 1996; Williams, 1997). However, species living in intermittent waters generally face less predation and competition than invertebrates in permanent waters (Wiggins et al., 1980; Williams, 1996).

Aside from environmental conditions, caddisflies in temporary habitats face aquatic threats similar to species that inhabit permanent systems. These factors include diseases (Kohler and Hoiland, 2001), flooding (Lytle, 2003), predation (Boyero et al., 2006), limited food availability, cannibalism (Wissinger et al, 2004), and parasitism (Wisseman and Anderson, 1984).

Predators of caddisflies in temporary habitats may include large aquatic insects (dragonflies, beetles) or amphibians (frogs, salamanders) (Batzer and Wissinger, 1996; Wellborn et al., 1996; Wissinger et al., 1999). In permanent systems, fish are the dominant invertebrate predators (Wissinger et al., 1999). However, not all temporary systems are fishless habitats, as some backwater areas and intermittent water bodies serve as important nursery grounds for fish species (Sheaffer and Nickum, 1986). Fish were observed at the type locality for the PRCF and included the plains topminnow, *Fundulus sciadicus* Cope, common carp, *Cyprinus carpio* L., and the brassy minnow, *Hybognathus hankinsoni* Hubbs (Whiles et al., 1999). Sites with the PRCF now contain the Western mosquitofish, *Gambusia affinis* Baird and Girard, an invasive species introduced to Nebraska from the southeastern United States (Hurlbert et al., 1972, Haynes, 1993).

Mosquitofish have reduced abundances of fairy shrimp that are typically found in fishless waters (Leyse et al., 2003). Bence (1988) reported mosquitofish to significantly alter the invertebrate community in study enclosures by reducing microcrustaceans (copepods, cladocera, and ostracods). Among invertebrates, mosquitoes, microcrustaceans and zooplankton have been reported as food sources for mosquitofish (Hurlbert and Mulla, 1981; Crivelli and Boy, 1987; Blanco et al., 2004). No studies have examined the effects of mosquitofish on Trichoptera or other benthic dwelling insect orders, such as Ephemeroptera.

This study tested water quality parameters at unoccupied (none to few PRCF) and occupied (PRCF) sites; feeding on caddisflies by the mosquitofish, and the plains topminnow, *Fundulus sciadicus* Cope, a minnow native to Nebraska; aestivation by larvae in response to the presence of shade; PRCF behavior when exposed to a heat lamp, and mortality associated with immersion in a laboratory setting.

MATERIALS AND METHODS

Study Area

Nine sites were chosen for measuring water quality and were located between Elm Creek and Havens, Nebraska. Boundaries for each site were set at a length of 100 meters. Occupied sites were those that have a PRCF population. Unoccupied sites were those that have been surveyed and did not contain a PRCF population but are similar in appearance, vegetation structure, and hydroperiod. The five occupied sites chosen for this study were: Bombeck, Havens, Hord Lake, McCormick 1, and the Trust Forested site (Trust) (Appendix A). The four unoccupied sites chosen were: Blue Hole, TNC Wood River 1, Cottonwood Ranch, and McCormick 2 (Appendix A).

Water Quality

At each site, a Troll 9500 (In-Situ, Inc.; Fort Collins, CO) was used to measure rugged dissolved oxygen (RDO; mg/l), conductivity (µS/cm) and pH approximately weekly between 12 January and 30 March 2010. Between 31 March and 10 June 2010, pH, conductivity, and total dissolved solids (TDS) were measured using a HANNA® COMBO Tester (HI 98129; Woonsocket, RI) at each site. Dissolved oxygen (D.O.; mg/l) was measured using a HANNA® dissolved oxygen meter (HI 9143; Woonsocket, RI) from 31 March to 10 June 2010. Small water samples (~100 ml) were collected once weekly at each site for subsequent turbidity measurement (Nephelometric Telemetry Units (NTU)) in the laboratory using a LaMotte® 2020e (Chestertown, MD). All water quality information was collected from the same location at each site.

Statistical Analysis

All data for each measured variable were combined despite differences in instrumentation. A student's T-test ($p = 0.05$) was used to compare mean values of each measured variable between occupied and unoccupied sites. Two time periods, between 12 January 2010 and 10 June 2010 and between 14 April 2010 and 10 June 2010, were tested independently because of the PRCF life cycle. Dissolved oxygen and N.T.U. were compared for the entire sampling period using a Mann-Whitney test, because the data were non-parametric.

Fish Predation Trials

Mosquitofish were collected from a backwater area below the Harlan County Dam in Harlan County, NE (40° 04'17"N, 99° 12'28"W) or from a slough south of Kearney, NE south of the main channel of the Platte River (40° 39'28"N, 99° 00'56"W). Plains topminnow, *Fundulus sciadicus*, were collected from a brood stock pond at the Sacramento-Wilcox Wildlife Management Area (WMA) near Holdrege, NE (40° 22'44"N, 99° 15'05"W) and were only used in one experiment. Mosquitofish and plains topminnow were kept in a 150 liter (L) aquarium filled with water collected from Cottonmill Lake State Recreation Area near Kearney (40° 42'16"N, 99° 08'44"W). Fish were fed frozen bloodworms (Omega One®, Ferndale, NY) *ad libitum* until 24 hours before each trial. Unless otherwise noted, only female fish were used in each trial and were only used once. For all experiments, at least one control tank had five caddisfly larvae and no fish.

To quantify fish predation on the PRCF, a series of experiments was conducted at the University of Nebraska at Kearney (Kearney, NE). For each laboratory trial, four liters of water was placed into 9.5 L aquaria. Five caddisflies were placed into the tank and then one fish was introduced. Case-less caddisflies of the family Hydropsychidae were used to determine if mosquitofish would feed on benthic caddisflies. Hydropsychidae were collected from the Kearney Canal in Kearney, NE (40° 40'34"N, 99°04'56"W). Each tank was placed in a Percival® environmental chamber (Percival Scientific, Inc., Perry, IA). Chambers were set to a 14:10 light-dark (L:D) cycle, at 50 % relative humidity, and a daytime temperature of 20 degrees Celsius (°C) and a nighttime temperature of 10°C. The number of caddisflies remaining was determined after 24, 48, and 72 hours.

Remaining experiments were conducted using third and fourth instars of a caddisfly obtained from a backwater area of the South Fork of the Elkhorn River near Chambers, NE (42° 12'06"N, 98° 53'06"W). This species closely resembles the PRCF in size and morphology.

Two experiments, one with mosquitofish and one with plains topminnow, were run at a 14:10 L:D cycle at 50% relative humidity (RH), and at a daytime temperature of 15°C and a nighttime temperature of 5°C (n = 15) to simulate spring conditions. In the experiment with plains topminnow, nine male fish and one female fish were used. In a second experiment, to test the effects of temperature on fish predation, three sets of 12 tanks were tested at constant temperature of 15, 20, or 25°C (n = 12). In this experiment, all environmental chambers were set to a 12:12 L:D cycle at 50% RH. The number of larvae consumed at different temperatures was analyzed using a one-way ANOVA.

Shade Cover Study

To determine if PRCF larvae aestivate in areas based on the amount of moisture and shade along slough banks, habitat was surveyed at three locations that support PRCF populations. Sites chosen were Bombeck, Trust Forested, and Hord Lake (Appendix A). Average aestivating PRCF larval density was 116 ± 54.72, 26 ± 5.29, and 14 ± 4.16 larvae per m^2 in 2010 at these sites, respectively. At each location four transects five meters in length were set perpendicular to the water line on the north and south banks of the slough. Each transect was set 20 meters apart, except at the Trust were two transects were separated by 40 meters, because of previous habitat disturbance.

At each one-meter interval along all transects, the lower left corner of a 0.25 m^2 quadrat was placed against the ground and the following was completed: 1) if present, number of PRCF larvae were counted; 2) canopy cover was estimated above the tallest understory layer by using a spherical densitometer. The number of crosses not occupied by vegetation was counted and subtracted from 96 to obtain an estimate of canopy cover. Canopy cover was estimated four times for each quadrat and averaged; 3) percentages of forb (flowering plants), woody vegetation, graminoids (grasses, sedges, rushes) and bare ground were estimated for each quadrat; 4) distance of quadrat from the water line, and 5) percent soil moisture measured in water fraction by volume (wfv) and temperature using a Stevens® Water Monitoring Systems (Portland, OR) soil salinity meter. This was done four times at each quadrat, and then all values were averaged. Soil temperature and moisture was measured in each quadrat across all transects before going back to each quadrat and completing the other measurements.

Statistical Analysis

The average densities of caddisfly larvae per m^2 at each site were compared using a one-way ANOVA ($p = 0.05$). A Spearman correlation was run to test for significant correlations between each measured variable and the number of aestivating PRCF larvae ($p = 0.05$). Comparisons were made for each individual site and across all sites.

Behavioral Experiments

Aestivating larvae collected from a slough near Chambers, NE (42° 12' 02"N, 98° 53'06"W) were used to assess whether or not caddisfly larvae similar in appearance to the PRCF would select among available substrate material when exposed to a 250 Watt heat lamp. A piece of plywood (60 cm x 60 cm) was divided into four sections around a 10 cm circle at the center of the board. One subsection of the plywood had nothing on the surface. The remaining three areas had sand only, soil only, or soil with a leaf layer on top. Sand material used in the trials was collected from Bassway Strip WMA near Kearney, NE (40° 41'09"N, 98° 56'55"W), soil was collected from Chambers, NE and loose leaf material, primarily consisting of fallen cottonwood leaves, was collected from the Bombeck site. Before each trial, the entire board, except for the circle and the subsection with nothing, was misted with water.

Before beginning any trial, the heat lamp, placed 40 cm above the center of the plywood, was turned on for 15 minutes. Thirty larvae were used ($n = 30$) in the experiment, and for each trial, one larva was placed at the center of the plywood directly under the heat lamp. Once each larva was placed on the board, a timer was started and the time at first movement and the section chosen after five, 15, and 30 minutes were recorded. Each trial was stopped after 30 minutes, because little movement was observed in the caddisfly after fifteen minutes during preliminary trials. Nine larvae ($n = 9$) were used as controls and were observed in the same manner as experimental larvae, except control larvae were not exposed to a heat lamp.

Average temperatures at the center of the plywood, at 25 and 50 cm away from the heat lamp on each substrate, and at 25 and 50 cm away from the heat lamp under the leaf layer were measured using an Onset® HOBO light/temperature pendant.

