POTENTIAL FACTORS INFLUENCING POPULATIONS OF THE PLATTE

RIVER CADDISFLY (*IRONOQUIA PLATTENSIS*)

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By

Michael Christopher Cavallaro

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POTENTIAL FACTORS INFLUENCING POPULATIONS OF THE

PLATTE RIVER CADDISFLY (*IRONOQUIA PLATTENSIS*)

Michael Christopher Cavallaro

University of Nebraska at Kearney, 2012

Advisor: W. Wyatt Hoback

Abstract

The Platte River caddisfly, *Ironoquia plattensis*, is a facultative benthic macroinvertebrate endemic to central Nebraska where it is listed as a Tier One, or at-risk, species. First described in 1999, *I. plattensis* was documented as the primary aquatic insect component (~50%) at the type locality, Mormon Island, near Grand Island, NE. The type locality is in a wet meadow that is devoid of trees, which resembles the historic conditions in the Platte River valley.

Between 2009 and 2011 extensive surveys were conducted along the Platte, Loup, and Elkhorn river systems; this added to the known range of *I. plattensis*. These survey efforts brought the cumulative total to 35 sites across three river systems occupied by *I. plattensis* or a closely related species. Five populations of caddisflies were selected to determine the amount of genetic variability and gene flow among three areas on the Platte River and two recently discovered populations on other river systems. Genetic variability was assessed using amplified fragment length polymorphism (AFLP). Adequate gene flow was found among all five populations, and relatively high genetic variability was determined by G_{st} and F_{st} values.

I. plattensis exhibits a one-year lifecycle and is adapted to a seasonal cycle of flooding and drying. Larvae migrate to land along the edge of backwater sloughs in late April or early May and aestivate, or maintain low levels of activity, until pupation in early September. A decrease in flooding disturbances has permitted the encroachment of exotic vegetation in riparian areas and catalyzed the drying of side channels. Subsequently, these modifications have altered the allochthonous leave litter input. A growth and survival study testing the effects of native and nonnative plants on aquatic fifth instars revealed no significant differences in weight gain but showed a significant increase in head capsule width in caddisflies that consumed cottonwood, *Populus*. Survival was highest for larvae that fed on native species and lowest for specimens that consumed common reed, *Phragmites*.

Variation in weather patterns can lead to hypoxia of aquatic environments and flooding of terrestrial environments. Immersion tolerance experiments compared aquatic fifth instars, terrestrial fifth instars, and pupae and showed pupae to be sensitive to hypoxia; as a result to prolonged exposure to hypoxic conditions both aquatic and terrestrial larvae accumulated lactate over time.

Predation by vertebrates often alters aquatic insect community structure. Compared to most predators tested, the brook stickleback (*Culaea inconstans*), is native to Nebraska but not to the historic range of *I. plattensis*. In a series of laboratory based feeding trials, brook stickleback demonstrated the ability to consume *I. plattensis* larvae of all sizes.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE: INTRODUCTION TO TRICHOPTERA, THE PLATTE RIVER,

AND THE PLATTE RIVER CADDISFLY

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Introduction to Trichoptera

The caddisflies (Trichoptera) are only outnumbered by Diptera in ecological diversity among insect orders which are have aquatic life stages (Mackay and Wiggins, 1979; McCafferty, 1998; Holzenthal et al., 2010), and are found on every continent except Antarctica (Morse, 2012). Caddisfly larvae are adapted for aquatic habitats and occur in nearly every type of freshwater aquatic ecosystem, lentic and lotic, as well as marine tidal pools (Triplehorn and Johnson, 2005; Holzenthal et al., 2010). Except for a few species which have a terrestrial larvae phase (e.g. *Ironoquia*), all immatures are aquatic (Banks, 1900; Flint, 1958; Williams and Williams, 1975). Caddisfly larvae are considered an important and large component of benthic communities in most aquatic ecosystems (McCafferty, 1998). This is credited to their ecological role as major components of secondary production in aquatic ecosystems (Merritt et al., 1996).

Although caddisflies are known to occur in all freshwater ecosystems, they are most abundant in lotic freshwater systems (Loeb and Spacie, 1994). Other common residents to these lotic systems are Ephemeroptera (mayflies) and Plecoptera (stoneflies); all three of these orders are sensitive to poor water quality and are widely accepted as bio-indicators (Lenat, 1988; Loeb and Spacie, 1994). Together, Ephemeroptera, Plecoptera, and Trichoptera, constitute the EPT index which provides a general biological assessment of water quality (Lenat, 1988; Loeb and Spacie, 1994). In addition to acting as biological indicators, caddisflies have been used in forensic entomological studies (Byrd and Castner, 2009). A case study from a south central Michigan stream revealed the use of Limnephilidae and Hydropsychidae larvae to determine postmortem

submersion interval (Wallace et al., 2008). Investigators used the varying case making behaviors to determine the overlap between time of immersion and time of disappearance of the victim (Wallace et al., 2008).

Caddisflies are holometabolous, and are most closely related to Lepidoptera (butterflies and moths) (McCafferty, 1998; Triplehorn and Johnson, 2005). The scientific name of the caddisflies, Trichoptera, is derived from the Greek *trichos* meaning hair(s) and *pteron* meaning "winged" or possessing wings (McCafferty, 1998; Triplehorn and Johnson, 2005; Holzenthal et al., 2010). "Trichos" describes the hair like structures covering adult caddisfly wings and most of their body (McCafferty, 1998). This is unlike Lepidoptera ("scale-wings"). Adult Lepidoptera often display colorful, vibrant patterns on their wings (Voshell Jr., 2002) while adult caddisflies typically exhibit drab, dull coloration (Voshell Jr., 2002). Together, Trichoptera and Lepidoptera comprise the superorder Amphiesmenoptera (Nel et al., 2007). Phylogenetically, this superorder diverged from the basal lineage: "Mecopterida" (Nel et al., 2007). Trichoptera first appeared during the Triassic period during the Mesozoic era (Grimaldi and Engel, 2005), and total phylogenetic divergence occurred late Jurassic, early Cretaceous (de Moor and Ivanov, 2008).

Morphologically, caddisfly larvae feature eruciform (caterpillar-like) and campodeiform (elongated, flattened) larval morphologies (Cushing, 1963); their mouthparts are downward facing, or hypognathous (Cummins and Merritt, 1978). The larvae have a sclerotized head and thoracic plate, and reduced antennae (Triplehorn and Johnson, 2005). In addition, they mostly resemble caterpillars but lack abdominal prolegs

with crochets (Peterson, 1962; Stehr, 1987; Voshell Jr., 2002). Similar to Lepidoptera larvae, spin silk from openings in the labium which originate from internal silk glands (Cummins and Merritt, 1978; McCafferty, 1998; Triplehorn and Johnson, 2005). The pupae display exarate morphology and are usually dectitious (Holzenthal et al., 2010). Adult caddisflies, or imagos, are somewhat similar to moths in general appearance (Voshell Jr., 2002; Triplehorn and Johnson, 2005), and are considered weak fliers (e.g. Ciborowski and Corkum, 1988; Greenwood and Bickerton, 2001; Kopp et al., 2001; Triplehorn and Johnson, 2005). Wings are held roof-like over the body, extend beyond the abdomen, and typically have parallel wing venation; adult wing spans can range from less than 3mm to as great as 100mm (Voshell Jr., 2002; de Moor and Ivanov, 2008). Also, unlike Lepidoptera, which possess a well-developed, coiled proboscis, adult caddisflies typically have deficient, poorly developed mouthparts (Crichton, 1957), and adults live for about a month (Stevens et al., 2000).

There are approximately 13,000 described caddisfly species belonging to over 45 families (Holzenthal et al., 2010; Morse, 2012); with total estimated diversity of nearly 50,000 species (Schmid, 1984). Ten out of the 620 known genera contain approximately 30% of the world's described species (de Moor and Ivanov, 2008). Diagnostic features which separate caddisfly families include presence of abdominal humps, thoracic sclerotization, position and length of antennae, shape of prolegs in relation to the anal claw, and case building, "caddis", substrate (Ross, 1944).

The relatively high diversity of caddisflies is attributed by their ability to occupy all four trophic categories as outlined by Cummins (1973): shredders, collectors, grazers,

and predators. The key feature that allows for this diversity lies within their silk glands. There are three accepted suborders of Trichoptera: Annulipalpia, Integripalpia, and Spicipalpia (Holzenthal et al., 2010); each of these suborders are characterized by the method of silk use (Wiggins, 1996; Wiggins, 2004; de Moor and Ivanov, 2008). Caddisflies have several methods for silk utilization, including safety lines (Odontoceridae and Hydropsychidae), attachment to substrate for support, prey restraint (Rhyacophilidae, Polycentropodidae and Arctopsychidae), building protective cases (Lepidostomatidae, Leptoceridae, and Limnephilidae), and nets or filters (de Moor and Ivanov, 2008). However, variation in silk utilization is even seen among caddisflies with similar strategies. Malas and Wallace (1977) found three different species of net-spinning caddisflies, *Parapsyche cardis*, *Diplectrona modesta*, and *Dolophilodes distinctus*, coexisting in southern Appalachian streams. Gut content analysis revealed that each species was targeting a specific particle size and was also reflected in the net mesh size constructed by each species (Malas and Wallace, 1977).

Caddisfly feeding strategies offer substantial value to the productivity of aquatic ecosystems. Cummins and Klug (1979) outline seven general food resources available to stream invertebrates: based on size detritus (particulate organic matter; POM), coarse particulate organic matter (CPOM), fine particulate organic matter (FPOM), ultra-fine particulate organic matter (UPOM), periphyton, macrophytes, and animals. These feeding strategies facilitate nutrient cycling in aquatic systems which govern the river continuum (Cummins and Merritt, 1978; Vannote et al., 1980).

Limnephilidae is the dominant caddisfly family; with roughly 300 species across 50 genera in North America (Cummins and Merritt, 1978). Occasionally referred to as "Northern Case-Makers", limnephilid larvae occur in a variety of aquatic systems and vary in size (8-35 mm) (Ross, 1944). Some characteristics of limnephilid larvae include heavily sclerotized pronotum and mesonotum, antennae located midway between eyes and manidibles, and a sclerotized plate present on top of ninth abdominal segment (Ross, 1944; Triplehorn and Johnson, 2005).

The *Ironoquia* genus is comprised of a total of six species: *I. plattensis, I. punctatissima, I. parvula, I. dubia, I. lyrata,* and *I. kaskaskia* (Holzenthal et al. 2010). Each member from the genus exhibits some terrestrial larval behavior (Ross, 1938; Flint, 1958; Williams and Williams, 1975; Whiles et al., 1999; Ćuk, R. and Vučković, 2010). Terrestrial behavior is unusual among caddisflies but is not unique to the genus *Ironoquia*. This terrestrial phase is exhibited in different capacities across several genera. *Philocasca demita* Ross was described in 1941, and is known as the first caddisfly species in North America described to be entirely terrestrial (Anderson, 1967). Investigators discovered every larval instar in pitfall traps and Berlese funnels indicating a fully terrestrial life cycle (Anderson, 1967). Diel movements have also been observed in the larvae of *Desmona bethula* Denning, another semi-terrestrial caddisfly species. Larvae migrate to land to feed and then return to the water as air temperatures become unfavorable ($<0^{\circ}$ C). Based on mark-recapture data, Erman (1981) estimate 14%-16% of the population make the journey to land over a span of two nights (Erman, 1981). Similar to *I. plattensis*, *Desmona bethula* is a univoltine Autumn-emerging Trichoptera (Erman,

1981). Pre-pupal and pupal terrestrial stages of *Archithremma ulachensis* Martynov were described by Levanidova and Vshivkova (1984). The genus *Nothopsyche*, also has varying terrestrial stages with members of the group exhibiting entirely terrestrial immature stages to partially, or conditionally induced, terrestrial immature stages (Nozaki, 2002).