Statistical Analysis

A Mann-Whitney U test was used to test for differences between the average time of first movement in control and experimental larvae ($p = 0.05$). Because larvae had a choice to either remain on the selected substrate or leave and find a new substrate material, a Chi-square analysis was used to test whether or not the choice to remain on the selected substrate was greater than expected due to chance.

Immersion Experiment

An experiment was conducted to test the effects of immersion on aestivating larvae. Thirty larvae from Chambers, NE were placed in water obtained from Cottonmill Lake in separate compartments of two ice cube trays. The number of larvae alive was determined after 24, 48, and 72 hours. Larvae were kept in a Percival® environmental chamber on a 14:10 L:D cycle, 50% relative humidity, and a daytime temperature of 23°C and a nighttime temperature of 20°C. Ten control larvae were kept in the same conditions, except were kept in a moist environment inside a plastic vial. Larvae were considered dead if they did not crawl or move in ten minutes after removal from water.

RESULTS

Water Quality

Water quality parameters were similar between occupied and unoccupied sites when analyzed between 10 January and 10 June 2010 and between 14 April to 10 June 2010 ($p > 0.05$) (Table 3.1, Table 3.2, and Table 3.3). Turbidity at occupied sites was generally less than turbidity at unoccupied sites (Table 3.1, Table 3.2).

Fish Predation Trials

Mosquitofish fed upon caseless Hydropsychidae with an average of 1.3 ± 0.31 larvae consumed after 24 hours, 1.9 ± 0.31 larvae consumed after 48 hours, and 1.9 ± 0.29 larvae consumed after 72 hours ($n = 29$). Mosquitofish consumed zero third and fourth instar surrogate larvae after 24 and 48 hours at a daytime temperature of 15°C and nighttime temperature of 5°C. Increased temperatures resulted in slightly more surrogate larvae being consumed by mosquitofish, with a maximum of one of five larvae consumed (Figure 3.1). There were no differences in the mean number of caddisflies consumed at 15, 20, and 25°C after 24 ($P = 0.7979$, $n = 12$) or 48 hours ($P = 0.8012$, $n = 12$).

The mean number of caddisfly larvae consumed by the plains topminnow after 24 and 48 hours was 0.5 ± 0.31 and 0.8 ± 0.39, respectively ($n = 10$). The single female used consumed four larvae after 48 hours, while all other fish consumed a maximum of one larva. No mortality of control caddisflies occurred among all experiments.

Shade Cover Study

There was a large range in the number of caddisflies per 0.25 m^2 at the Bombeck site, with between 0 and 62 individuals observed per 0.25 m^2. The average number of caddisflies aestivating at the Bombeck site was 6.94 ± 1.85 larvae per m^2, compared to 0.23 ± 0.85 larvae per m^2 at the Trust, and 0.45 ± 0.11 larvae per m^2 at Hord Lake. Significantly more larvae were observed at the Bombeck site compared to the Trust and Hord Lake sites ($p < 0.01$), which were not significantly different ($p = 0.0694$, $n = 48$, $n = 42$, respectively).

Using a Spearman correlation, no measured variable correlated with the number of PRCF larvae across sites ($r_s = 0.011$, $p = 0.7392$, $n = 124$) (Table 3.4). Within each site, no predictor variables explained variation in PRCF aestivation at Hord Lake ($p = 0.8538$, $n = 42$). At the Bombeck site, the number of PRCF larvae was positively correlated with percent bare ground ($r_s = 0.343$, $p = 0.047$, $n = 34$). At the Trust site, the number of PRCF larvae was positively correlated with soil moisture ($r_s = 0.328$, $p = 0.023$, $n = 48$) and negatively correlated with percent forb composition ($r_s = -0.308$, $p = 0.033$, $n = 48$).

Behavioral Trials

Temperatures directly under the heat lamp were approximately 49.0°C, while temperatures on the soil, sand, and nothing sections ranged from 30.9-34.3°C. Temperatures under the leaf litter layer ranged from 27.3-28.2°C.

When exposed to a heat lamp, aestivating caddisfly larvae initiated movement away from the heat source after 21.8 ± 2.77 seconds ($n = 30$). Control larvae moved after

51.4 ± 17.83 seconds ($n = 9$). These data were not significantly different ($p = 0.147$). Among experimental larvae, the leaf substrate was most often chosen, whereas sand was the preferred substrate among control larvae (Figure 3.2). When exposed to a heat lamp, the number of caddisfly larvae selecting the leaf substrate and remaining there as a last choice was greater than expected due to chance ($X^2 = 20.83$, $v = 3$, $p < 0.05$). When not exposed to a heat lamp, the number of larvae selecting the sand substrate and remaining there as a last choice was greater than expected due to chance ($X^2 = 10.03$, $v = 3$, $p < 0.05$). No other data were significant.

Immersion Trials

No mortality was observed in aestivating larvae submerged in water after 48 hours. Mortality increased to 6.7 percent of tested larvae after 72 hours. Ten percent mortality was observed among control larvae during the same time period.

DISCUSSION

Data analysis of dissolved oxygen, conductivity, total dissolved solids, pH, and turbidity measured over a five-month period failed to detect any significant difference between occupied and unoccupied sites. In general, turbidity was higher at unoccupied sites than at occupied sites. At the Bombeck site, which has some of the highest larval densities and adult activity, the water is clear; and the benthos can be seen from the water surface, which is not always the case at other sites. Similar conditions are frequently observed at the Havens, Trust, and Hord Lake sites.

The lack of statistical differences in the mean water quality parameters between occupied and unoccupied sites demonstrates that unoccupied sites have some similarities to sites with the PRCF. During sampling, there were instances where dissolved oxygen. levels were below one part per million (ppm), at occupied and unoccupied sites. D.O. levels below two ppm are considered low (Dauer et al., 1992), and low dissolved oxygen is a common characteristic of temporary waters. Macroinvertebrates in temporary wetlands must be adapted to withstand changes in water chemistry and hypoxic water conditions associated with habitat drying (Wiggins, 1973; Williams, 1996). Variations in the level of tolerance to pollution and poor water quality by macroinvertebrates have previously been documented (Resh and Unzicker, 1975). Some caddisflies, including *Hesperophylax occidentalis* Banks (Limnephilidae), can tolerate hypoxic conditions and poor water quality (Kohlar and Rahel, 1993). Thus, average water chemistry does not appear to be a limiting factor to the range and abundance of the PRCF.

During its aquatic life stage, the PRCF may face predation threats from fish, frogs and tadpoles, dragonfly larvae, giant water bugs (Hemiptera), and other predators (Batzer and Wissinger, 1996; Wellborn et al., 1996; Wissinger et al., 1999). Native fish were present at the type locality for the species (Whiles et al., 1999). The invasive mosquitofish is now abundant in backwaters that also contain the PRCF (Vivian, personal observation). In laboratory trials, the mosquitofish readily preyed upon case-less caddisflies but ate very few surrogate PRCF larvae.

During preliminary trials with mosquitofish and the plains topminnows, neither species consumed fifth instar PRCF larvae, even after some fish had been starved for 48 hours or 72 hours. Mosquitofish provided third/fourth instars ate a maximum of two larvae in one tank after a 48 hour period. The plains topminnow consumed slightly more third/fourth instars than mosquitofish in the feeding trials. The lack of predation on fifth instars by both species suggests there may be a maximum-sized PRCF larva both species are willing to accept as prey. Caddisfly species in large cases have been shown to be less palatable to fish than caddisflies in small cases (Wissinger et al., 2003).

Predation by plains topminnow on caddisfly larvae suggest that native fish species may have evolved with this potential prey species. The plains topminnow is native to nine states in the Midwest, with the largest distribution being in Nebraska (Rahel and Thel, 2004). During the feeding trials with plains topminnow, the single female used in the experiment consumed four larvae, whereas male fish consumed a maximum of one larva. In other fish species, such as northern pike and lake trout, it has been found that female fish consume more than males because of energy needs for gonad development (Rowan and Rasmussen, 1996; Trudel et al., 2000).

In caddisflies, fish predation can affect the selection of materials used to construct either organic or inorganic cases (Wissinger et al., 1999; Boyero et al., 2006). Some species of caddisflies will switch from organic material to mineral material for case building through different instars (Otto, 1976; Otto and Svensson 1980). Because mineral cases are thought to be less palatable to fish (Zamora-Muñoz and Svensson, 1996), the PRCF may have adapted to fish predation by constructing a case of mineral rather than organic material.

Fish predation on PRCF may be limited by a lack of temporal overlap between the caddisfly and predatory fish. By the time fish numbers start increasing in late spring in backwater sloughs, the PRCF is a fifth instar and may be too big for mosquitofish or plains topminnow to consume. Also, the PRCF emigrates from slough waters as early as mid-April (Vivian, personal observation), before fish numbers increase. When PRCF larvae are second and third instars, mosquitofish may be present in low numbers, and water temperatures in central Nebraska sloughs between February and April are less than 15°C. Some fish have lower consumption rates in cooler water than in warmer water (Wurtsbaugh and Cech, 1983).

In addition to facing threats in the aquatic environment, the PRCF is exposed to threats on land while aestivating. Attempts to quantify microhabitat use by Trichoptera on land have not been reported in the literature. A study quantifying microhabitat use by four rodent species in a forest measured 29 microhabitat variables including stem density, shrub height, fallen log density, litter-soil depth, and others. This data was gathered in a systematic fashion (Dueser and Shugart, 1978), as was the data in this experiment; however this study measured only eight habitat variables.

During the shade cover study, at the Bombeck site, between 0 and 62 individuals were present per quadrat. This large variation in caddisfly numbers may indicate that the PRCF has a preference for certain habitat variables. However, no strong correlations were observed between measured habitat variables and larval densities.

During the shade study, a positive correlation was found between the number of PRCF larvae and percent bare ground at the Bombeck site. At the Bombeck site, in the presence of a large shade canopy, the presence of understory vegetation is restricted. This contrasts to areas without a shade canopy, where monotypic stands of reed canarygrass occupy slough banks. Thus, more PRCF larvae were found in areas without reed canarygrass. It seems the thick root masses at the base of large patches of reed canarygrass deter PRCF aestivation.

At the Trust Forested site, PRCF larval densities were positively correlated with soil moisture and negatively correlated with percent forbs. Higher soil moisture was most often observed in the shaded areas immediately adjacent to the water line at the Trust site. However, the negative correlation with percent forbs was unexpected, because higher percentages of forbs are often observed under the shade canopy at the Trust, whereas outside the shade canopy, graminoids dominate the vegetation matrix.

No correlation between PRCF aestivation and any independent variable was observed at the Hord Lake site. This may have been because the slough banks at the Hord Lake were wetter in July 2010 than normal.

During this study, a positive correlation between PRCF density and proximity to the water line was expected, but was not observed. The high water line throughout the summer fluctuates, and depending on the time of year, this could make a difference in whether or not a positive correlation is observed. This study was conducted towards the end of July after the PRCF had been aestivating for about two months and the water line had receded about one meter from June levels. Because PRCF larvae retain the ability to move, aestivating larvae may follow the water line during the summer. If, however, larvae follow the water line, then a correlation between densities and distance from the water line would have been observed.

When compared across sites, there were no measured variables significantly correlated to PRCF aestivation. While each site appears grossly similar, the sites are different. For instance, at the Trust site, the shade canopy is more dense and homogenous across the north slope and relatively thin on the south slope. The Bombeck and Hord Lake sites are more open and differ in overstory composition.

Observations made during 2009 and 2010 terrestrial surveys and the shade cover study matches results of behavioral trials in the laboratory. When given a choice of four substrates, PRCF larvae that were exposed to high temperatures consistently moved to and remained in the section with soil and leaves. Trials in the laboratory and results from the shade cover field experiment indicate that searches for larvae in most sloughs are best conducted in shaded areas. This is likely because PRCF larvae are prone to desiccation during aestivation. Desiccation may be one of the greatest threats faced by PRCF during aestivation, because PRCF retain the gills during this time (Vivian, personal observation). Species living in intermittent aquatic habitats may seek protection by burrowing into moist substrate (Wiggins et al., 1980). This has also been observed in the PRCF.

In July 2009 during terrestrial surveys, one PRCF larva was observed with its head in the soil and abdomen oriented perpendicular to the ground. In May 2010, PRCF larvae were observed to burrow about five cm underground during aestivation (Vivian, personal observation; Geluso et al., unpublished data). Searches for aestivating larvae aboveground at this site did not detect any PRCF.

During preliminary behavioral trials in the laboratory eight out of ten larvae buried in moist sand when exposed to a heat lamp. Furthermore, caddisfly larvae left out of a moist environment in the laboratory died quickly (in a matter of minutes to hours), likely as a result of desiccation. Having evolved in a largely treeless plain (Eschner et al., 1981; Johnson and Boettcher, 2000) where summer air temperatures can exceed 37°C, PRCF are likely to possess adaptations to avoid desiccation during the summer months. Episodic floods and droughts are characteristic of prairie streams (Dodds et al., 2004), and burrowing may also enable PRCF to survive these events.

Because PRCF retain their gills during the aestivation period, the survival of most larvae after 72 hours of submersion in water is not unexpected. During late May to early June 2010, water levels at sites with the caddisfly overflowed the banks because of several rain events and subsequent flooding. Immediately after flood waters receded, the McCormick 1 and Bombeck sites were visited to determine if aestivating larvae remained near the average high water line. Aestivating larvae were observed at each location less than one meter from the water's edge, and had thus likely had been submerged during the flood event.

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FIGURES

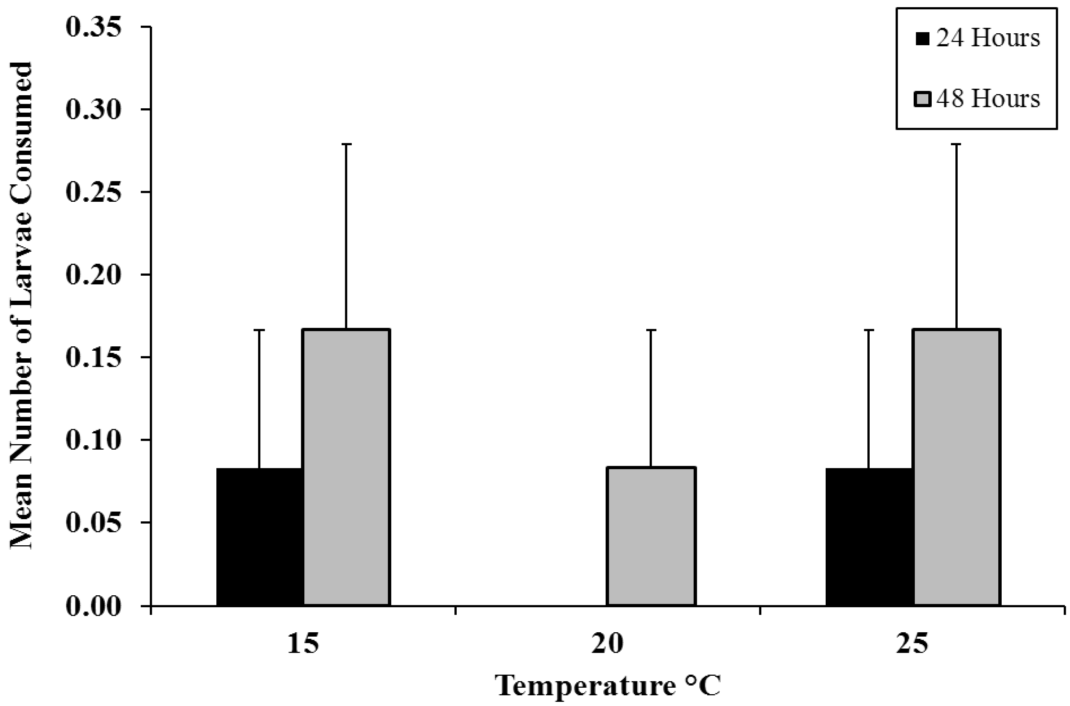

Figure 3.1. Mean number of surrogate caddisfly larvae (± 1 S.E.) consumed by mosquitofish after 24 and 48 hours at a constant temperature of 15, 20, or 25°C. Mean number of larvae consumed at different temperatures was not significantly different ($p > 0.05$).

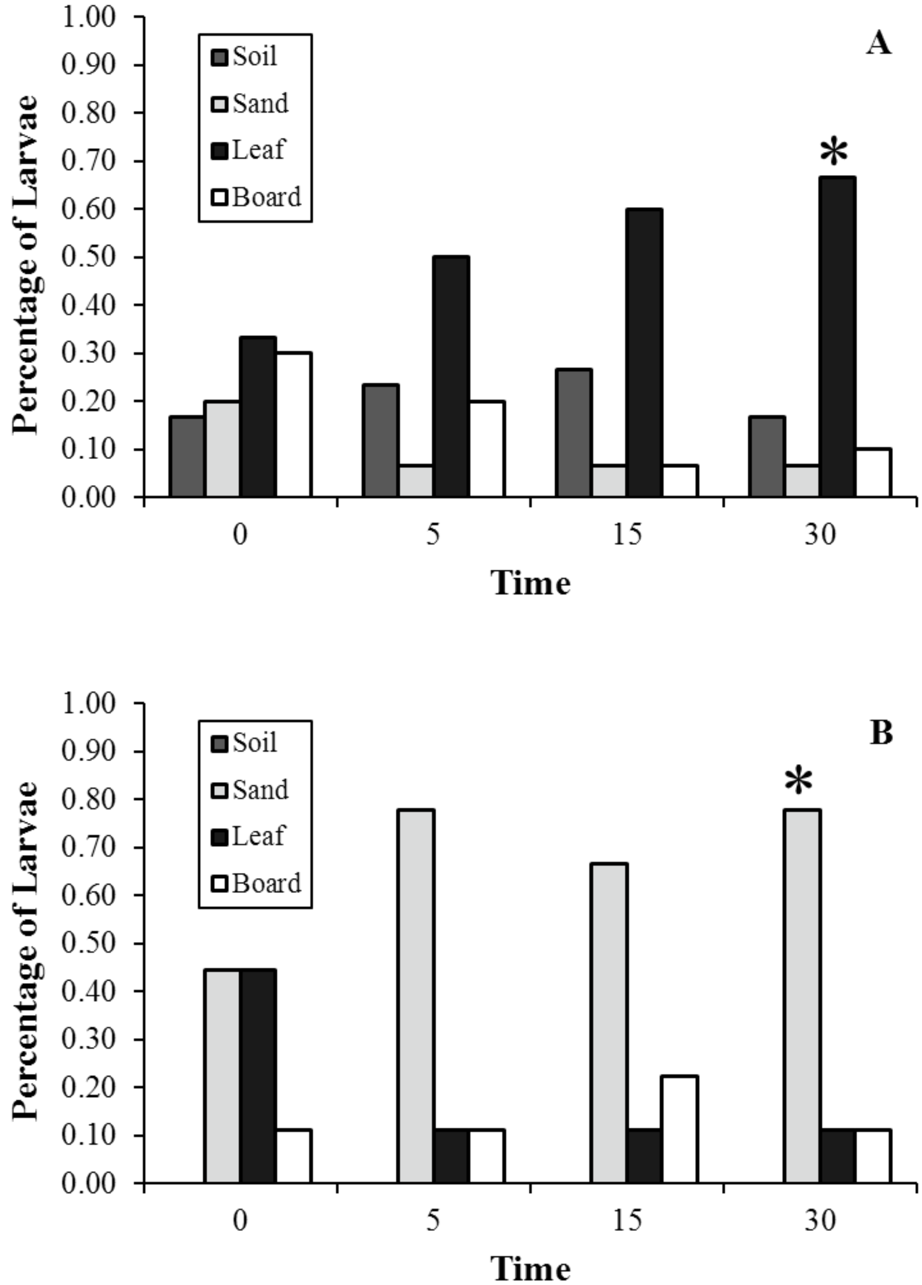

Figure 3.2. Percentage of control larvae (n = 9) (A) and larvae exposed to a heat lamp (n = 30) (B) that selected soil, sand, leaf, or board as a first choice and after five, 15, and 30 minutes. Asterisk (*) indicates significance (P < 0.05) based on Chi-squared goodness of fit.

TABLES

Table 3.1. Average pH, D.O, μS, T.D.S., and N.T.U. values (± 1 S.E.) at each occupied and unoccupied site from 10 January and 10 June 2010. No water quality parameters at occupied sites were significantly different from those at unoccupied sites ($p > 0.05$).