The Platte River

Aquatic ecosystems within the Great Plains are highly dynamic and responsive to variable environmental fluctuations (Covich et al., 1997). The aquatic ecosystems within the prairies of North America are subjected to hydrologic variability and landscape pressures in addition to residing in a portion of an already endangered biome (Dodds et al., 2004). The Platte River is a part of the Missouri River basin and stretches roughly 530 kilometers across the state of Nebraska (Eschner et al. 1981; Liske, 2001); it is also one of the most important river systems in the Great Plains (Williams, 1978). The central Platte River, between Lexington and Chapman, is described as a continuously braided river system; caused by meandering reaches from several channels (Johnson, 1994). Nebraska also features many other characteristic Great Plains aquatic ecosystems including ox-bow lakes, springs, ephemeral streams, sloughs, and wetlands (Dodds et al., 2004). These ecosystems are threatened and continue to dwindle throughout North America as more of the Great Plains transitions to anthropogenic purposes, such as agriculture and urbanization (Dodds et al., 2004).

Historically, the Platte River featured few trees because of seasonal maintenance by wildfires, pulsed flooding, and ice scouring (Liske, 2001; Whiles and Goldowitz,

2001). Over the past twenty years multiple restoration projects have been implemented to

reduce trees and restore prairie biodiversity (Sidle et al., 1989; Currier, 1998; Liske,

2001). Piegay et al. (2009) describes the difficulties coupled with managing a braided

river system where the natural modifications which can occur rapidly create variable

geometric stream forms and boundaries. The organisms that rely on these habitats are

adapted to these conditions (Dodds et al., 2004); however, human intervention has

stabilized the channel consequently creating extreme environmental conditions in an

already variable habitat.

Since the arrival of European settlers, the Platte River valley has lost 75% of its

original wet meadows (Liske, 2001). Over the past 100 years; some examples include as

the construction of dams, irrigation canals, reservoirs, and water diversions have occurred

(Eschner et al. 1981). Irrigation is the primary use of the Platte River and the USGS

estimates nearly 70% of the flows at Grand Island, NE have been depleted by water

diversions and agricultural use (Aiken, 1999). Wilcove et al. (1998) states the greatest

threat to imperiled species is habitat degradation from agricultural operations (e.g.

irrigation diversions); in addition to habitat degradation, other mechanisms which cause

species to decline include pollution, harvesting, disease, and exotic species establishment.

Changes to the hydrology have affected many aspects of the Platte River, most

notably the riparian plant communities (Johnson, 1994). In the past 60 years, the river

corridor has become heavily forested and open areas have been invaded by exotic plants

including reed canary grass (*Phalaris arundinacea* L.), *Phragmites* Cav., russian olive

(*Elaeagnus angustifolia* L.), and purple loosestrife (*Lythrum salicaria* L.). The second

greatest threat to native biodiversity in an ecosystem is invasion by exotic species (Wilcove et al. 1998).These invasive plants severely reduce riparian vegetation diversity; Naeem et al. (2000) discuss the value of high native plant biodiversity as it creates an ecosystem which is more resistant to invading plant species.

The "Big Bend" region of the Platte River valley is considered critical habitat for many bird species including the whooping crane (*Grus americana*), the Great Plains population of the piping plover (*Charadrius melodus*), and the interior least tern (*Sterna antillarum athalassos*) (Norling et al., 1989). In addition, the shallow reaches of the lower Platte River provide important habitat for the endangered pallid sturgeon, *Scaphirhynchus albus* (National Research Council, 2005). Over the past 25 years, complications have arisen between the Endangered Species Act (ESA) and water management within the state of Nebraska. Conservation actions have prohibited total exploitation, to some degree, of the Platte River (Aiken, 1999).

Life History and Biology of the Platte River Caddisfly

The Platte River caddisfly, *Ironoquia plattensis* Alexander and Whiles (Trichoptera: Limnephilidae), was discovered in 1997 and described in 1999 from an ephemeral, wet meadow depression on Mormon Island in south-central Nebraska (Alexander and Whiles, 2000). The species was named after the location it was discovered—the Great Plains and Platte River (Alexander and Whiles, 2000). Morphologically and phylogenetically, it is most similar to *Ironoquia parvula* (Banks, 1900). The two can be easily separated by examining the parameres and shape of inferior

appendages (Alexander and Whiles, 2000). Alexander and Whiles (2000) hypothesize this divergence occurred during the Pleistocene glaciations.

I. plattensis larvae have five larval instars (Figure 1.1). As aquatic larvae, *I. plattensis* is a shredder of coarse particulate organic matter which facilitates the breakdown of leaf litter (Whiles et al., 1999). Larvae have been observed grazing or scraping algae from submerged logs (Cavallaro, personal observation). Atypical to most caddisfly species, *I. plattensis* features a terrestrial phase during the fifth and final instar life stage; it appears adapted to a seasonal cycle of flooding and drying (Whiles et al. 1999). Habitats supporting *I. plattensis* retain water 75-90% of the time (Goldowitz, 2004). *I. plattensis* moves to land in late April or early May, typically concurrent with seasonal hydrological shifts. Once larvae reach the slough bank they enter a state of aestivation, maintaining low metabolic activity, until pupation in September (Whiles et al. 1999).

Whiles et al. (1999) suggested that *I. plattensis* is likely an important component of energy transfer between the aquatic and terrestrial environments. Observations during this study showed *I. plattensis* larvae remain relatively active through the summer months (Cavallaro, personal observation). In addition, terrestrial larvae appear sensitive to abiotic stimuli (Cavallaro, unpublished data). Observations in the field (Geluso et al., 2011) and laboratory settings have revealed a subterranean behavior where *I. plattensis* larvae are found to adhere to the roots of fibrous monocots (Figure 1.3). Fifth instars of Lepidoptera enter a wandering phase where they feed very little, if at all, and search for a site to

pupate (Dominick and Truman, 1986; Stefanescu, 2000); I find this Lepidoptera "wandering phase" analogous to *I. plattensis* larvae terrestrial phase.

Upon pupating, *I. plattensis* larvae will collect detritus and leaf material which is integrated in a "door" to enclose themselves in their case (Figure 1.2). Pupation lasts an estimated 3-4 weeks (Whiles et al., 1999). After emergence, adults oviposit following fall rains and slough inundation (Whiles et al., 1999). Geluso et al. (2011) documented a "mass emergence" event in the fall of 2009 which included hundreds of individuals swarming in sync. Examination of the hydrology at the type locality showed that aquatic systems too ephemeral or permanent may be unsuitable for *I. plattensis* (Whiles et al., 1999; Whiles and Goldowitz, 2001). Whiles and Goldowitz (2005) delineated the macroinvertebrate assemblages across several wetlands within the central Platte River. Only one study site contained *I. plattensis*; its presence was significant when compared to other macroinvertebrates found (Whiles and Goldowitz, 2005). *I. plattensis* accounted for 9% of the total macroinvertebrate biomass and was the overwhelming dominant source of insect emergence (>50%) (Whiles and Goldowitz, 2005).

Platte River Caddisfly Conservation Status and Monitoring Efforts

I. plattensis is listed as a Tier-One, or at risk species although the United States Fish and Wildlife Service, "Service" hereafter, found it did not merit federal protection in 2012 (USFWS, 2012). Between 1999 and 2004, 49 surveys were conducted to identify sites occupied by *I. plattensis* and only six sites were found to support the species, in addition to the type locality (Goldowitz, 2004). When described, *I. plattensis* attained average aquatic densities of 802 larvae per m^2 ± 194 (Whiles et al. 1999). Initial *I.*

plattensis surveys were conducted with the use of a stove-pipe corer which offered a sampling area of 314 cm^2 (Whiles et al. 1999). Presently, among these original sites only one site supported densities similar to those historically found at the type locality while most other sites support less than 10% of historic numbers. In 2007, the Service was petitioned by the WildEarth Guardians to list *I. plattensis* as a federally endangered species due to its limited range, declining numbers, and sensitivity to habitat alterations.

Until 2009 it was believed *I. plattensis* range was limited to the Platte River. Between 2009 and 2010, 104 additional sites with potential habitat were visited, and 21 new populations were identified (Vivian, 2010). *I. plattensis* was found in sloughs adjacent to main channel of the Platte, Loup, and Elkhorn Rivers (Vivian, 2010); the habitat surrounding the backwaters ranged from native, vacant grasslands to sloughs with patches of heavy forest.

Continued surveys were conducted on Platte River Recovery Implementation Foundation properties. Current survey reports for these properties can be found in the Appendix A. Headwaters Corp. are a natural resources systems management consulting firm. Currently, the largest restoration project is focused around the Big Bend region of the Platte River valley. This restoration work is designed to improve staging habitat for migratory bird species including sandhill cranes (*Grus canadensis*), whooping cranes (*Grus americana*), the piping plover (*Charadrius melodus*) and the interior least tern (*Sterna antillarum athalassos*). This staging habitat used by these birds overlaps a large portion of *I. plattensis* known range. Different from the original life history paper, all of

the surveys conducted for the aquatic phase are completed with a 30 centimeter diameter D-frame dip net; searches for the terrestrial larvae follow the methods of Vivian (2010).

Recently, changes to the Platte River including invasion by native and exotic vegetation have required extensive habitat management and restoration through mechanical and chemical removal of vegetation (Pfeiffer et al., 1999). Because there is a lack of historic data, the impacts of vegetation removal on *I. plattensis* are unknown. In an effort to manage this habitat while respecting the potentially sensitive conservation status of *I. plattensis*, future work may include quantifying *I. plattensis* response to vegetation removal.

The Service lists 95 species of caddisfly that have merited an evaluation of their conservation status. Of those 95, only one, *Glyphopsyche sequatchie*, currently warrants protection. The Sequatchie caddisfly occupies two spring runs in Marion County, Tennessee. *I. plattensis* and *G. sequatchie* have similar reasons for their apparent decline (Vivian, 2010; USFWS, 2011). Limited distribution and small population sizes make them vulnerable to habitat alterations. For *I. plattensis*, changes in the Platte River's hydrology, invasive plant encroachment, and aquatic vertebrate predators coupled with these baseline concerns have caused their apparent decline. In addition to habitat change, the Sequatchie caddisfly experiences siltation from industrial practices and both point and nonpoint pollution (USFWS, 2011).

There are currently 3 species of Trichoptera on The Xerces Society for Invertebrate Conservation Red List: *Ochotricia susanae*, *Agapetus montanus*, and *Sericostriata surdickae*. These three species face threats from impaired water quality

which can be caused by increased siltation via erosion and runoff, low dissolved oxygen levels, high temperatures, and pollutants. All listed species share similar conservation needs: general survey information (abundance, distribution, and range) along with a better understanding of their life history and potential threats (Xerces.org).

There are several potential threats to *I. plattensis* and the goals of this research are to elucidate the factors influencing *I. plattensis* populations. I address the genetic diversity, microhabitat requirements, and deleterious effects of invasive species on *I. plattensis* populations.

OBJECTIVES

The objectives of my thesis project were to: 1) assess the genetic variation and gene flow among *I. plattensis* populations, 2) investigate the hypoxia tolerance in the immature stages and pupae of *I. plattensis*, 3) determine the effects of exotic riparian vegetation on individual *I. plattensis* larvae, and 4) assess the potential threats of vertebrate predators on early *I. plattensis* instars through laboratory studies.

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FIGURES

Figure 1.1 *Ironoquia plattensis* larvae II-V instars left to right (Photo by Cavallaro).