Occupied	pH	D.O (mg/L)	μS	TDS	NTU
Bombeck	7.47 ± 0.13	8.49 ± 0.83	673 ± 37	316 ± 16	0.63 ± 0.11
Havens	7.31 ± 0.12	6.90 ± 0.72	771 ± 39	409 ± 15	1.28 ± 0.16
Hord Lake	7.37 ± 0.14	7.21 ± 1.04	764 ± 41	417 ± 15	2.30 ± 0.49
McCormick 1	7.73 ± 0.15	12.13 ± 1.32	943 ± 43	453 ± 14	8.06 ± 0.53
Trust Forested	7.24 ± 0.08	7.26 ± 0.87	1096 ± 49	565 ± 24	2.37 ± 0.59
Mean	**7.42 ± 0.09**	**8.40 ± 0.97**	**849 ± 76**	**432 ± 40**	**2.93 ± 1.32**
Unoccupied	pH	D.O (mg/L)	μS	TDS	NTU
Blue Hole	7.61 ± 0.17	6.09 ± 1.22	821 ± 31	398 ± 8	3.79 ± 0.69
Cottonwood Ranch	8.03 ± 0.10	14.49 ± 1.46	988 ± 47	521 ± 18	6.02 ± 2.53
McCormick 2	7.22 ± 0.08	7.12 ± 0.75	914 ± 39	457 ± 15	1.93 ± 0.64
TNC Wood River 1	7.15 ± 0.13	7.16 ± 1.37	828 ± 74	482 ± 22	3.48 ± 1.25
Mean	**7.50 ± 0.20**	**7.72 ± 2.55**	**886 ± 38**	**465 ± 26**	**3.68 ± 0.87**

Table 3.2. Average pH, D.O, µS, T.D.S., and N.T.U. values (± S.E.) at each occupied and unoccupied site from 14 April and 10 June 2010. Values at occupied sites were not significantly different from values at unoccupied sites during this time period ($p > 0.05$).

Occupied	pH	D.O (mg/L)	µS	TDS	NTU
Bombeck	7.30 ± 0.14	6.36 ± 0.71	673 ± 23	326 ± 19	0.64 ± 0.09
Havens	7.41 ± 0.13	5.40 ± 0.76	832 ± 32	418 ± 17	1.41 ± 0.20
Hord Lake	7.40 ± 0.17	5.41 ± 0.79	863 ± 56	431 ± 13	2.71 ± 0.88
McCormick 1	7.92 ± 0.27	11.63 ± 2.44	944 ± 30	470 ± 12	3.32 ± 0.84
Trust Forested	7.21 ± 0.10	4.91 ± 0.62	1182 ± 38	590 ± 19	2.62 ± 0.89
Average	**7.45 ± 0.12**	**6.74 ± 1.24**	**899 ± 83**	**447 ± 43**	**2.14 ± 0.49**
Unoccupied	pH	D.O (mg/L)	µS	TDS	NTU
Blue Hole	7.27 ± 0.12	1.95 ± 0.77	825 ± 7	409 ± 6	2.20 ± 0.29
Cottonwood Ranch	8.03 ± 0.15	13.45 ± 2.01	1068 ± 36	531 ± 18	4.50 ± 2.07
McCormick 2	7.31 ± 0.09	5.28 ± 0.63	943 ± 26	472 ± 14	1.29 ± 0.25
TNC Wood River 1	7.24 ± 0.13	4.42 ± 1.00	1011 ± 26	509 ± 12	2.46 ± 0.50
Average	**7.46 ± 0.18**	**6.28 ± 2.49**	**962 ± 52**	**480 ± 27**	**2.61 ± 0.68**

Table 3.3. P-values of all statistical tests used to compare water quality parameters at occupied and unoccupied sites ($n = 5$, $n = 4$, respectively). Values with an "a" indicate results were analyzed using an un-paired T-test. Values with a "b" indicate results were analyzed using a Mann-Whitney U test.

Parameter	10 January to 10 June	14 April to 10 June
pH	0.710^{a}	0.905^{b}
D.O.	0.556^{b}	0.413^{b}
µS	0.703^{a}	0.568^{a}
T.D.S.	0.544^{a}	0.557^{a}
N.T.U.	0.413^{b}	0.579^{a}

Table 3.4. Spearman (r_s) correlation coefficients between PRCF larval density and measured parameters and associated P-values from the shade cover study. Significant correlations marked with an asterisk (*).

	Trust		**Bombeck**	
	PRCF larvae per m^2		PRCF larvae per m^2	
	Coefficient	P-value	Coefficient	P-value
Distance from water line (m)	-0.013	0.930	-0.165	0.347
% Canopy	-0.100	0.496	0.297	0.087
Soil Moisture (wfv)	0.328	0.023*	-0.110	0.531
Soil Temperature (°C)	0.038	0.797	-0.161	0.361
% Bare Ground	-0.105	0.476	0.343	0.047*
% Forb	-0.308	0.034*	0.165	0.349
% Woody	0.055	0.711	0.046	0.793
% Graminoid	0.187	0.202	-0.243	0.166

APPENDIX A

All collection dates prior to 2004 pertain to data and observations gathered by Goldowitz (2004). All observations from 2007 are from Riens and Hoback (2008). It is unknown at this time whether or not the caddisflies observed outside the Platte are the Platte River caddisfly (see text).

Site Name	**Dates**	**Present or Absent**	**County; River Basin**
Site Description			
6th and C	6 May 2009 22 May 2009 15 September 2009	Absent (w/cases) Absent (w/cases) Absent (w/cases)	Merrick; Platte
This site was discovered while driving on C Road and was dry on each visit. Willow trees, *Salix sp.*, and cottonwoods, *Populus deltoides,* were the dominant shade species. Only cases have been observed at this location. This site appears to have been dry for a while, as determined by looking at historical imagery in Google Earth®.			
96 Ranches 1	20 July 2010	Absent	Dawson; Platte
This area was visited due to word of mouth that the PRCF had been collected at this location in 1996 (M. Peyton, personal communication, 2010). The area was an isolated, shallow slough pocket with duckweed, *Lemna* sp. on the surface. Cottonwoods shaded the slough banks, which were mostly dry above the water line. Cattails, *Typha sp.* was the predominant emergent vegetation. Water was about 0.3 to 0.5 meters deep. No Western mosquitofish, *Gambusia affinis*, were observed.			

96 Ranches 2	1 September 2010	Absent	Dawson; Platte
This site was identified using Google Earth® satellite imagery. This area formed part of an old irrigation ditch but also connected to the main river channel futher downstream. Water depth along the slough channel was about 0.3 to 0.5 meters deep. The substrate was soft and thick in slough channel, and a clay soil was present along slough banks. The predominant vegetation had recently been common reed, *Phragmites*, but spraying of exotic vegetation had occurred at this site. Large shade trees were absent. The area appeared similar to East Odessa WMA and Marshall sites. The search for larvae at this location included digging into the substrate. Mosquitofish were absent.			
Arcadia Diversion Dam	1 September 2010 17 August 2010	Absent Absent	Custer; Middle Loup (potentially PRCF or closely related species)
This area was visited after identifying potential habitat using Google Earth®. The water was light brown in color and turbid and likely too deep for the PRCF. The banks leading away from the water line were shallow. The marsh was directly connected to the river channel.			
Bader 1	27 May 1999 4 October 2004 12 October 2007 16 September 2008 22 May 2009 4 October 2009 11 May 2010	Present Present Present Present Present Absent Present	Merrick; Platte

Site was one of six historical PRCF populations. One adult was collected here in 1999 (Goldowitz, 2004). A few adults were observed in 2007. Three caddisfly pupae were observed in 2008. Fewer than ten adults were observed at this site in 2008. One larva was observed both May 2009 and 2010. No adults were seen at this site in October 2009. This site was excavated, and large shade trees were cleared in 2007 as part of a United States Fish and Wildlife Service (Service) restoration project, and this could have had an adverse impact on the PRCF (personal communication, Harms, 2009). The PRCF has not been observed at any other restored slough at Bader. This site had willows, fogfruit (*Lippeoa lanceolata*), and threesquare (*Schoenoplectus pungens*) in 1999 (Goldowitz, 2004). The area was open with willows and sedges, *Carex* sp., present on the edges in 2009-2010. Duckweed and cattails comprised macrophytes in slough. Mosquitofish, *Gambusia affinis*, were abundant in slough. Water depth ranged from 0.5 to 0.75 meters.

Bader 2	27 May 1999 4 October 2004 6 October 2009	Present Absent Absent	Merrick; Platte

Slough was dry when sampled but showed signs that it may be wet for part of the year. Cattails and willows were present. Cottonwoods lined slough edges. No cases or larvae were collected in 2004 or 2009. A few larvae were found near the culvert in 2004 (Goldowitz, 2004).

Bassway Strip WMA 1	29 July 2009 13 May 2009	Present	Buffalo; Platte

Area was visited after identifying potential habitat in Google Earth®. Area sampled was a side channel. Two aestivating caddisfly larvae were observed on a shallow bank in 2009. Two larvae were collected during aquatic sampling in 2010. The area was dominated by a monotypic stand of *Phragmites*.					
Bassway Strip WMA 2	29 July 2009		Absent		Buffalo; Platte
Site was visited after identifying potential habitat in Google Earth®. Area sampled was similar to Bassway Strip WMA 1 but also contained willow trees.					
Bell	15 September 2009 6 October 2009		Absent	Absent	Merrick; Platte
This site was identified during site recoinassance at Silver Creek WMA (no slough habitat) near the parking lot on the same road. Slough was dry in 2009, but the substrate was moist. Site appeared suitable for the PRCF on both visits. Slough was shaded by willows, dogwoods, *Cornus sp.,* and cottonwoods. *Polygonum* sp. was also present.					
Binfield 1	21 May 1999 October 2007 28 May 2009 3 September 2009 28 September 2009 18 May 2010	12	Present Absent Present Present Absent	Absent	Hall; Platte
Site was one of six historic PRCF populations. This slough sat amongst a stand of cottonwoods, green ash, *Fraxinus pennsylvanica*, grape vines, *Vitis* sp., and poison ivy, *Toxicodendron radicans*. Duckweed was abundant on slough surface. Emergent vegetation mostly consisted of cattails and willows. The slough did not hold much water in 2009 or 2010. Two larvae were observed in 2009 through aquatic sampling. No larvae were observed during terrestrial sampling in September 2009. One adult was observed during night trapping in September 2009. No larvae were observed in 2010, but the site was likely still active.					