Figure 1.2 *Ironoquia plattensis* pupae completely enclosed (Photo by Cavallaro).
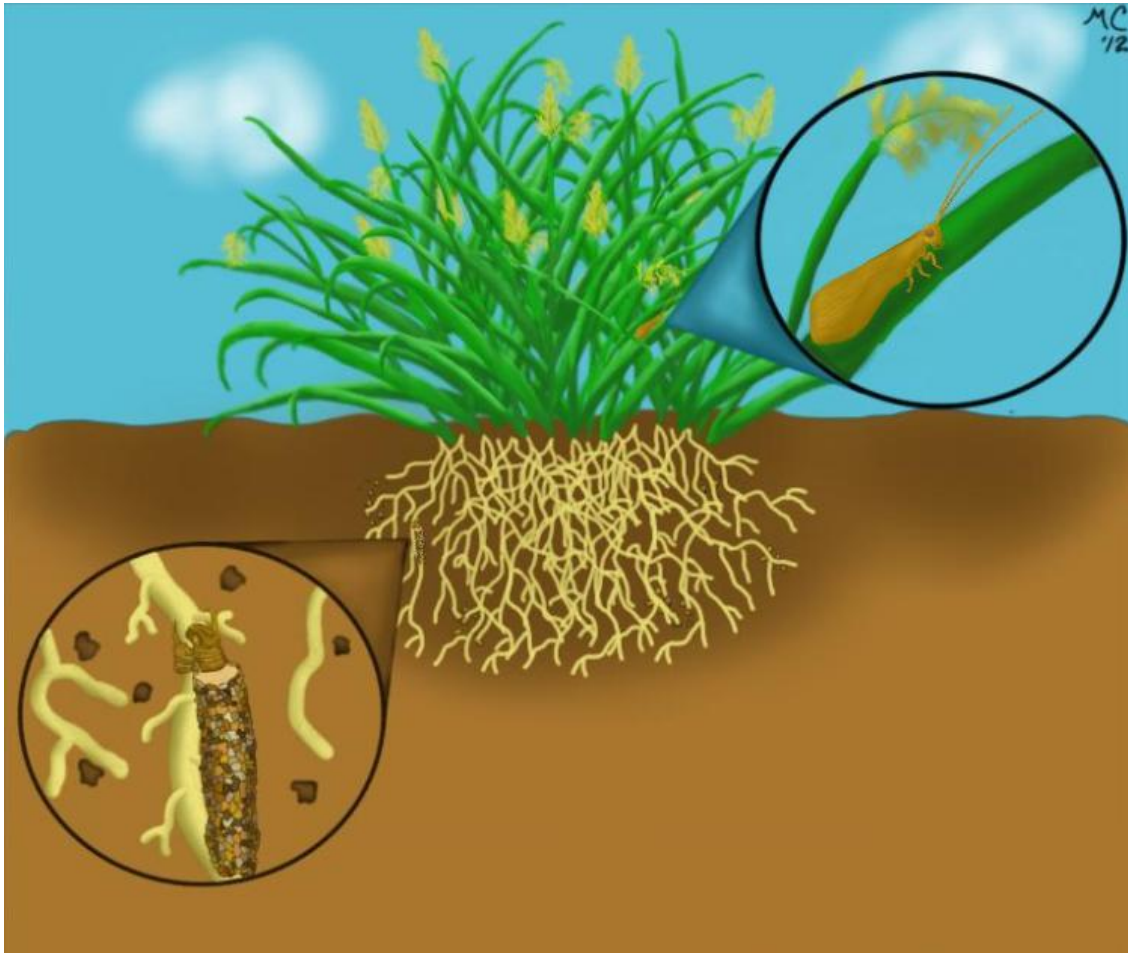
Figure 1.3 *Ironoquia plattensis* larvae can be found adhered to the fibrous root systems of monocots (e.g. Reed Canary grass) along slough banks. *I. plattensis* adults are exposed to windy conditions upon emergence in late-September and typically find refuge in the low lying vegetation.

CHAPTER TWO: PLATTE RIVER CADDISFLY RANGE, POPULATION ASSESSMENT, AND GENETIC VARIABILITY

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ABSTRACT

The Platte River caddisfly (*Ironoquia plattensis* Alexander and Whiles 2000) was described from a warm-water slough along the Platte River in central Nebraska and was considered abundant at the type locality. Surveys of 48 sites in 1999 and 2004 found five additional sites with this species. The caddisfly was found to be extirpated at the type locality in 2004 and an additional site in 2007. Because of its apparent rarity and decline, the Platte River caddisfly is a Tier I species in Nebraska and is being considered for listing under the Endangered Species Act. For this project, field surveys were conducted for new populations between 2009 and 2011 and identified 28 sites with the caddisfly out of 96 sites surveyed along the Platte River. Larval densities were quantified at a subset of sites with a population. At most sites, densities were lower than observed at the type locality. Six sites on other Nebraska drainages were found to support morphologically similar caddisflies. Because of the discovery of populations outside the Platte River drainage, amplified fragment length polymorphism (AFLP) were used to determine the amount of genetic variability among sites on the Platte, Loup, and Elkhorn rivers. Analysis of molecular variance (AMOVA) showed adequate gene flow among the three river systems. Differentiation, but not total divergence, was exhibited by the most northern population from the Elkhorn River. Low dispersal ability and disjunct populations with low densities suggest that all populations of this species should be conserved.

KEY WORDS Aestivation; intermittent hydroperiod; *Ironoquia plattensis*; slough; Trichoptera

INTRODUCTION

The Platte River caddisfly (*Ironoquia plattensis* Alexander and Whiles) was formally described in 2000 from an intermittent, warm-water slough in central Nebraska on Mormon Island, which is owned and maintained by the Crane Trust, Inc. (previously the Platte River Whooping Crane Maintenance Trust). Mormon Island has not been topographically altered for agriculture like the majority of the Platte River Valley (Whiles et al., 1999; Alexander and Whiles, 2000). Like other members of *Ironoquia*, the Platte River caddisfly emigrates from the water to aestivate in surrounding upland areas for about four months during the summer (Flint, 1958; Williams and Williams, 1975; Wiggins, 1977; Alexander and Whiles, 2000). This dormant stage likely represents an adaptation to withstand summer dry periods in intermittent wetlands (Williams, 1996). During a life history study in 1997-1998, the Platte River caddisfly was found to be an abundant component of the type locality and represented 57 percent of aquatic insect emergence production from the slough (Whiles and Goldowitz, 2001). After conducting the life history study, Whiles et al. (1999) reported 805 ± 194 larvae per m^2 were present in the aquatic environment, while 219 larvae per m^2 were observed during the aestivation period; thereby representing an important transfer of biomass from the aquatic to terrestrial environment.

After it was described, surveys for the Platte River caddisfly were conducted in areas with potentially suitable habitat in 1999 and 2004 (Goldowitz, 2004). Surveys in 48 locations identified five additional sites with the Platte River caddisfly on the Platte River along a 100-km stretch between Gibbon and Central City, Nebraska, and 10

locations, occurred on the Loup River. However, results for the Platte River caddisfly on the Loup were negative (Goldowitz, 2004).

Monitoring efforts in 2004 and 2007 did not detect the Platte River caddisfly at two of the nine previously surveyed locations, including the type locality (Goldowitz, 2004). Because of its apparent rarity, the Platte River caddisfly is listed as a Tier I species (those at risk of extinction across their range) in Nebraska (Schneider et al., 2005) and is being considered for federal protection under the Endangered Species Act (USFWS, 2009).

In this study, field surveys were conducted for the Platte River caddisfly in Nebraska, quantified aquatic and/or terrestrial larval densities at a subset of sites with Platte River caddisflies, and compared these data to historic numbers from the type locality. While conducting surveys in 2009, sites were found with morphologically similar caddisflies on the Loup and Elkhorn River. Sites with the caddisfly on all three drainages appeared similar in hydroperiod and vegetation composition and large differences were not observed in morphology among individuals collected on all drainages. However, it has been noted that morphologically comparable populations that are reproductively isolated can represent cryptic species (Martin and Bermingham, 2000; Parsons and Shaw, 2001). There are sites between the Platte, Loup, and Elkhorn Rivers that are a minimum of 50 km apart, and the absence of suitable habitat between isolated sites can represent a barrier to dispersal and subsequent breeding for species with poor dispersal abilities (Myers et al., 2001; Blakely et al., 2006). The Platte River caddisfly appears to be a weak flier (Vivian, personal observation), and genetics techniques can

provide insight into a species' dispersal ability in the absence of directly observing significant dispersal events (Clobert et al., 2001; Kelly et al., 2002; Blakely et al., 2006).

Amplified fragment length polymorphism (AFLP) was used to assess the following: 1) if caddisfly populations outside the Platte River exhibit gene exchange with populations along the Platte River, or if they represent an undescribed species or subspecies, and 2) if populations on the Platte River exhibit gene flow, or if breeding isolation has occurred.

MATERIALS AND METHODS

Study Area

To locate populations of the Platte River caddisfly, sloughs along the Platte, Loup, and Elkhorn Rivers in Nebraska were surveyed (Figure 2.1). The Platte River is formed at the confluence of the South Platte and North Platte Rivers east of the city of North Platte, Nebraska. Platte River flows are tied to snowmelt from Colorado and Wyoming and local precipitation events. The Loup and Elkhorn Rivers are tributaries of the Platte River; they drain portions of the Nebraska Sandhills, and are more dependent on input from the Ogallala Aquifer than precipitation events. Elevations range from 980 m above sea level at the western sites of the study area to 580 m at the eastern locations.

Each river surveyed contained adjacent side channels (sloughs) from past meanderings that either had a direct surface connection to the main river channel or were separated from the main channel by natural berms. These sloughs had little or no flow and a presence of organic sediment that had accumulated as a result of being cut off from the flow of the main channel. For this study, sloughs were characterized as lentic bodies

of water with stands of emergent vegetation and typical wetland flora, including cattails (*Typha*), willows (*Salix*), and duckweed (*Lemna*). These sloughs are subject to fluctuations in the groundwater table and surface water inputs (Whiles et al., 1999) and typically have an intermittent hydroperiod, holding water about 75-90% of the time (Goldowitz, 2004).

Identification of New Populations

Google Earth® (Google, Inc., Mountain View, CA) satellite imagery was used to locate potential Platte River caddisfly habitat. Potential habitat was considered to be any side channel or linear depression near the main river channel with signs of emergent aquatic vegetation and presence of water. After identification of potential habitat, landowner information was obtained for the purpose of obtaining permission to conduct surveys on private land. Surveys were also conducted at Wildlife Management Areas (WMAs) managed by the NGPC, rights-of-way along roadsides, and land owned by non-governmental organizations (NGO) including: the Nebraska Public Power District (NPPD), Central Nebraska Public Power and Irrigation District (CNPPID), Platte River Recovery Implementation Foundation, The Nature Conservancy (TNC), and the Crane Trust.

For the purposes of this study, a site was defined as a slough that was potentially suitable to Platte River caddisfly with at least 100 meters of upland area between it and another such area. Searches for terrestrial larvae were conducted for 30 minutes, and if no Platte River caddisfly were found, the site was considered to be unoccupied.

In 2009-2011, 96 sites were sampled along the Platte River, 13 sites on the Loup River, and six sites along the Elkhorn River. A Garmin® (Olathe, KS) GPSMAP® 60CSx was used to record the coordinates of each location sampled and only considered sites with live larvae as occupied.

Aquatic Sampling

Aquatic sampling was conducted in May 2009 and May 2010 at six known sites and 17 new sites to compare Platte River caddisfly population densities with numbers observed at the type locality by Whiles et al. (1999). In 2010, five sites were sampled twice. In each slough, four one-meter passes within the top three cm of substrate were made using a 30-cm, D-frame net. Four passes were made at all sloughs sampled, except at the Havens site in 2009 and the Trust Grassland site in 2010 (Geluso et al., 2011). The highest average larval density observed at each site was then divided by 410.67, the number observed at the type locality in May 1998 as reported in Whiles et al. (1999). On the non-Platte drainages, only presence/absence data were collected.

Terrestrial Sampling

A protocol was developed to conduct terrestrial sampling at all but two sites in 2009 and six sites in 2010 to establish Platte River caddisfly presence or absence at a given location and to quantify larval density on slough banks during the aestivation period (Whiles et al., 1999). If no individuals were observed within 30 minutes, the caddisfly was considered absent. Cases with larvae were 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 a larva was found, the timer was stopped, a 0.25 m^2 quadrat was placed on the ground with the larva at the center, and all aestivating larvae inside the quadrat were counted. This was repeated four times if larvae were found in four separate instances during the thirty-minute search. If larvae were only detected in one instance, then the remaining samples were recorded as zeroes. Counts were expressed as a percentage of historic numbers by dividing the highest average larval density obtained at each site by 219 (Whiles et al., 1999).