Binfield 2	18 May 2010	Present	Hall; Platte
This slough was identified while sampling Binfield 1 in 2010. This area held about 0.5 meters of water and was surrounded by willow trees. Two larvae were collected during aquatic sampling in 2010.			
Binfield 3	28 May 2009 3 September 2009 28 September 2009	Present Present Absent	Hall; Platte
The slough at this location was identified while driving on Shoemaker Island Road. The abundance of emergent vegetation, particularly cattails, made this slough easy to locate. Identification of a population occurred through terrestrial sampling. Water level was shallow and slough was nearly dry in May 2009. The site was heavily shaded on both the north and south slough banks by cottonwoods, green ash, and willows.			
Blede	21 July 2010	Absent	Lincoln, Platte
Google Earth® was used to identify potential habitat at this location. Slough sampled was adjacent to the main channel of the Platte River and near the Gothenburg Canal. Area appeared suitable for the PRCF. Banks were searched extensively, and a shovel was not incorporated into survey efforts. In late May 2010, it was first observed that some PRCF individuals will bury about five centimeters below the soil surface when aestivating. Macrophytes in water column mostly consisted of softstem bulrush, *Schoenoplectus tabernaemontani*, and cattails. Willows lined slough edges. Duckweed was present on water surface. An intermittent shade canopy was present and included Russian olive, *Elaeagnus angustifolia*, green ash, and cottonwoods. Water depth was about 0.3 to 0.5 meters.			
Blue Hole	7 August 2009 29 September 2009 21 May 2010	Absent Absent Absent	Buffalo; Platte

Site was visited after identification of suitable habitat during site reconnaissance. Slough sampled was long, shallow wetland with little emergent vegetation. When present, vegetation included cattails and *Phragmites.* Duckweed was abundant on slough surface through spring and summer. Area was shaded with cottonwoods, dogwood, and green ash. Smooth brome, *Bromus inermis*, and reed canary grass (RCG), *Phalaris arundinacea*, were abundant along banks. Brook stickleback, *Culaea inconstans*, was dominant fish in slough, and mosquitofish were absent. A shovel was not used during survey efforts. Hydroperiod of site was closely associated to that of river. Site could be active 100 meters beyond sampled reach, as slough approaches river.

Blue Hole WMA 1	30 March 2010	Absent	Buffalo; Platte

Site was visited after identification of potentially suitable habitat using Google Earth®. Slough sampled was back channel to sandpit lake. Channel was unshaded and lacked emergent vegetation. Filamentous algae and small amounts of duckweed were present in water column. Area supported mosquitofish and did not initially seem suitable for the PRCF.

Blue Hole WMA 2	30 March 2010	Absent	Buffalo; Platte

Small slough sampled contained little emergent vegetation. Cottonwood trees were dominant shade species present. Some algae was present on water surface. Water depth was about 0.5 meters, but seemed too shallow to support the PRCF.

Bombeck	25 May 1999 13 October 2007 20 May 2009 21 May 2009 16 August 2009 23, 28-30 September 2009 1, 4, 6 October 2009 13 May 2010 26 May 2010 14 July 2010 18 August 2010	Present Absent Present Present Present Present Present Present Present Present Present	Kearney; Platte

Site was one of six historic PRCF populations. Slough was old south channel of the Platte River. Site was dominated by RCG in and out of channel. Other species present included *Polygonum* sp., cattails, rushes*,* *Juncus* sp., *Verbena sp.,* duckweed, purple loosestrife, *Lythrum salicaria, Ludwigia palustris*, and poison ivy. In the absence of large stands of RCG on slough banks, stands of cottonwoods, green ash, elm trees, *Ulmus sp.*, dogwoods, and maple trees, *Acer* sp., were present. Water in slough channel was clear with low turbidity, which was often less than one Nephelometric Telemetry Units (NTU). Slough did not dry in 2009 or 2010. Water flowed through site on most collection dates, whereas other sloughs with the PRCF are more lentic. Area likely had a groundwater connection. Mosquitofish were present during the mid-to-late summer, although the fish was not abundant. Several larvae from channel and along banks and adults have been consistently collected from this location. Only cases were observed in 2007 (Riens and Hoback, 2008).

Bowman Lake SRA	9 August 2004 17 August 2010 5 October 2010	Absent Present Present	Sherman; Middle Loup (potentially PRCF or closely related species)

<table>
<tr><td colspan="4">This site was identified after using Google Earth® to locate potential habitat. The slough was a tail race of Bowman Lake but was also in close proximity to the Middle Loup River. One larva was collected along slough bank in 2010. Six adults were collected from this site in October 2010, and the site was nearly dry at the time. This site was previously declared absent by Goldowitz (2004). The slough did not hold water in several locations during sampling. The slough banks were shaded by green ash, cottonwoods, elm, black locust, *Robinia pseudoacacia,* and dogwoods. RCG was present in the slough bottom and was an abundant component of the understory. River bulrush, *S. fluviatilis*, was also present in patches. Mosquitofish were absent.</td></tr>
<tr><td>Brooks</td><td>18 May 1999
14 October 2004
12 October 2007
27 May 2009
23, 28 September 2009</td><td>Present
Absent
Absent
Absent
Absent</td><td>Hall; Platte</td></tr>
<tr><td colspan="4">Site was one of six historic PRCF populations. Area was former north channel of Platte River. Site was dry in 2004 and 2007 (Goldowitz, 2004; Riens and Hoback, 2008) and held water in 2009 during each site visit. Slough had no emergent vegetation. Slough banks were shaded by a diversity of grasses. Shade trees were sparse.</td></tr>
<tr><td>Buffalo Bill SHP</td><td>21 July 2010</td><td>Absent</td><td>Lincoln; North Platte</td></tr>
<tr><td colspan="4">Site was visited after potential habitat was identified using Google Earth®. This area was mostly a matrix of intermittent sloughs running through a large grassland. Many of the sloughs were dry during sampling and may have been too dry to support the PRCF. A shovel was not used during sampling.</td></tr>
<tr><td>Caveny</td><td>2 October 2004
14 August 2009</td><td>Absent
Absent (w/cases)</td><td>Hall; Platte</td></tr>
</table>

Site was visited due to word of mouth that area may be suitable for PRCF (C. Helzer, personal communication, 2010). Site was dry when sampled, but one larval case was observed. Slough connected to Trust Forested site downstream.			
Cedar 1	17 July 2010 4 October 2010	Absent (w/cases) Absent	Cedar; Wheeler
Area was identified as potential habitat while driving along Highway 281 and was subsequently sampled. Two cases were found at this site within first five minutes of visit. Area sampled was slough along dirt road. Vegetation in slough and along banks was predominately willows, cattails, *Polygonum* sp., and prairie cordgrass, *Spartina pectinata*, were also present. Wind may have hampered collection of adults in 2010.			
Chambers 1	19 June 2010 12 September 2009 17 April 2010 30 July 2010 4 October 2010	Present Present Present Present Present	Holt; Elkhorn
Area sampled was slough near the South Fork of the Elkhorn River and was visited due to identification of possible PRCF presence (S. Butler, personal communication, 2009). Site was likely spring fed and straddled both sides of road. Site experienced seasonal fluctuations of water table elevation. When present, emergent vegetation was largely cattails and RCG where slough had dried. Duckweed was abundant on slough surface. RCG and poison ivy were the predominant plant species on slough banks. Large cottonwoods were present on sides of slough furthest from road. Cattle grazing occurred beyond fence line. Larval densities of more than 400 individuals per square meter have been observed at this site.			
Chambers 2	12 September 2009	Present	Holt; Elkhorn

Slough sampled was in roadside ditch and was visited due to identification of possible PRCF presence (S. Butler, personal communication, 2009). Willows and RCG lined slough banks. *Polygonum* sp. and cattails were present in slough bottom. Slough did not hold much water when visited.			
Chambers 3	12 September 2009	Absent	Holt; Elkhorn
This area was visited after identifying potential habitat while driving in area. Slough sampled was along highway in ditch/right-of-way. Cattails, river bulrush, and *Polygonum sp.* were all present in slough bottom. Large shade trees including green ash and cottonwoods were present. Area sampled seemed highly suitable to the PRCF.			
CNPPID 1	22 July 2010	Absent	Lincoln; North Platte
Google Earth® was used to identify potential PRCF habitat at this location. This area was part of Fremont Slough and looked suitable for the PRCF. Cottonwoods comprised the dominant portion of the shade canopy. Cattails were abundant in the channel. The area had been sprayed for *Phragmites*. Mosquitofish were present in the channel during sampling. The presence of *Ironoquia punctatissima* was documented.			
CNPPID 2	22 July 2010	Absent	Lincoln; North Platte
Google Earth® was used to identify potential habitat at this location. This slough sat behind a dike and may have been artificial. Duckweed was present on the water surface during sampling, and emergent vegetation was absent. A large shade canopy covered the slough. The area appeared suitable for the PRCF.			
Cottonwood Ranch 1	11 August 2009	Absent	Dawson; Platte
Site was visited after land reconnaissance tour with NPPD personnel. Area sampled was small slough with willows and little emergent vegetation. Site contained evidence of cattle grazing.			

Cottonwood Ranch 2	28 August 2009	Absent	Dawson; Platte

Site was visited after land reconnaissance tour with NPPD personnel. Site was side channel of Platte River that resembled the Bombeck site in some aspects. Channel flowed for most of year but slack water areas were present. There was no emergent vegetation in the channel. RCG dominated slough banks. Russian olive was the dominant shade tree. Cattle grazing occurred at this location.

Cottonwood Ranch 3	7 August 2009	Absent	Dawson; Platte

Site was visited after land reconnaissance tour with NPPD personnel. Area sampled was small slough just off north channel of Platte River with cattails and RCG. Slough banks were very sandy with dry soil. A few willows lined the slough banks. Area appeared to be impacted by cattle grazing.

Davis	10 August 2010	Absent	Howard, Middle Loup

Site was visited due at the request of NGPC staff. Area sampled was restored wetland with no emergent vegetation. Duckweed and filamentous algae were present on slough surface. Shade trees were largely absent.