Genetics Analysis

Caddisfly larvae were collected using a metal sieve (25-cm diameter) and adults using a standard sweep net and a 75-watt mercury vapor light trap at five locations (Figure 2.3). Twenty individuals were preserved from each site for genetic analysis by placing them directly into 95% ethanol following collection. Samples were stored at -80°C upon reaching the laboratory. Thirty several genetic protocols were used to provide insight into a species' dispersal ability in the absence of directly observing significant dispersal events (Clobert et al., 2001; Kelly et al., 2002; Blakely et al., 2006). AFLP was used to determine gene flow within a species (Clark et al., 2007; Kneeland, 2011). Therefore, it is appropriate that the AFLP be used to test the hypothesis that the Platte River caddisfly is one breeding population. Thirty archived stable flies, *Stomoxys calcitrans* (Diptera: Muscidae), were used as the out-group.

DNA Extraction

All samples were washed in 70% ethanol and rinsed with nanopure® (Thermo Scientific, Waltham, MA) water prior to DNA extraction. DNA was extracted from the

thorax of each individual following a modified cetyl trimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). Extracted DNA samples was suspended in 1x TE (10 mM Tris-HCL; 0.1 mM EDTA) buffer and used a nanodrop® spectrophotometer (Nanodrop 2000, Thermo Scientific, Wilmington, DE) to determine the quality and quantity of the DNA, after which samples were stored at -20°C. Prior to initiating the AFLP procedure, DNA was diluted to 20-100 ng/µl.

Amplified Fragment Length Polymorphism

For the AFLP procedure, a modified protocol was followed from Vos et al. (1995) and optimized by Kneeland (2011). DNA was cleaved using common (*Eco*RI) and rare (*Mse*I) site restriction enzymes. Oligonucleotide adapters were ligated onto the sticky ends of the restriction fragments, and the resulting fragments were amplified non-selectively using primers that matched the sequences of the adapters. Finally, selectively amplified processed the DNA using primers with a three base pair extension sequence in addition to the adapter sequence (Table 2.1). The resulting AFLP product was run on a 6.5% polyacrylamide gel and visualized on a GeneReadIR infrared laser scanner (LI-COR Biosciences, Lincoln, Nebraska).

Data Scoring and Analysis

Gels were calibrated using an IRD-700 labeled 50-700 bp sizing standard and scored using the software program SAGA MX 3.2 (LI-COR Biosciences). The data were converted to a Boolean vector for further analysis, with a "1" indicating band presence and a "0" indicating absence.

Popgene 1.32 (Yeh and Boyle, 1997) was used to assess genetic diversity at the

population level with assumed Hardy-Weinberg equilibrium. The percentage of

polymorphic loci and Nei's gene diversity were calculated for each location. The

Popgene software was also used to estimate G_{st} which measures the amount of genetic

variation in multiple populations and the total population among populations sampled

(Nei 1973).

Arlequin v. 3.5 (Excoffier et al., 2005) was used to conduct the analysis of

molecular variance (AMOVA) and to calculate F_{st}, where F = the inbreeding coefficient,

S = subpopulations and T = the total population (Wright, 1950). The AMOVA tested for

genetic structure between and within populations. Significance was tested by running

1,023 permutations of the data.

RESULTS

Surveys

Between 2009 and 2011, field surveys were conducted for the Platte River

caddisfly at 108 new sites on the Platte, Loup, and Elkhorn Rivers, and six historic sites

were resurveyed for the caddisfly; Geluso et al. (2011) identified one additional site at the

Crane Trust. Along the Platte River, I identified 23 new sites that supported the Platte

River caddisfly out of 96 sites sampled, and five of the six historic sites still support a

caddisfly population (Figure 2.2). Geluso et al. (2011) also found the caddisfly to be

present at the type locality after six years of it being undetected there (Goldowitz, 2004;

Vivian, 2010). During surveys, 12 sites were found to only contain caddisfly cases and

no live individuals and found 54 sites to be unoccupied (Figure 2.2). Of the 54 locations

without the Platte River caddisfly, seven had evidence of cattle grazing (compacted soils, reduced vegetation cover), ten were sloughs that had been restored within the last ten years, and ten were sites with vegetation and water characteristics that visually differed from sites with extant populations. The remaining 27 areas contained apparently suitable habitat but did not support a caddisfly population.

Out of 18 sites surveyed on the Loup and Elkhorn Rivers, I found six sites with a caddisfly population, two sites contained cases only, and ten sites contained neither cases nor caddisflies (Figure 2.2). Among these sites, two sloughs were previously restored, one had evidence of cattle grazing, and two areas represented marsh habitat rather than slough habitat and were deeper than other sites occupied by the caddisfly.

Aquatic and Terrestrial Sampling

Because of differences in methodology, the aquatic sampling data in this study cannot be directly compared with those of Whiles et al. (1999). Nonetheless, Whiles et al. (1999) represents the best baseline for comparing caddisfly population densities. During the 2010 aquatic sampling, the highest mean larval density observed was 125.7 ± 95.47 per m^2 at the Patrick site, or about 31 percent of what was observed at the type locality (Table 2.2). Out of 16 sites sampled for larvae, 14 represented less than 10 percent of numbers observed at the type locality in 1997-1998 (Whiles et al., 1999; Table 2.2) and again in 2010 at the Trust Grassland site by Geluso et al. (2011; Table 2.2).

A direct comparison to our terrestrial larval density data cannot be made with those of Whiles et al. (1999) again because of differences in methodology. However, among all terrestrial sampling efforts, the Bombeck site contained the highest densities of

aestivating larvae (Table 2.3). One quadrat sampled at this 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. Results from terrestrial sampling across 13 sites with extant

populations resulted in only 12 percent or less of historic densities observed (Table 2.3).

I did not observe terrestrial larvae at one historic site, Brooks, and it appears the caddisfly

is extirpated from this location (Figure 2.2).

Genetics Analysis

Site restriction enzymes produced 218 informative markers. The AMOVA results

showed most of the variation to be within populations (62.15%) (Table 2.4). Statistical

analysis of the data revealed a gene flow of Nm = 1.2728 among the caddisfly

populations (Larson et al., 1984) as an Nm value >1.0 infers high gene flow (Clark et al.,

2007). Nm is the unit assigned to denote the amount of gene flow between populations

and is derived from the result of the sampled population number (N) and rate of

migration among those populations (m) (Larson et al., 1984; McDermott and McDonald,

1993; Clark et al., 2007). This can be estimated from F_{ST} ($F_{ST} \sim 1/(4Nm+1)$) (Allendorf

and Luikart, 2007). F_{ST} (0.37854) and G_{ST} (0.2820) were calculated with Arlequin and

Popgene, respectively, indicating that the majority of the genetic variation is within

populations (Clark et al., 2007).

A dendrogram was generated displaying genetic distances among the populations

tested using the UPGMA method (Figure 2.4) modified from the Neighbor-joining

method (Sneath and Snokal, 1973; Clark et al., 2007). Genetic distance measures the

amount of gene substitutions per locus and is an indication of differentiation (Nei, 1972).

In this case, early divergence was exhibited by the "Elkhorn1" population. "Elkhorn1" shared 89%-92% of common bases, whereas the remaining caddisfly populations sampled shared 91%-96% (Table 2.5).

DISCUSSION

With the identification of 23 new caddisfly populations on the Platte River between 2009 and 2011, the known range of the Platte River caddisfly is now approximately five times larger than the range previously reported for the species (Goldowitz, 2004). Survey efforts also identified six populations of a similar caddisfly on other river systems, which I further examined using AFLP because of the separation of sites by geographic distance.

Although the identification of new caddisfly populations has led to an increase in the known range of this species, the distribution of the Platte River caddisfly is disjunct (Figure 2.2). Currently, a 155-km gap in sites with the Platte River caddisfly occurs between Elm Creek, Nebraska and Hershey, Nebraska along the Platte River. Within this range, I searched 21 sites for the caddisfly, but only two sites were found with cases only, and no live individuals were observed. The absence of the caddisfly within this area of the Platte River could be a result of habitat loss and/or degradation. Low numbers of the caddisfly may also remain undetected by our sampling methodology or be impacted by the time of year that the surveys were conducted.

Within the 155-km gap, several canals divert water for irrigation, and this water diversion has resulted in the loss of several wetlands along the Platte River (Currier et al.

1985; Sidle et al. 1989). Six mainstream dams along the Platte River upstream of the gap have also resulted in wetland losses along the river corridor (Currier et al., 1985; Sidle et al., 1985). During this study, I observed several depressions at Dogwood Wildlife Management Area, and these areas were dry but showed signs of previous beaver (*Castor canadensis*) activity indicating that changes in water levels had occurred. Meanwhile, the Platte River in this area is incised because of a diversion return, and this may have resulted in the loss of sloughs as channel incision is known to lead to adjacent wetland deterioration and losses (Fischenich and Morrow, 2000).

The methodology used to detect new Platte River caddisfly populations in 2009-2011 was to search the ground surface for discarded larval cases and aestivating larvae. However, in May 2010, aestivating larvae were observed buried in the ground 5 -10 cm below the ground surface. This was previously an undocumented behavior in Trichoptera (Geluso et al. 2011) and may be a means to avoid desiccation (Wiggins et al., 1980). This behavior has subsequently been observed at two additional locations.

In 2009 and 2010, terrestrial surveys for sites with the Platte River caddisfly occurred after mid-July. By this time of year, mortality may occur to aestivating larvae, potentially as a result of desiccation (Vivian, personal observation). Thus, surveys for aestivating larvae should be conducted in early summer or avoided all together. Because of variability associated with terrestrial sampling, aquatic sampling offers the most standardized way of quantifying Platte River caddisfly larval densities.

Population Assessment

Geluso et al. (2011) reported the presence of a population with larval densities approximately 35% greater than those observed at the type locality (Whiles et al., 1999). This site at the Crane Trust is about five km upstream of the type locality, and both sites consist of mixed grass prairie with wet meadow habitat and a lack of tree cover and similar hydroperiods (Whiles et al., 1999). Two other sites, Bombeck and Patrick, supported populations that were 24 and 30 percent, respectively, of what was observed at the type locality (Whiles et al., 1999). Eight other sites sampled aquatically were found to have densities of less than two individuals per m^2. Given the abundance of the caddisfly at the type locality in 1997-1998 and at the Trust Grassland site in 2010, it appears the caddisfly achieves greater abundance in some locations compared to others. A related species, *I. punctatissima*, has been observed with similar population characteristics and is locally abundant in certain systems (Gray and Johnson, 1988).

Low densities compared to those of the type locality may indicate that some sites with the caddisfly represent marginal or degraded habitat. One factor affecting habitat quality could be the lack of emergent vegetation such as cattails at some sites, which would reduce the amount of autochthonous material in the slough. In other wetlands and streams, shredders, like the caddisfly, are generally absent from areas without sufficient autochthonous or allochthonous material (Vannote et al., 1980). Permanent waters may also reduce caddisfly abundance at some locations, as sites with water depth greater than one meter and with permanent hydroperiods had fewer larvae, such as at the McCormick site. Permanent waters may be more favorable to fish and amphibians that can prey on caddisfly larvae (Wissinger et al., 2003; Whiles and Goldowitz, 2005; Tarr and Babbitt,

2007). Differences in habitat type may also explain the differences in densities observed at the type locality and all other sites. The sites at the Crane Trust are dominated by grasses and sedges and lack a tree canopy, whereas most other sites are surrounded by woody riparian vegetation.

One other concern regarding surveys was the discovery of 12 sites with only cases and no live larvae or adults. Laboratory data suggest that Platte River caddisfly cases degrade slowly as has been observed with other species (McCabe and Gotelli, 2003), and I recommend that cases not be used to established caddisfly presence (Vivian, 2010). Sites with only cases may represent recent extirpation events or populations with very low larval densities.