Dearking 1	30 July 2009 11 May 2010	Present	Polk; Platte

Google Earth® was used to identify potentially suitable habitat at this site. Area sampled was a patchwork of sloughs that sat amongst a stand of Eastern red cedar, *J. virginiana*. Slough banks supported a variety of grass species when shade was absent. Emergent vegetation was absent. Mosquitofish were abundant in each slough. Larvae were mostly detected under the shade of a large tree along bank of slough closest to the river. Six aestivating larvae were observed in 2009, and one larva was observed during aquatic sampling in May 2010.

Dearking 2	10 August 2009	Absent (w/cases)	Polk; Platte

Google Earth® was used to identify potential habitat at this location. Area sampled was open slough network among large stand of Eastern red cedar. Slough was dry in 2009 when PRCF cases were found. No live individuals were collected, and the site has not been revisited. This area is upstream of the Havens site. Aside from sampling, a site reconnaissance of this area was conducted. In walking 1.6 km through the area, it appeared many former sloughs had been encroached upon by RCG, which seems to have reduced the amount of available habitat at this site.

Dinan	11 August 2009	Absent	Hall; Platte

Site sampled was area of future restoration activity and was sampled at the request of the Service. Area contained typical wetland plants like sedges, rushes, small amount of cattails, and purple loosestrife. Ragweed, *Ambrosia sp*., was abundant along banks. Slough was dry during sampling and seemed to not hold much water or hold water long enough during the year to support the PRCF.

Dogwood WMA 1	2 April 2010	Absent	Dawson; Platte

Google Earth® was used to identify potential habitat at this location. Slough contained stand of *Phragmites* that had recently been sprayed. Duckweed was present. Surrounding slough banks were sandy and moist. Slough seemed to be connected to groundwater. Shovel was not used during survey. Other depressional features surrounding the slough had signs of former beaver activity, which indicated area may have at one time supported slough habitat. Majority of upland area was dry and supported smooth brome rather than wetland plants.

Dogwood WMA 2	2 April 2010	Absent	Dawson; Platte

Google Earth® s was used to identify potential habitat at this location. Area sampled was small slough in tail race of sandpit lake. Cattails, duckweed, and RCG were all present.

Dowse	1 September 2009	Absent	Custer, Middle Loup

This area was identified as potential habitat through a conversation with one of the landowners. Sampled banks on the north side of the slough/marsh near camping trailer just off the road. Area was more marsh-like than slough habitat. Water may have been too deep to support the PRCF.			
ELauby	20 July 2010	Absent	Dawson; Platte
This site was visited after identifying potential habitat in Google Earth®. Area sampled was small kettle hole adjacent to stream. Side channel adjacent to hole was also sampled. Stream edges were devoid of grasses and shade.			
East Odessa WMA	2 April 2010 14 May 2010	Absent (w/cases) Present	Buffalo; Platte
Site was sampled after identifying potential habitat using Google Earth®. This site was a side channel to the Platte River. Willow trees were dominant along slough banks. Dead stands of *Phragmites* were present. One aestivating larva was observed on 14 May 2010. Mosquitofish were present, and other unidentified fish species were observed.			
East Willow Island WMA	12 August 2010	Absent	Dawson; Platte
Google Earth® was used to identify potentially suitable habitat at this location. Site sampled was large and appeared to be highly suitable PRCF habitat. Slough had little emergent vegetation, but duckweed was abundant on water surface. Shade canopy was present. Survey efforts included digging for PRCF.			
Gail	10 August 2010	Absent	Howard, Middle Loup
Slough was sampled at request of NGPC agency staff. Slough sampled had been grazed by cattle and contained little shade. Duckweed was present on slough surface. Habitat did not seem suitable for PRCF.			

Glasser 1	6 August 2009 18 May 2010	Present	Merrick; Platte
Google Earth® was used to identify potential habitat at this location. Small slough sampled was situated amongst a stand of Eastern red cedar. RCG was predominant species in slough where water was shallow. Majority of slough lacked emergent vegetation. Water depth was around 0.2 meters when sampled in 2009 and 0.5 meters in 2010. Four aestivating larvae were observed in 2009. One larva was collected during aquatic sampling in 2010.			
Glasser 2	6 August 2009	Absent	Merrick; Platte
Google Earth® was used to identify potential habitat at this location. Small depression present was nearly dry when sampled. Horsetail, *Equisetum* sp., and RCG were predominant plant species present. Substrate of slough banks and slough bottom was sandy.			
Glasser 3	18 May 2010	Present	Merrick; Platte
Google Earth® was used to identify potential habitat at this location. Area sampled was a long slough with little emergent vegetation. Slough coursed through grassland area close to main channel of Platte River. Water depth was about 0.5 meters in May 2010.			
Harold Anderson WMA	20 August 2010 10 September 2010	Absent (w/cases) Absent (w/cases)	Howard, Middle Loup
Potential habitat was identified at this site using Google Earth®. The slough was at the bottom of a small, steep canyon wall. Arrowhead, *Sagittaria latifolia*, cattails, and river bulrush, were the dominant emergent plants. Water level was about 0.3 meters during sampling, and the slough was dry in spots. Two cases were observed.			

Havens	11 May 2010 21 May 2010	Present		Polk; Platte

Potential habitat was identified at this site using Google Earth®. The Havens location was a series of sloughs that stretched over a half mile adjacent to the main channel of the Platte River. In 2009 the area appeared to contain several separate sloughs. In 2010, each depression remained connected through September. Most depressions contained minimal amounts of emergent vegetation. When present, emergent vegetation largely consisted of cattails and *Phragmites*. *Polygonum* sp. and *L. palustris* were also present. Duckweed was abundant on the water surface. This site supports one of the largest known PRCF populations along the Platte River.

Hoback	25 August 2009 21, 23, 30 September 2009 13 May 2010	 Present Present	 Present	Buffalo; Platte

This area was incidentally located during a visit to the the Platte River. The area was revisited after finding discarded cases at this site in June 2009. Eight larvae and four adults have been observed at this site. Fifteen larvae were observed during aquatic sampling in 2010. Fewer than 10 adults were observed in September 2009. This site contained duckweed, cattails, and RCG, which was abundant. Eastern red cedar was the predominant tree species on the slough banks, and the area was heavily shaded. It appeared the slough had been reduced in size in recent years due to encroachment by RCG and subsequently smooth brome.

Hord Lake	4 October 2004 13 October 2007 23 September 2008 1 October 2008 8 October 2008 16 October 2008 22 May 2009 13 May 2010 21 May 2010 13 July 2010 1 September 2010 30 September 2010	Present Present Present Present Present Present Present Present Present Present Present Present	Merrick; Platte

Site was one of six historic PRCF populations. Slough sampled was between recreational lake and cornfield. Hydroperiod at site was intermittent and loosely associated with that of the nearby Platte River. Slough was connected to adjacent lake and seemed to have groundwater connection. Hord Lake site supports one of largest PRCF populations known. Site supported a mix of emergent vegetation, including RCG, cattails, purple loosestrife, river bulrush, and *Polygonum* sp. Vegetation on slough banks consisted of: Kentucky bluegrass, *Poa pratensis*, Virginia creeper, *Parthenocissus quinquefolia*, wild grape, poison ivy, smooth brome, *Sporobolus* sp., *Aster* sp., sedges, fog fruit, dogwoods, Eastern red cedar, cottonwoods, and green ash. Both sides of slough were intermittently shaded. Water depth varied across site but was 0.5 meters in most locations.

Hostetler	12 March 2010	Absent	Buffalo; Platte

Area was sampled at request of the Headwaters Corporation. Area sampled was directly adjacent to the Platte River and lacked wetland plants. Lack of wetland vegetation indicated ponded area did not remain wet enough during year to support a PRCF population. Area was wet during site visit and had distinct sloping banks.			
Kent Diversion Dam	17 April 2010	Present	Loup; North Loup (potentially PRCF or closely related species)
This site was visited after identifying potential habitat in Google Earth®. The sloughs next to the diversion were dominated by willows and were mostly dry. Grab sample was taken from a separate slough near the diversion. Two larvae were collected from the slough, which was shaded by large trees. Some emergent vegetation was present. The water in the slough was likely groundwater fed.			
Kosmicki	11 August 2010	Absent (w/cases)	Lincoln; North Platte
Site was sampled after identifying potential habitat using Google Earth®. The slough was an old side channel of the North Platte River. The water was lentic and duckweed was abundant on slough surface. The water was likely 0.75 meters or more in some locations and did not seem to fluctuate. A mixture of grass species was present on slough banks. The alluvium was sandy gravel. Two cases were observed by using a shovel during the survey. Area seemed suitable for the PRCF.			
Kuhl 1	10 August 2010	Absent	Howard, Middle Loup
Site was sampled at request of NGPC staff. Area sampled was restored slough, which had been restored in 2008. Filamentous algae was the dominant macrophyte present. Slough was mostly open water. A diverse mix of grasses and sedges was present along slough edges. Shade species were absent.			
Kuhl 2	10 August 2010	Absent	Howard, Middle Loup

Site was sampled after identifying potential habitat en route to Kuhl 1. Area sampled was a natural slough with a large shade canopy. RCG was abundant along slough margins. Duckweed was present on water surface. Slopes leading away from water's edge were shallow. A shovel was not incorporated into survey efforts, but slough banks were scoured for discarded cases. Area appeared suitable for PRCF.			
Lauby 1	12 August 2010	Absent	Dawson; Platte
Area examined was a restored slough and was visited in conjunction with Lauby 2. The restoration work was two years old at the time of the survey, and very little vegetation was present.			
Lauby 2	12 August 2010	Absent	Dawson; Platte
Google Earth® was used to identify potential habitat at this location. This area was a side channel to the Platte River with low flow. The water depth was less than 0.3 meters in most places. Hydroperiod seemed intermittent. Substrate along slough edges was sandy. Slough banks supported a diversity of grasses. Shade canopy was patchy and largely consisted of cottonwood trees. A shovel was used during the survey.			
Lone Tree WMA 1	20 July 2009	Absent	Merrick; Platte
Google Earth® was used to identify potential habitat at this location. Area sampled was large, open slough in a grassland setting. Cattails were abundant in slough, and a diverse mix of grasses was present along slough banks. Cottonwood trees were present but did not abut sloughs, and shade was patchy. A shovel was not used during the survey, and the slough seemed suitable for the PRCF.			
Lone Tree WMA 2	20 July 2009	Absent	Merrick; Platte
Google Earth® was used to identify potential habitat at this location. Slough was similar to Lone Tree WMA 1.			