Genetics Analysis

Using AFLP enables comparisons for assessing genetic diversity among closely related species and populations (Mueller and Wolfenbarger, 1999; Kelly et al., 2002). Our genetic analyses indicate the Platte River caddisfly occurs on the Platte, Loup, and Elkhorn Rivers and is not endemic to the Platte system as was previously thought. The data show that this caddisfly is more widespread than previously reported; some populations are isolated from one another by distances of up to 100 km or more. Gene flow among caddisfly populations appears to be relatively high. However, even species with larger Nm values may contain populations that are completely isolated from the remaining populations (Larson et al., 1984). This seems to be the case with the Elkhorn 1 site, which was the most divergent from the other populations tested and is the site that is the most distant from the others sampled.

According to Williams (1996), aquatic insects that occur in temporary freshwaters most likely disperse as adults as opposed to drifting as larvae. It has also been reported that Limnephildae are strong fliers (Svensson, 1974; Kelly et al., 2002) with body size and wing length corresponding to dispersal ability (Kovats et al., 1996). Platte River caddisfly adults are small compared to many other Nearctic limnephilid species (Alexander and Whiles, 2000). Based on observations of Platte River caddisfly adults, this species appears to be a poor flier and likely has low dispersal ability. Platte River caddisfly adults are also active for a short period of time (Whiles et al., 1999), and this likely limits its dispersal ability compared to other caddisflies with longer adult lifespans (Svensson, 1972).

AMOVA results showed that most of the genetic variation is within populations (Kelly et al., 2002; Clark et al., 2007; Krumm et al., 2008). The positive correlation between genetic distance and F_{ST} value further demonstrates the sampled Platte River caddisfly populations remain isolated from one another and that gene flow is low with at least some of the sites sampled. Our value is similar to that reported by Kelly et al. (2002) for *Wormaldia tagananana*, a species endemic to the Canary Islands and one with a narrow geographic range. Kelly et al. (2002) attributed the narrow range of *W. tagananana* to its poor dispersal ability. Although Kelly et al. (2002) found *W. tagananana* to have high genetic structuring, they reported that the species was subject to loss of heterozygosity through genetic drift. The high degree of isolation in sites currently occupied by the Platte River caddisfly indicates the species is also likely subject to this loss.

Site isolation is likely a result of habitat loss resulting from land use change and water development in central Nebraska. During pre-settlement times, overland dispersal would likely have occurred more frequently because of the presence of more sloughs across the landscape. Because a majority of Nebraska's wetlands have been lost and converted for other uses, remaining Platte River caddisfly populations will likely remain isolated from one another and be subject to inbreeding depression and localized extirpation events, particularly when one considers the small size of some populations (Ruggeiro et al., 1994; Brook et al., 2002).

MANAGEMENT IMPLICATIONS

Two recent surveys indicated no detection of Platte River caddisfly populations, including the type locality (although the Platte River caddisfly was found again at the type locality in 2010), and 12 sites with cases-only suggesting recent extirpation, the species appears to be threatened by ongoing landscape-level changes. Landscape-level changes in hydrology including water development and irrigation projects likely pose the greatest threat to this species.

The cause of the decline of the caddisfly at sites along the Platte River is unknown; if the cause is intrinsic (natural), there may be little that can be done to prevent further decrease (Flather and Sieg, 2007). However, if extrinsic (anthropogenic) causes can be identified, the possibility for recovery is greater, and the species should be a high priority for conservation (Flather and Sieg, 2007). Understanding the population trends for this species will be essential to determining the best means of conserving the species and assessing the risk of its extinction (Reynolds, 2003).

Reynolds (2003) identifies vulnerable populations as those with either a small/declining range size or low/declining abundances. Our results suggest that the Platte River caddisfly has a larger range than was initially known for the species, although this is likely the result of increased sampling rather than range expansion. Further monitoring of its distribution will be necessary to determine whether changes in range size are occurring. Monitoring changes to its habitat will also be important in the conservation of this species.

The differentiation between the Elkhorn River population ("Elkhorn1") and the other caddisfly populations suggests that these groups should be viewed separately and that conservation of all populations is important to preserve genetic variation in the species. Further genetics analysis of additional sites could help identify whether or not the caddisfly is vulnerable to habitat fragmentation and isolation of sites (Tscharntke et al., 2002).

The Platte River caddisfly may be in decline across its range, and human alterations that affect temporary wetlands such as diversion projects or impoundments could further jeopardize the species. Efforts to restore or improve flows in the Platte River, as is being done under the Platte River Recovery and Implementation Program, could benefit the caddisfly; however, care should be taken to not create permanent waters that would allow colonization by fish which may act as predators on the caddisfly (Vivian, 2010).

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FIGURES
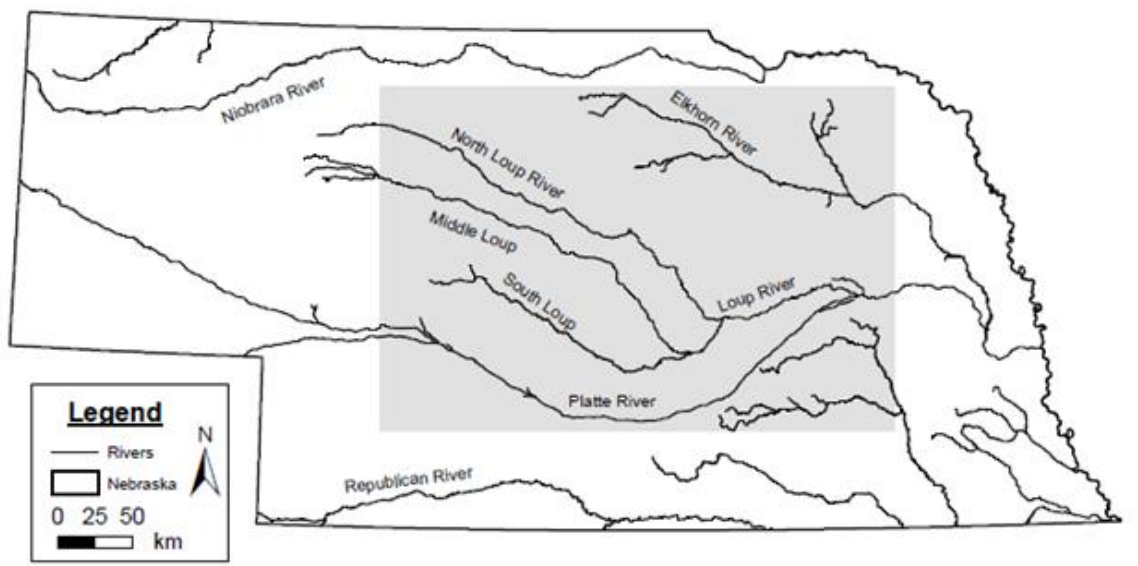
Figure 2.1 Map of Nebraska with major rivers labeled. Shaded portion represents survey area and study area for this project.

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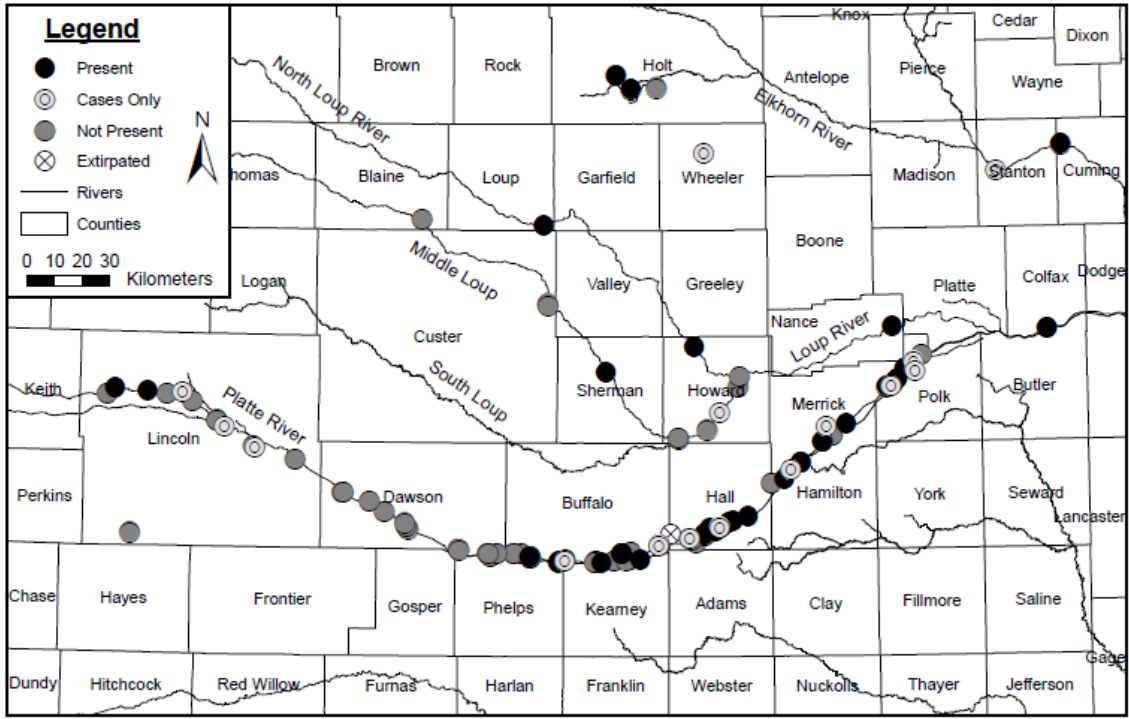
Figure 2.2 Map depicts 2009-2011 survey efforts conducted in search of sites with the Platte River caddisfly, *Ironoquia plattensis*, along the Platte, Loup, and Elkhorn River in Nebraska. Black circles indicate caddisfly presence, dark gray circles indicate caddisfly not present, double gray circle indicates caddisfly cases only, and the circle with a black "X" indicates caddisfly apparently extirpated
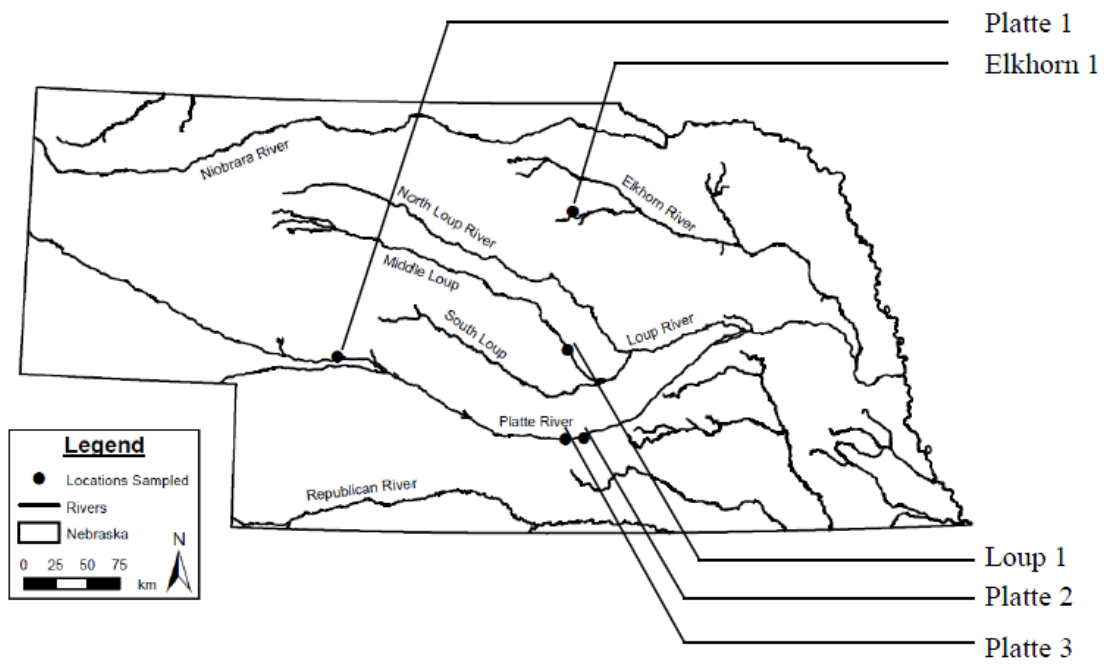
Figure 2.3 Map of sampling locations of populations represented in the study.

```
                              +-----------Platte3
                              !
                      +--3          +-----Platte1
                      !  !      +--1
                      !  +----2  +-----Platte2
  +-----------------------------------------4          !
  !                           !          +-------Loupe1
--5                           !
  !                           +-------------Elkhorn1
  !
  +--------------------------------------------------- Stable Fly Out Group
```
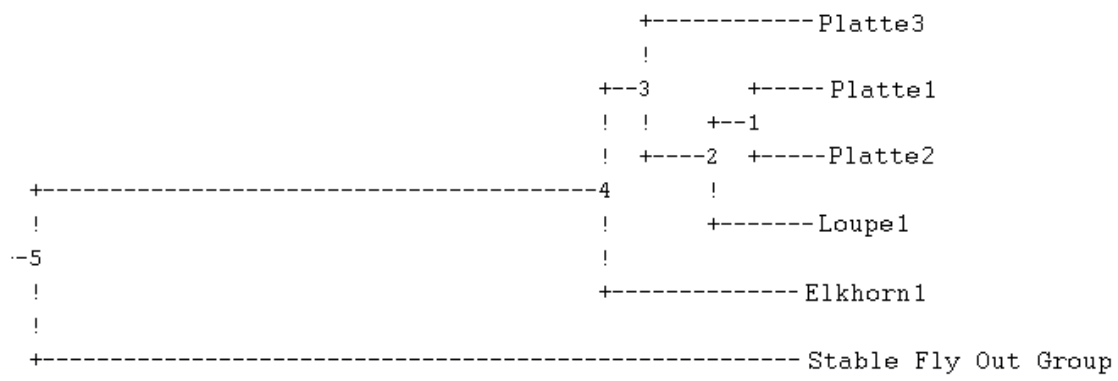
Figure 2.4 UPGMA dendrogram (Nei 1972) with genetic distances *Ironoquia plattensis* populations and out group.