Loups Bottom WMA	1 September 2009 10 September 2010	Absent (w/cases) Present	Loup; North Loup (potentially PRCF or closely related species)
This area was visited after identifying potential habitat in Google Earth®. The slough was a former side channel to the North Loup River and was dominated by arrowhead, rushes, and cattails. Duckweed was abundant on slough surface. Water depth was likely more than 0.75 meters at its deepest point. Shade canopy was dense and mostly consisted of Eastern red cedar.			
Loup Junction WMA	10 August 2010 20 August 2010	Absent Absent	Middle and North Loup Junction, Howard
Site was sampled after identifying potential habitat using Google Earth®. Area surveyed was large slough near junction of North and Middle Loup Rivers. Water appeared that it could be more than 0.75 meters deep at the center. Macrophytes, particularly arrowhead, were abundant. A dense shade canopy was present. Slough banks were gradual and shallow. A shovel was incorporated into survey efforts.			
Lichtenwalter	22 July 2010	Absent	Dawson; Platte
This area was visited after identifying potential habitat using Google Earth®, although through satellite imagery, it looked as if the area may be impacted by cattle grazing. Evidence of grazing was present during the survey. Mosquitofish were present. Dead willows and other hardwoods were present, and the banks were not well shaded. The area was heavily denuded and seemed unsuitable for the PRCF. No emergent vegetation was present.			
Marshall	2 Apr 2010	Absent (w/cases)	Buffalo; Platte

Site was sampled after identifying potential habitat using Google Earth®. Area sampled appeared much like East Odessa. One case was observed. Because sampling occurred during April and no aquatic sampling was done, it was not expected that any live individuals would be observed. PRCF are likely present at this location			
Martin's Reach 1	18 August 2009	Absent	Hall; Platte
Site was sampled after identifying potential habitat using Google Earth®. Area sampled was intermittent slough-type habitat with an abundance of willows. Area was dry during sampling, despite most other sloughs remaining wet through 2009. Hydroperiod may have been too ephemeral to support a PRCF population.			
Martin's Reach 2	24 May 1999 16 April 2010 18 May 2010	Absent Absent (w/cases) Absent	Hall; Platte
This area was identified as potential habitat using Google Earth®. The slough was a former side channel to the Platte River and was likely deep (>1 meter) in some areas. Several beaver dams interspersed the channel. The slough was long and wide open when sampled. The banks were largely exposed and unshaded. Eastern red cedar was the dominant shade species. One case was found during the first site visit, and no live individuals were collected or located during aquatic sampling. This area may be active due to its permanence and undisturbed state. Large fish may reduce the ability of the PRCF to thrive at this location.			
Matson 1	20 August 2009	Absent	Dawson; Platte
Site was sampled after identifying potential habitat using Google Earth®. No macrophytes were present, and evidence of cattle grazing was present. Russian olive was the dominant canopy species.			
Matson 2	20 August 2009	Absent	Dawson; Platte

Site was sampled after identifying potential habitat using Google Earth®. Area sampled was a restored slough near Matson 1 and also seemed to be impacted by cattle.			
Matson 3	20 August 2009	Absent	Dawson; Platte
Area sampled was dry area near Matson 1 and 2 that supported a wetland plant community and appeared to hold water for some of the year, but likely not long enough to support a PRCF population.			
McCloud	22 July 2009	Absent	Merrick; Platte
Site was sampled after identifying potential habitat using Google Earth®. Area sampled was a side ditch to slough channel that coursed to the north, north east. Area was dry when sampled, but had been wet. Vegetation stand in slough and along banks was dense. Instream vegetation was mostly cattails. Area seemed suitable for PRCF.			
McCormick 1	29 September 2009 15 April 2010 14 May 2010 26 May 2010 14 July 2010	Present Present Present Absent Present	Buffalo; Platte
This site was sampled at the request of the Headwaters Corporation. Five adults were observed in 2009. One larva was observed emigrating from the slough on 15 April 2010, which was earlier than previously reported in the literature (Whiles et al., 1999). Nine individuals were observed on 14 May 2010, but none on 26 May 2010, possibly because aestivation had already occurred by that time. Nine larvae were observed on 14 July 2010 through terrestrial sampling. This slough appeared similar to Martin's Reach 2, except for the presence of willows. Spraying for *Phragmites* in autumn 2009 left several patches of dead vegetation in 2010. Large fish, including Centrachidae, were present in slough. Mosquitofish was the dominant fish species present while sampling.			

McCormick 2	25 August 2009	Absent	Buffalo; Platte
This site and remaining McCormick sites were sampled after identifying potential habitat during reconnaissance of property. Area sampled was small slough dominated by willows along edges. Mosquitofish were abundant in slough and duckweed was abundant on water surface. Macrophytes were largely absent. Slough was initially thought to be separate entity from McCormick 1 but remained connected to McCormick 1 through 2010.			
McCormick 3	18 August 2009	Absent	Buffalo; Platte
Area sampled was ponded area with no emergent vegetation. Fallen leaves dominated pond substrate. Banks leading away from wetland were shallow. Cattle grazing occurred at this location.			
McCormick 4	18 August 2009	Absent	Buffalo; Platte
Area sampled was very similar to McCormick 3. Fish were absent from both wetlands.			
McCormick 5	18 August 2009	Absent	Buffalo; Platte
Area sampled was linear depression with *Equisetum* and RCG. Cottonwoods and green ash were dominant shade species. Area was dry during sampling but was surveyed, because area seemed to hold water for some portion of year. Water may have been too ephemeral for PRCF.			
McGee 1	6 August 2009	Absent (w/cases)	Buffalo; Platte
Site was sampled after identifying potential habitat using Google Earth®. The slough at this site was small and was an isolated depression. The upland area was covered in smooth brome and largely dry at the time. RCG was also present along the edges. There was no emergent vegetation, but duckweed was present on slough surface. Fish were absent. One case was collected, but a shovel was not used during survey. Site seemed suitable for PRCF.			

McGee 2	18 August 2009	Absent	Buffalo; Platte
Site was sampled after identifying potential habitat using Google Earth®. Area sampled was impounded slough and cattails were dominant macrophyte in slough.			
Milburn Diversion Dam WMA	20 August 2010	Absent	Middle Loup; Blaine
Site was sampled after identifying potential habitat using Google Earth®. Slough sampled was dominated by willows and was largely dry during visit. Cottonwoods were abundant. A shovel was not used during sampling.			
Morgan 1	17 June 2010	Present	Kearney; Platte
Site was sampled after identifying potential habitat using Google Earth®. Area sampled was long slough with intermittent breaks. Substrate was sandy and macrophytes were mostly absent, except for the presence of duckweed. Shade trees did not abut slough and largely consisted of cottonwoods, when present. More than ten larvae were observed.			
Morgan 2	17 June 2010	Present	Kearney; Platte
Site was sampled after identifying potential habitat using Google Earth®. Area was very similar to Morgan 1.			
Mormon Island	1997-April 2004 13 October 2007 16 September 2008 24 September 2008 21 May 2009 April 2010	Present Absent Absent Absent Absent Present	Hall; Platte

Type locality for species. Area was inside a large cattle exclosure when visited in 2008. No adults or larvae were found in 2004, 2007, or 2008 fall sampling (Goldowitz, 2004; Riens and Hoback, 2008; unpublished data). Area supported a diverse plant community in 2008-2009. Several macrophytes were present in 2008-2009, but water column was largely open with some duckweed present on slough surface. One case was found during aquatic sampling in May 2009, and some larvae were observed during 2010 (K. Geluso, unpublished data, 2010).			
Muskrat Run WMA	21 July 2010 11 August 2010	Absent	Lincoln; North Platte
Site was sampled after identifying potential habitat using Google Earth®. There were a couple of sloughs at this location, but the PRCF was absent. This area had been cleared of trees and sprayed for control of *Phragmites*, and the area was devoid of shade. Emergent vegetation was mostly cattails. Thistles were abundant along banks, which hampered search efforts. Survey efforts at this location included digging for larvae.			
Newark	12 April 2010 17 May 2010	Absent (w/cases) Present	Buffalo; Platte
Site was sampled at request of Headwaters Corporation. This site was a former side channel when visited. Slough banks supported an abundance of willows. RCG was dominant in the slough and along banks. Duckweed was present on the slough surface. Two cases, collected within the first minute of the site visit, were observed on 12 April, and two larvae were collected during aquatic sampling on 17 May. Mosquitofish were present.			
North River WMA	21 July 2010	Present	Lincoln; North Platte
Site was sampled after identifying potential habitat using Google Earth®. This area was likely a former channel to the North Platte River. The slough held about 10 centimeters of water. Canopy cover primarily consisted of cottonwood trees. Smooth brome was abundant on slough banks, and emergent vegetation was largely absent. One pupa was observed at this site (TJ Walker, NGPC).			

Patrick	11 August 2010 06 October 2009 11 May 2010	Present Present Present	Merrick; Platte
Site was sampled after identifying potential habitat using Google Earth®. Slough was an old side channel that fed into larger, restored area to the east. RCG was abundant in the slough and along slough banks. Shade species largely consisted of Eastern red cedar and cottonwoods. Restored area did not have any PRCF. Several larvae (151) were collected from this slough in 2010. One grab sample from a small pocket of the slough contained 123 larvae, and these larvae were collected from an area amongst several fallen cottonwood leaves.			
Platte State WMA	11 August 2010	Absent (w/cases)	Lincoln; Platte
Site was sampled after identifying potential habitat using Google Earth®. Area was dry during visit, but had recently been wet, as indicated by duckweed on wet substrate surface. Slough was situated amongst an abundance of shade trees, including Eastern red cedar and cottonwood trees. Several cases were observed.			
Prairie Wolf WMA	20 August 2010 07 October 2010	Absent (w/cases) Present	Loup; Nance
Site was sampled after identifying potential habitat using Google Earth®. Area sampled was a large, open slough, with little canopy vegetation. Slough may have been more than 0.75 meters at its deepest location. Substrate was mostly sand. Slough seemed to have a more permanent than intermittent hydroperiod. Cases were collected after digging in bank substrate for larvae, and one adult was observed in October 2010.			
Rowe 1	9 July 2009	Absent	Buffalo; Platte
Site was visited after conversation with Rowe personnel that sloughs on property could be potential PRCF habitat. Area sampled was restored slough across main channel of Platte River. Restoration work was more than five years old. Filamentous algae was dominant macrophyte in slough. Duckweed was present on slough surface and mosquitofish were present. Banks were largely unshaded.			

Rowe 2	9 July 2009	Absent	Buffalo; Platte

Site was visited after conversation with Rowe personnel that sloughs on property could be potential PRCF habitat. River bulrush, cattails and RCG were abundant in slough. RCG was abundant along slough edges. Slough was dry during sampling, but slough had been wet. A shovel was not used during sampling. Habitat seemed suitable.

Rowe 3	29 July 2009	Absent	Buffalo; Platte

Site was visited after conversation with Rowe personnel that sloughs on property could be potential PRCF habitat. Area sampled was restored slough. Restoration had occurred two years prior to sampling. Filamentous algae was dominant macrophyte present. Mosquitofish were present in water. Slough banks were largely unshaded.

Sherrerd	22 July 2009	Absent	Buffalo; Platte

Site was sampled after identifying potential habitat using Google Earth®. Slough was a restoration project that was conducted about eight years prior to the survey. Bank vegetation contained some purple loosestrife and sedges, and macrophytes were largely absent.

Silver Creek 1	06 May 2009 23 May 2009	Absent (w/cases) Absent (w/cases)	Merrick; Platte

Site was sampled after identifying potential habitat while driving in area. Slough appeared to be an old impoundment, but contained an abundance of cattails, and cottonwoods were present on slough banks. Duckweed was present on slough surface. Only cases have been observed at this location, and the amount of emergent vegetation made aquatic sampling difficult. Difficulty was encountered in trying to obtain landowner information.

Silver Creek 2	06 May 2009 23 May 2009	Absent (w/cases) Absent (w/cases)	Polk; Platte

Site was sampled after identifying potential habitat while driving in area. There was minimal flow at this site when visited, and 10 to 20 centimeters of standing water was present. Area was impacted by cattle grazing upstream and downstream of area sampled. PRCF cases were found amongst cottonwoods and green ash. Large trees were absent from slough on east side of highway.			
Sock	30 July 2009 6 October 2009 11 May 2010	Present Absent Present	Merrick; Platte
Slough sampled was relatively open. Shade species were present, but did not abut slough. There was a small amount of emergent vegetation in slough channel. Duckweed was present on water surface, and mosquitofish were present in the slough. A diverse mix of grasses lined the slough edges. Three larvae were observed at this location in 2009. No adults were observed in 2009. Several larvae (37) were collected here in May 2010.			
Sommer 1	31 August 2010	Absent	Lincoln; Platte
Site was sampled after identifying potential habitat using Google Earth®. Area was side channel habitat similar in appearance to Odessa WMA and Marshall. Side channel was dominated by dead, standing Phragmites and willows. Shovel was used during survey.			
Sommer 2	31 August 2010	Absent (w/cases)	Lincoln; Platte
Site was sampled after identifying potential habitat using Google Earth®. Two caddisfly cases were found at this location. Duckweed was abundant on the water surface. A shade canopy was present, and emergent vegetation consisted of cattails and rushes. Site seemed suitable for PRCF.			

TNC Sutherland 1	26 August 2009 29 August 2009 05 September 2009 31 August 2010	Present Present Present Present	Lincoln; North Platte

Site was visited after conversation with TNC staff about potential PRCF habitat at this location. Area sampled was a side channel to the North Platte River. The water was flowing slightly upon each visit and was consistently about 0.25 to 0.75 meters in depth. The area was dominated by *Polygonum* sp., willows., and RCG in and out of channel. Large cottonwoods were present away from the slough banks but provided shade to the channel. More than ten larvae/pupae were observed on both 29 August and 05 September 2009. Eight pupae were collected in 2010.

TNC Sutherland 2	26 August 2009	Absent	Lincoln; North Platte

Site was visited for same reason as TNC Sutherland 1. Site may have been connected to positive site upstream (TNC Sutherland 1). Area lacked distinct banks surrounding slough edge, which hampered search efforts at this site. Water edge was lined with rushes. Water depth was about 0.3 meters. A few willows were present, but large shade trees were absent.

TNC Wood River 1	5 August 2009	Absent	Hall; Platte

This site and rest of Wood River sites were visited at request of TNC staff. Area sampled was small pond with sandy substrate. Pond lacked macrophytes and other emergent plants. Pond banks were shallow.

TNC Wood River 2	5 August 2009	Absent	Hall; Platte

Area sampled was similar to TNC Wood River 1. Before sampling, area seemed unsuitable to PRCF.

TNC Wood River 3	5 August 2009 4 October 2009 21 May 2010 27 May 2010	Absent Absent Present Present	Hall; Platte
Area sampled was large slough adjacent to a pond and near the main river channel. Willows dominated slough banks and edges. Duckweed was abundant on slough surface. Cattails and mosquitofish were abundant in slough. When first surveyed, the PRCF was considered absent. Area was subsequently surveyed for adults in October 2009 and none were observed. One larva was collected in May 2010 while sampling for other macroinvertebrates in water column. One larva was collected from one of five subsequent samples on 27 May 2010.			
TNC Wood River 4	5 August 2009	Absent	Hall; Platte
Area sampled was restored grassland slough. Slough restoration was less than five years old.			
TNC Wood River 5	5 August 2009	Absent	Hall; Platte
Area sampled was small creek. Other caddisfly species in aggregated clump was observed on fallen log. Creek had minimal flow at time of sampling, but may attain velocities unsuitable for PRCF. Water depth was up to 0.75 meters in most locations, and slough may also have been too deep for PRCF.			
Tooley Park 1	22 Jun 2009	Absent	Hamilton, Platte
This site and Tooley Park 2 were visited after conversation with Prairie Plains Resource Institute staff about potential suitable PRCF habitat at this location. Area sampled was small slough that was bisected by trail through park. RCG was dominant species in and around slough.			
Tooley Park 2	22 June 2009	Present	Hamilton, Platte

Area sampled was side channel to the Platte River. *Phragmites* was the dominant vegetation along channel banks. Two larvae were observed.			
Trust Forested	28 September 2009 14 May 2010 27 May 2010 13 July 2010 26 July 2010	Present Present Present Present Present	Hall; Platte
Site was sampled after identifying potential habitat using Google Earth®. First observed larvae upstream of headquarters on 14 August 2009. Four adults were observed at this location on 28 September 2009 during night sampling. Twelve larvae were collected on 14 May 2010 and one larva collected on 27 May 2010. Twenty-six larvae were observed on 13 July 2010. Area sampled was a long slough with a dense canopy along the banks. Canopy species included cottonwoods, Eastern red cedar, dogwoods, white mulberry, *Morus alba*, and green ash. Water level at this site varied throughout the year and was near 1.3 meters at the deepest location, but about 0.3-0.5 meters in most places. Site had an intermittent hydroperiod but remained wet through 2010. Mosquitofish were abundant in slough from late spring through fall. Other minnow species were present in the water. Slough was mostly open, but duckweed was abundant on the slough surface. When present, macrophytes included *L. palustris* and river bulrush. RCG, sedges, and prairie cordgrass were abundant on slough banks.			
Trust Grassland	29 September 2009 01 October 2009 25 April 2010 30 May 2009 30 September 2010 01-03 October 2010	Present Absent Present Present Present Present	Hall; Platte

Slough was long and narrow with a water depth up to 0.75 meters. Some flow was observed at this location in May 2010 and was dry in October 2010, thus the hydroperiod was intermittent. A diverse mix of grasses, forbs, and sedges was present on slough banks, and the area was treeless. Mosquitofish were abundant in the slough from late spring through fall. Other fish species have been observed at this location, including the plains topminnow, *Fundulus sciadicus* Cope (M. Harner, personal communication, 2010). Duckweed was present in small amounts on water surface. Slough had few other macrophytes. This population was first observed in 2009 when PRCF adults were active at this site and were described as "swarming" (K. Geluso, personal communication, 2009). No adults were observed here on 01 October 2009, possibly because the PRCF was past its peak abundance. Over 500 larvae per square meter were observed at this location in May 2010 (Geluso et al., unpublished data). First observations that the PRCF burrows about five centimeters below the soil surface during aestivation were made at this location in May 2010. Over 100 adults were observed at this site on 30 September 2010. About thirty adults were observed on 01 October, four on 02 October, and zero on 03 October. Numbers observed indicated the peak abundance likely occurred on 29 or 30 September 2010.

Wallace	23 June 2010	Absent		Lincoln, unnamed creek

Site was sampled after identifying potential habitat using Google Earth®. Area sampled was a small creek with apparent intermittent hydrolperiod. Macrophytes and shade species were largely absent. Mosquitofish and other minnows were present in creek/slough. Rushes, sedges, and RCG were abundant on creek banks. PRCF may have been overlooked, because a shovel was not used during survey efforts. Area appeared to be suitable for PRCF.

Willhoft	30 July 2009 4 October 2009	Absent (w/cases)	Absent	Merrick; Platte

Site was sampled after identifying potential habitat using Google Earth®. This area was part of Warm Slough that ran through Central City and to the east. The area was covered in a large, monotypic stand of RCG. The water depth was about 15 centimeters, and duckweed was present on the surface. No cases or larvae were observed on the first visit. During a second visit to search for adults, one case was found. Habitat seemed suitable for PRCF.

Wyoming 1	14 July 2009	Absent	Buffalo; Platte

This site and all Wyoming sites were sampled at the request of the Headwaters Corporation. Area sampled was small, shallow wetland. Purple loosestrife was most abundant plant in and around slough. Mosquitofish were present in water pools that remained, as area was almost dry during sampling effort. Slough banks were shallow.

Wyoming 2	14 July 2009	Absent	Buffalo; Platte

Area sampled was a large, restored wetland that looked more like a pond than a slough. Algae and duckweed were present on the slough surface, and mosquitofish were abundant. Area likely had permanent hydroperiod. Cattails and sedges were present around slough edges. Slough banks were shallow. Water appeared deep in center (>1 m).

Wyoming 3	14 July 2009	Absent	Buffalo; Platte

Area sampled was a large, restored slough. Slough was starting to dry out when sampled, so area likely has an intermittent hydroperiod. Willows were abundant around slough banks. Filamentous algae and duckweed were present on surface. Purple loosestrife was abundant on slough banks, which were gradual.

Wyoming 4	14 July 2009	Absent	Buffalo; Platte

Area sampled was large, restored slough with little aquatic vegetation, except for some filamentous algae. Slough banks sloped gently away from water and may have been too gradual for PRCF. Mosquitofish were present.