TABLES

Table 2.1 Primers and adapters used in selective amplification. Primers with three basepair extension sequence in addition to the adapter sequence are indicated with (*).

Oligonucleotide	Purpose	Sequence (5'-3')
*Eco*RI-forward adapter	Adapter ligation	CTCGTAGACTGCGTACC
*Eco*RI-reverse adapter	Adapter ligation	AATTGGTACGCAGTCTAC
*Mse*I-forward adapter	Adapter ligation	GACGATGAGTCCTGAG
*Mse*I-reverse adapter	Adapter ligation	TACTCAGGACTCAT
*Eco*RI primer	Pre-Amplification	GACTGCGTACCAATTC
*Mse*I primer	Pre-Amplification	GATGAGTCCTGAGTAA
M-CTG	Selective Amplification	GACTGCGTACCAATTC + CTG*
M-CAC	Selective Amplification	GATGAGTCCTGAGTAA + CAC*
E-ACT	Selective Amplification	GACTGCGTACCAATTC + ACT*

Table 2.2 Mean number (± 1 S.E.) of Platte River caddisfly larvae collected through aquatic sampling along the Platte River. Sites with one asterisk (*) denote 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 Vivian (Geluso et al. 2011).

Site Name	Larvae per m^2 2009	Larvae per m^2 2010	Larvae per m^2 2010 - 2	Percent of 1998 (410.67)
Bader	0.83 ± 0.83	0.83 ± 0.83	--	0.20
Bassway	--	1.7 ± 1.67	--	0.41
Binfield	1.7 ± 0.96	0 ± 0	--	0.41
Binfield3	--	1.7 ± 0.97	--	0.41
Bombeck	54.0 ± 14.14	45.8 ± 8.95	98.2 ± 43.53	23.96
Dearking	--	0.83 ± 0.83	--	0.10
East Odessa*	--	0 ± 0	--	0.00
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	18.50
McCormick	--	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*	--	0 ± 0	--	0.00
Trust Forested	--	10.0 ± 5.61	0.83 ± 0.83	2.45
Trust Grassland**	--	553.0 ± 284.0	--	134.67

Table 2.3 Mean number (± 1 S.E.) of terrestrial Platte River caddisfly larvae observed using the quadrat protocol along the Platte River.

Site name	Larvae/m^2 2009	Larvae/m^2 2010	Percent of 1997 (219 larvae/m^2)
Bassway	1 ± 1.0		0.46
Binfield1	0 ± 0.0		0.00
Binfield3	5 ± 1.0		2.28
Bombeck	9 ± 3.0	116 ± 54.7	52.97
Brooks	0 ± 0.0		0.00
Dearking	3 ± 1.9		1.37
Glasser	3 ± 1.0		1.37
Havens		25 ± 3.4	11.42
Hoback	9 ± 5.7		3.65
Hord Lake	5 ± 2.5	14 ± 4.2	6.39
McCormick		11 ± 4.4	5.02
North River		1 ± 1.0	0.46
Patrick	2 ±1.2		0.91
Sock	3 ± 3.0		1.37
Trust Forested	1 ± 1.0	26 ± 5.3	11.87

Table 2.4 Two-level AMOVA fixation indices and results. Significance tests were accomplished with 1,023 permutations.

Source of Variation	D. F.	Sum of Squares	Variance Components	Percentage of Variation
Among Groups	2	570.073	5.03503 V_a	17.83
Among Populations Within Groups	2	252.201	5.6567 V_b	20.03
Within Populations	92	1614.871	17.55294 V_c	62.15
Total	96	2437.144	28.24468	
Fixation Indices	V_c and F_{st}	0.37854	P-value	0.057 ± 0.0070

Table 2.5 Genetic identity (above diagonal) and genetic distance (below diagonal) (Nei, 1978; Kelly et al. 2002) of Platte River caddisfly populations and out group.

Population ID	Platte3	Elkhorn1	Platte1	Platte2	Loup1	Stable Fly Out Group
Platte3	****	0.9152	0.9347	0.9273	0.9118	0.7301
Elkhorn1	0.0886	****	0.9263	0.9138	0.897	0.6796
Platte1	0.0675	0.0766	****	0.9663	0.9528	0.6863
Platte2	0.0755	0.0902	0.0343	****	0.9583	0.6863
Loup1	0.0923	0.1087	0.0483	0.0426	****	0.7068
Stable Fly Out Group	0.3146	0.3863	0.3835	0.3765	0.3471	****

CHAPTER THREE: HYPOXIA TOLERANCE IN LARVAE AND PUPAE OF THE SEMI-TERRESTRIAL PLATTE RIVER CADDISFLY (*IRONOQUIA PLATTENSIS*)

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ABSTRACT

The Platte River caddisfly, *Ironoquia plattensis*, is a benthic macroinvertebrate that is only found associated with river side channels in central Nebraska. These areas are subject to pulsed spring rains which wane to subsequent drying. *I. plattensis* larvae are adapted to this hydric cycle, partitioning time as larvae in water and on land where they eventually pupate. Flooding, especially soil flooding, can cause severe hypoxia and most terrestrial organisms that are trapped underwater drown in a short period of time. Shallow, warm waters ($> 30°$ C) and aquatic environments that receive nutrients can also experience severe hypoxia as a result of algal blooms and decomposition. I exposed aquatic larvae, terrestrial larvae, and pupae of *I. plattensis* to severely hypoxic water and found that pupae were most sensitive having a time to 50% mortality (LT_{50}) of 3.14 and 7.67 hours, at $20°$ C and $10°$ C respectively. Aquatic fifth instars had an LT_{50} of 44.17 and 74.21 hours and terrestrial larvae survived 61.68 and 89.89 hours, at $20°$ C and $10°$ C respectively. Both aquatic and terrestrial larvae accumulated lactate in response to hypoxia. The hypoxia tolerance of terrestrial larval stages suggests an adaptation to flooding while the sensitivity of the pupal stage suggests that fall flooding could cause significant mortality.

INTRODUCTION

The aquatic ecosystems in the Great Plains of North America undergo frequent

hydrologic changes as a result of highly variable weather patterns (Dodds et al., 2004).

These seasonal changes present a challenging environment for even the most adapted

organisms (Naiman and Decamps, 1997). The effects of prolonged immersion or

exposure to hypoxic environments on metabolically active insects has been extensively

studied (reviewed by Hoback, 2011), but relatively overlooked with regards to species

conservation (Sei, 2004; Brust et al., 2006).

The yearly lifecycle of the endemic Platte River caddisfly, *Ironoquia plattensis*,

aligns with the historic hydrologic cycles associated with the Platte, Loupe, and Elkhorn

drainages (Whiles et al., 1999; Vivian, 2010). Between 1999 and 2004, 49 surveys

identified only six sites supporting *I. plattensis*, including the type locality (Goldowitz,

2004). Presently, among these original sites only one site supports comparable densities

to those historically found at the type locality while most other sites support < 10%

(Vivian, 2010) which prompted the United States Fish and Wildlife Service (Service

hereafter) to consider protection for the species.

The *I. plattensis* exhibits an uncommon life stage in trichopterans where it exits

the aquatic environment of the backwater areas (sloughs) and aestivates on land

beginning in May (Whiles et al., 1999). During this time larvae can move and burrow

into soil (Geluso et al., 2011). In August, larvae become immobile and pupate for 2-3

weeks (Whiles et al., 1999). Pulsed flooding events and variable weather patterns

commonly leave stagnant water which in turn generates hypoxic conditions (Baumgärtl et

al., 1994; Brinkman, 1994). Anthropogenic modifications to the Platte River and the surrounding riparian habitat have altered the quantity of water available and increased nutrient inputs (Eschner et al., 1981; Sidle et al., 1989; Dodds et al., 2004). Over the past 100 years, humans have utilized the rivers to construct dams, irrigation canals, and water diversions to control seasonal flooding and provide irrigation water (Eschner et al., 1981). Of these changes, irrigation use is the primary catalyst behind water availability in the river corridor; by the USGS estimates nearly 70% of the flows at Grand Island, NE have been depleted via water diversions and agricultural use (Aiken, 1999).

Microhabitat oxygen concentrations associated with aquatic ecosystems often dictate benthic macroinvertebrate assemblages (Hoback and Stanley, 2001; Garvey et al., 2007; van der Geest, 2007). Previous studies have recognized the importance of oxygen concentrations as it relates to caddisfly larval habitat selection, food processing, and spatial distribution (Becker, 1987; Ward, 1992; Nebeker, et al., 1996; Chapman et al., 2004; van der Geest, 2007). This study determined the immersion tolerance of different life stages of *I. plattensis*. A subsequent objective quantified the evidence of anaerobic respiration.

MATERIALS AND METHODS

Immersion trials

Aquatic fifth instars were collected with a D-frame dip net from a slough south of the Platte River near Gibbon, Nebraska. Terrestrial fifth instars and pupae were collected with a metal sieve using the methods of Vivian (2010) from the slough banks adjacent to where aquatic larvae were collected. Following the methods of Hoback et al. (1998),

larvae and pupae were submerged in hypoxic water to simulate hypoxic conditions which may occur in backwater sloughs due to decomposition and respiratory demands or the flooding of soils surrounding the slough. Aquatic and terrestrial fifth instars were placed in individual 35-ml screw cap glass vial followed by deoxygenated water. Spring water was bubbled with nitrogen gas for 3 minutes per half liter of water in a 1000-mL Erlenmeyer flask. To release any air bubbles trapped in their sand cases or adhered to the glass, all vials were gently tapped.

All vials were subsequently placed into Percival® environmental chambers set at either 10° C or at 20° C in darkness. Larvae were removed from hypoxic conditions in subsets of 5 individuals at 48, 54, 60, 66, 72, 78, 84, and 90 hours. Controls consisted of ten individuals in 35-ml screw cap vials with normoxic water for aquatic instars and ten individuals in vials with a moist cotton ball for terrestrial instars, which were observed for activity at each time interval. Aquatic larvae were placed in 60-mL plastic containers filled with aerated water and terrestrial larvae were placed in plastic container with a moist cotton ball, returned to the environmental chamber, and given 24 hours to recover. A full recovery for larvae was defined by walking upright after the allotted 24 hour recovery period. *I. plattensis* pupae, like many holometabolous insects, roll their abdomen when contacted. Fully recovered pupae were defined by this characteristic. All pupae were given 24 hours in recover in a Petri dish with a moist cotton ball. After 24 hours the pupal cases were cut at the posterior tip without harming the pupae, and then pupae were prodded to provoke an "abdominal roll" to determine survival.

Survival data were analyzed using Toxstat 3.4 software. Time to 50% mortality (LT_{50}) and 95% confidence intervals were calculated using probit analysis.

Lactate assays

A total of 15 individual aquatic and terrestrial larvae were immersed in severely hypoxic water and removed in subsets of five individuals at intervals of 50, 60, and 70 hrs. Five individuals not exposed to hypoxia were flash frozen and used as controls. Time intervals were determined by the LT_{50} of the previous experiment.

Upon removal, all individuals were flash frozen with liquid nitrogen and stored at -80° C until the lactate assay was performed. To prepare samples, specimens from each time interval were placed in 10µL of lysis buffer, ground with a plastic pestle in a 1.5 mL Eppendorf tube, and centrifuged at 4,000 x g for 4 minutes at 4° C. The supernatant was removed and then centrifuged in a 10kDa MWCO spin filter to deproteinize.

The lactate assay was conducted according to the protocol outlined by the manufacturer (Sigma Aldrich, Lactate Assay Kit, No. MAK064). Colormetric assay absorbance measurements were performed at 570 nm in a 96-well plate reader; each sample was analyzed in triplicate. Based on a standard curve, wavelength measurements were used to calculated lactate volume in µg/µL.

RESULTS

Survival of immersion in severely hypoxic water differed among the three life stages tested (Figure 3.1). At 20° C conditions, aquatic larvae and terrestrial larvae survived 44.17 and 61.68, respectively, while pupae only survived a significantly shorter

time of 3.14. At 20° C conditions, aquatic larvae and terrestrial larvae survived 74.21 and 89.89, respectively, while pupae survived 7.67 hrs, significantly less than larvae.

Lactate assays performed on both larval stages showed a comparable increase in accumulation over time at 20° C (Figure 3.2). The amount of lactate increase was significant when exposed to hypoxic conditions demonstrating *I. plattensis* performs anaerobic metabolism as both aquatic and terrestrial larvae.

DISCUSSION

A multitude of insects have adapted strategies to colonize, persist, and thrive in oxygen limited environments (Hoback and Stanley, 2001). Typically, aquatic insects are sensitive to severe hypoxia, with the exception of chironomid and chaoborid larvae (Knipling et al., 1961; Hoback and Stanley, 2001). *I. plattensis* is an interesting test subject, having morphologically identical larval during both their aquatic and terrestrial stage; oxygen-limitations are experienced in both aquatic and terrestrial environments during flooding. Hypoxia tolerance in insects has been measured over a wide array of groups (see review Hoback, 2011), and anaerobic respiration induced by oxygen deficient systems has been documented across several families (Nielsen and Christian, 2007; Hoback, 2011).

Although not statistically significant, terrestrial larvae survived longer than aquatic larvae. Differences among survival of life stages have previously been documented in holometabolous tiger beetles and hemimetabolous grasshoppers, and appear to correlate with likelihood of hypoxia exposure for some groups (Brust et al.,

2006; Brust and Hoback, 2009). The sensitivity of pupae may be a result of metabolic demands of this stage or changes in anaerobic capacity.

Van der Geest (2007) describes two tactics aquatic insects can employ when exposed to hypoxic conditions: limit all activities for minimal oxygen use or actively attempt to escape hypoxic conditions. The extended tolerance of hypoxic conditions by terrestrial larvae could be explained by the frequency of exposure or depressed metabolic rates associated with aestivation.

Vivian (2010) discusses a subterranean behavior which aestivating terrestrial larvae exhibit; further notes on this behavior are mentioned in Geluso et al. (2011). The soil organic horizon is the most hypoxia prone terrestrial environment (Baumgärtl et al., 1994; Hoback and Stanley, 2001). Soil anaerobiosis occurs in waterlogged soils (Drew and Lynch, 1980; Baumgärtl et al., 1994); the increased microbial productivity associated with fine root systems in soils found in riparian zones potentially compounds the severity of hypoxia when compared to other soils (Kiley and Schneider, 2005; Bingham, 2009). The exact mode of oxygen uptake during the terrestrial life stage is currently unknown; although, larvae typically reside along the slough banks were there is high relative humidity and low temperatures suggesting atmospheric gas exchange occurs over moist gills which are retained by terrestrial larvae (Cavallaro, personal observation).

Changes in the morphology of the Platte River indirectly affect the macroinvertebrate assemblages, side channels, and slough habitats, by altering slough banks via erosion (Milner and Gloyne-Phillips, 2005). Eroded slough banks with exposed roots provide important instream habitat for a myriad of aquatic insects (Bingham, 2009).

The functional role these root mats play in stream habitats is largely unknown (Bingham, 2009); conversely, these exposed roots may facilitate the transition from the aquatic to terrestrial phase in the *I. plattensis* lifecycle (Cavallaro, personal observation). Although *I. plattensis* incorporates this as a necessary stage in their development, the migration to land can also be viewed as a behavioral adaptation to changes in oxygen concentration (Heinis and Swain, 1986); we see adjustments in spatial distribution of some aquatic invertebrates based on hypoxic events—e.g. zygopterans (Apodaca and Chapman, 2004), Ephemeroptera (Bäumer et al., 1999), Hydropsychidae (van der Geest, 2007), Decapoda (Bell and Eggleston, 2005), etc. Under hypoxia induced environment stress, where their time to achieve fifth instar may be truncated, larvae may begin early aestivation as a preventative strategy allowing them to persist in sloughs which dry suddenly or more quickly than during common seasonal conditions.

Here, I report the first evidence of Trichoptera generating lactate via anaerobic metabolism. During its aestivation *I. plattensis* exhibits low metabolic activity and high lipid reserves—and they do not appear to eat once they exit the water (Cavallaro, personal observation). Aquatic fifth instars are more active than in their subsequent terrestrial phase potentially causing a slightly quicker accumulation of lactate.

Gill ventilation is an important aspect in oxygen uptake in aquatic insects. During hypoxic conditions, larvae of the stonefly (*Oyamia lugubris*) exhibit a "push-up" behavior to help facilitate respiratory demands (Genkai-Kato et al., 2000). Philipson and Moorhouse (1976) reported 60-70% of oxygen uptake of caddisfly larvae belonging to the family Polycentropodidae was achieved using ventilation. The species of this family,

unlike Limnephilidae, do not form a case. Cased caddisfly larvae ventilate their gills by undulating their abdomen to force water across their gills (Leader, 1971; Phillips and Moorhouse, 1974). The oxygen uptake mechanism in terrestrial larvae is unknown, but it is unlikely they exhibit any gill ventilating behaviors. During trials with aquatic larvae, nearly 30% of the larvae immersed remained active and continued to chew or shred parts of their case despite severely hypoxic conditions (Cavallaro, personal observation).

Aquatic larvae of *I. plattensis* are CPOM (coarse particulate organic matter) shredders, physically manipulating leaf litter (Whiles et al., 1999). Where they occur, *I. plattensis* is the dominant component of the macroinvertebrate community responsible for CPOM breakdown (Meyer and Whiles, 2008). Meyer and Whiles (2008) found higher shredder abundance and biomass was directly correlated by the occupation of *I. plattensis* at tested sites across the Platte River. Oxygen deficiency has been documented to severely alter foraging behaviors in other caddisfly larvae (Nebeker et al., 1996; Bjelke, 2005). Nebeker et al. (1996) found a delay in larval development, molting success, and time of molting in the caddisfly, *Clistoronia magnifica*, at dissolved oxygen concentrations below 4.6 mg/L. Bjelke (2005) compared the decomposition rates in CPOM shredding larvae and found zero consumption and consequent starvation at dissolved oxygen levels below 2.0 mg/L. Hypoxic conditions during the *I. plattensis* aquatic life stage in these backwater systems could inhibit growth and harshly impact populations with low densities. Future work should test the ability of aquatic instars to decompose coarse particle organic matter under hypoxic conditions.

ACKNOWLEDGEMENTS

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FIGURES
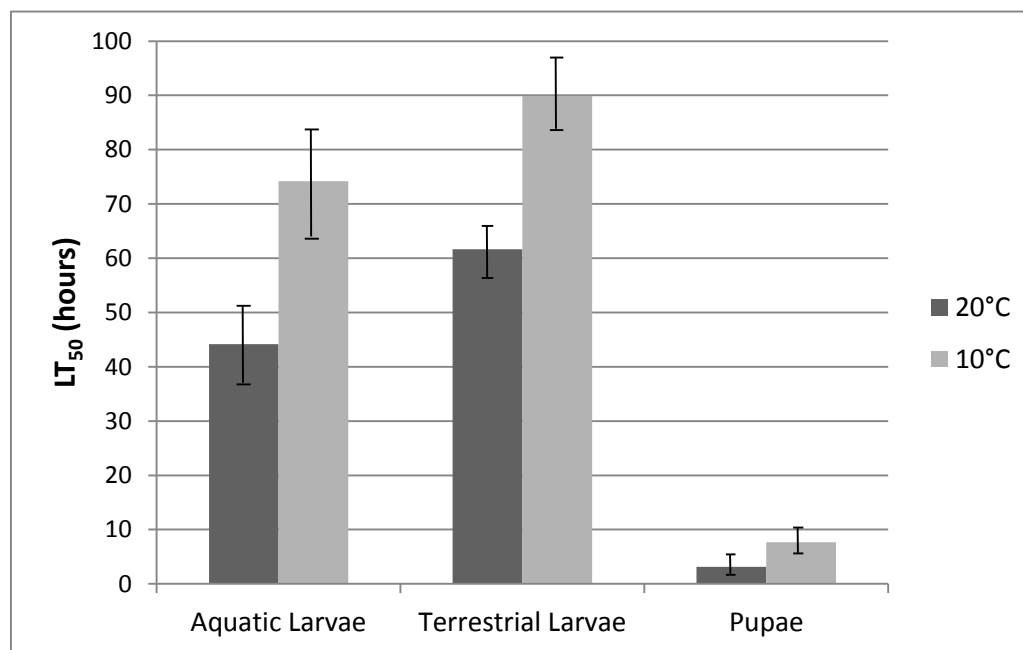
Figure 3.1 Mean survival times ($\pm$ 95% Confidence intervals) of three life stages of *Ironoquia plattensis*.
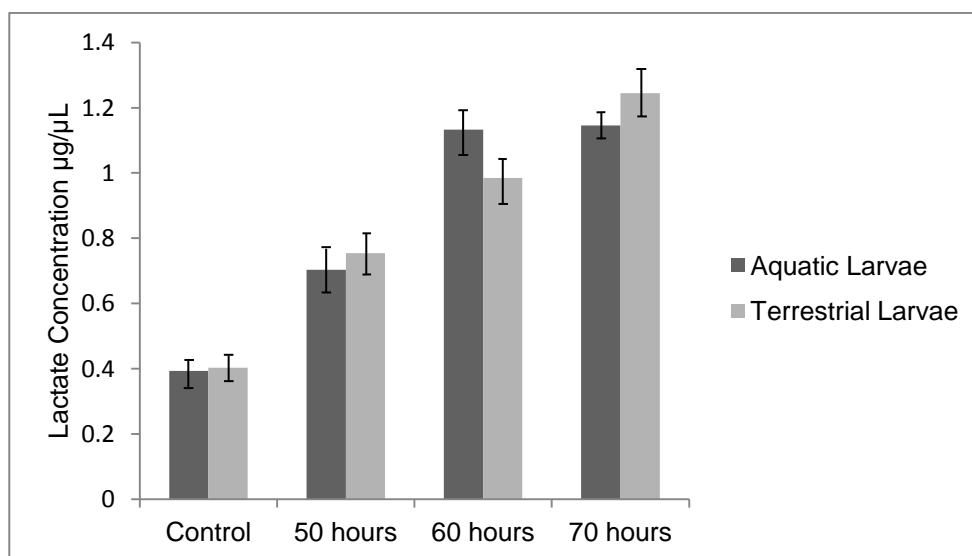
Figure 3.2 Mean ($\pm$ 1 S.E.) lactate concentration (ng/μL). Evidence of comparable lactate accumulation over time in both immersed aquatic and terrestrial larvae at 20ºC. Time 0 hours represents control larvae that were kept in normoxic conditions.

CHAPTER FOUR: EFFECTS OF NATIVE AND INVASIVE ALLOCHTHONOUS LEAF LITTER ON PLATTE RIVER CADDISFLY (*IRONOQUIA PLATTENSIS*) LARVAL GROWTH

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ABSTRACT

The Platte River caddisfly, *Ironoquia plattensis* Alexander and Whiles, is a

CPOM shredding limnephilid that is endemic to backwater sloughs associated with

Nebraska river systems. At the type locality, dominate plant communities included

lowland tallgrass prairies with occasional patches of riparian forest. As the dominate

component of the macroinvertebrate community, *I. plattensis* was estimated to consume

about 13% of the annual CPOM stock. Anthropomorphic alterations to Nebraska river

systems have disrupted the annual maintenance of these plant communities effectively

inviting the proliferation of nonnative plants and more extensive native woody plant

communities. Invasive plants severely reduce riparian vegetation diversity and

consequentially modify allochthonous input of leaf litter in adjacent waterways.

Controlled laboratory experiments examined the growth and food preference of *I.

plattensis* larvae exposed provided with leaf detritus. Growth was quantified after 30 days

using a no-choice test. Three native species, plains cottonwood, *Populus deltoides* Marsh,

broadleaf cattail, *Typha latifolia* L., and prairie cordgrass, *Spartina pectinata* Link, and

three nonnative species russian olive, *Elaeagnus angustifolia* L., common reed,

Phragmites australis Cav., and reed canarygrass, *Phalaris arundinacea* L. were tested.

Significant head capsule growth was observed for larvae that consumed native plant

detritus ($p = 0.02$) and *P. deltoides* alone ($p = 0.03$). *T. latifolia* was preferred in choice-

tests over three other plant species offered, although *T. latifolia* did not produce the

fastest relative growth. Nonnative plant leaf litter appears to limit growth of *I. plattensis*

potentially affecting production of this species that has a terrestrial phase after aquatic feeding.

KEYWORDS: riparian, Platte River, *Phragmites*, Trichoptera, feeding rates

INTRODUCTION

Prairie wetland systems are dynamic habitats yielding high productivity and biodiversity to the surrounding environments (Whiles and Goldowitz, 2005). These ephemeral systems are highly influenced by precipitation events, seasonal drying, and sediment deposits which dictate the plant communities (Helfield et al., 2012). Increasing concerns over the loss of prairie wetlands have prompted numerous management and restoration projects (Sidle et al., 1989; Currier, 1998; Liske, 2001; Whiles and Goldowitz, 2005). Unfortunately, the degradation of prairie wetland systems has been exacerbated by agricultural development (Meyer and Whiles, 2008). Moreover, impoundments to control seasonal flooding and provide irrigation water have resulted in increases of woody riparian vegetation.

In Nebraska, most restoration projects are associated with the Big Bend region of the Platte River valley (Kessler et al., 2011). Prior to these changes, June floods, summer drought, and ice scouring regulated the Platte River riparian communities (Johnson, 1994). Changes to the Platte River hydrology have allowed invasion by both nonnative vegetation and native woody plants and trees. Recently, much attention has been given to how the invasive riparian plants surrounding the braided Platte River channels is effecting native fauna (Rapp et al., 2012) with special concern to sandhill cranes, *Grus*

canadensis, (Johnsgard, 1980; Kessler et al., 2011) and river otters, *Lontra canadensis*, (Williams, 2011).

The endemic Platte River caddisfly, *Ironoquia plattensis* Alexander and Whiles, has a limited distribution and resides in palustrine wetlands along the Platte, Loup, and Elkhorn River systems (Vivian, 2010). Aquatic larvae are CPOM (coarse particulate organic matter) shredders (Newman, 1991), primarily physically manipulating leaf litter (Whiles et al., 1999). *I. plattensis* displays an uncommon terrestrial life phase where it will migrate to land in late May; this movement is associated with the flooding and drying of its habitat making it an important energy link to both aquatic and terrestrial systems (Whiles et al., 1999).Wetlands too ephemeral or permanent are typically not suitable for *I. plattensis* (Whiles et al., 1999); all sites retain water 75-90% of the time (Goldowitz, 2004). In the presented study, all of the native plant species (*Populus*, *Typha*, *Spartina*) used were documented at the *I. plattensis* type locality (Whiles et al., 1999). Previously studies have documented lower rates of CPOM decomposition in intermittent systems compared to more permanent systems (Tate and Gurtz, 1986; Hill et al., 1988; Whiles and Goldowitz, 2005). These regions are heavily impacted by changes in riparian plant communities and river morphology (Dodds et al., 2004).

Invasion by nonnative plants, or encroachment of native species into previously unoccupied habitats, in riparian ecosystems have potential to modify the structure of benthic macroinvertebrate communities through the input of allochthnonous vegetation (Moline and Poff, 2008). Such alterations affect habitat structure can directly influence

the benthic community diversity (Pidgeon and Cairns, 1981; Thompson and Townsend, 2003).

Previous research has shown nonnative allochthonous inputs to affect native macroinvertebrates. For example, the effects of nonnative *Elaeagnus* provides lower grade habitat and nutrition compared to cottonwood leaves, *Populus* (Moline and Poff, 2008). Royer et al. (1999) found higher macroinvertebrate diversity associated with *Populus* leaf packs when compared to *Elaeagnus* leaf packs after 30 days in an Idaho, U. S. A. stream. However, Moline and Poff (2008) conducted similar laboratory experiments using a cranefly shredder, *Tipula spp.*. Investigators found positive growth in all three conditions and highest survival with *Populus*.

Using a series of controlled laboratory experiments, I tested growth and survival of *I. plattensis* larvae fed various monospecific diets and given choices to determine their food preference.

MATERIALS AND METHODS

Plant material and larvae collection

In autumn of 2011, leaf material from six common riparian plants that inhabit the Platte River valley were directly collected post-senescence. Three of the selected species were nonnative (russian olive, *Elaeagnus angustifolia* L., common reed, *Phragmites australis* Cav., or reed canarygrass, *Phalaris arundinacea* L.) and three were native species (plains cottonwood, *Populus deltoides* Marsh, broadleaf cattail, *Typha latifolia* L., prairie cordgrass, *Spartina pectinata* Link). These species were chosen because of their abundance at currently active *I. plattensis* populations (Vivian, 2010). Materials

were dried and stored at room temperature until larvae were collected in spring of 2012. Caddisfly larvae were collected with a D-frame dip net from a detritus accumulation in a culvert south of the Platte River in Gibbon, Nebraska. Larvae were allowed to acclimate to laboratory conditions for a week prior to the experiment.

Monospecific diet growth

Testing methods followed Moline and Poff (2008), with slight modifications. All vegetative material was pre-leached two weeks prior to the start of the experiment. Late instar larvae ($n = 20$) were fed one of the six plant species. Larvae were placed in a mesh bag (mesh size < 2.0 mm) which was placed into an artificial living stream model and kept around 15.0 ° C ($\pm$ 1.5 ° C); larvae were fed a single plant species per treatment *ad libitum* for 30 days.

Before and after each trial, each larval total case length and head capsule width was measured to the nearest 0.01 mm using a micrometer. Larvae were then blotted dry and weighed to the nearest 0.001 g. Width of the larval head capsule was measured as the distance between the most distant lateral sides of head capsule margins; total case length was measured at the maximum length between the anterior and posterior ends. Larval mortality was also noted after each trial. Student's t-tests were used for statistical comparisons of before and after measurements.

Food choice assay

To test aquatic and terrestrial larvae preference among 6 plant species a cafeteria-style assay was conducted over the span of 24 hours following the methods of Dorn et al. (2001). Vegetative material was cut into 1 cm^2 squares and arranged on a randomized

2x2 grid in a Petri dish. Individual fifth instars were given 24 hours to feed. Aquatic

larval tests were conducted in aerated stream water and terrestrial larval tests were

conducted on moist paper towels to prevent desiccation. Petri dishes were 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 ambient temperature

remained constant at $20.0 \pm 1.0°$ C. Percent of leaf area consumed per larval was

measured using a leaf area meter (Li-COR 3100) and was compared using a

nonparametric Friedman rank test.

RESULTS

Monospecific diet growth

The 120 larvae maintained on the diets of six different plant species displayed

similar weight gain (Figure 4.1). No significant changes in total case length or wet weight

were observed across the six different treatments (Tables 4.1 & 4.2). Head capsule width

measurements were compared and found to differ significantly for native plant species (p

= 0.02) and *P. deltoides* treatments (p = 0.03). The highest mortality was observed in

trials with the nonnative *Phragmites* (60%). Cumulative mortality in nonnative plant

species was 30% compared to 7% among native plant species.

Food choice assay

Aquatic larvae consumed all of the plant species in the four-choice assay and

showed no significant preference. Nonparametric Friedman rank test ordered the

preference as *Typha latifolia > Populus deltoides > Phragmites australis > Elaeagnus*

angustifolia (Figure 4.2). Terrestrial larvae exhibited no measurable feeding on any plant species offered.

DISCUSSION

Overall, the results of this study were consistent with previous inquires involving the flexibility in aquatic macroinvertebrate diet (e. g. Sinsabaugh et al., 1985; Rong et al., 1995; Yeates and Barmuta, 1999; Rincon and Martinez, 2006; Moline and Poff, 2008). *I. plattensis* larvae were able to increase in size on all plant species. Positive growth was observed in all surviving larvae for each treatment (Table 4.2). The significance of the growth may have been influenced by the time and temperature provided. The duration of similar experiments on caddisfly larval growth ranged from 41 days (Hutchens et al., 1997), 7-21 days (Campos and Gonzalez, 2009), and 28-49 days (Gonzalez and Graca, 2003). In addition, Gonzalez and Graca (2003) tested the effects of a variety of deciduous plant species on the growth of *Sericostoma vittatum* larvae at four different temperatures. The results of their study found larvae had greater success and an increase in decomposition rates at specific temperatures depending on the plant species provided (Gonzalez and Graca, 2003). Both the environmental chambers and living stream were kept at a constant temperature of 15º C so that observed differences in feeding were not a result of temperature effects (Frouz et al., 2002).

The most significant increase was found in the head capsule width in the *P. deltoides* treatments; also, cumulative analysis of the head capsule width growth among native and nonnative head capsule widths found a significant increase in native treatments (Table 4.2). This suggests greater growth efficiency as a result of more

successful molts (Nijhout, 1975). Preliminary observations in the field utilizing leaf bags suggest that *I. plattensis* use *P. deltoides* as a dietary component (Figure 4.3). The mean head capsule width measurements of aquatic instars II-V generally shows the average increase in instars in each treatment (Figure 4.4). Further review shows three of the six treatments, *P. deltoides, T. latifolia, and P. australis*, on average showed an increase of one instar stage (Figure 4.5). The *P. deltoides* were the only treatments which saw significant head capsule width increases and *T. latifolia* was consumed in the greatest amount during the food-choice assays. Only 10% mortality occurred in *P. deltoides* and *T. latifolia* treatments, whereas larvae fed *P. australis* suffered >50% mortality. The limited sample size as a result of high mortality might account for the one instar stage achievement in *P. australis*. Previous studies on the breakdown of *P. australis* by macroinvertebrates have indicated a difference in processing leaf blades and shoots; no studies indicated increased mortality as a result of monospecific consumption of *P. australis*. In this study, leaf blades were used to feed *I. plattensis* larvae. Leaf blades have the highest percentage of nitrogen compared to the shoot and culm (Gessner, 2000). Nitrogen has been viewed as the most important nutrient in leaf decomposition (Webster and Benfield, 1986). High nitrogen content typically correlates with a higher decomposition rate (Mullholland et al., 1985) suggesting *I. plattensis* larvae ran out of food resources during the experiment and consequently starving, but there was no evidence of this. Other monocots tested had varying results as well. Grass litter input can be the dominant detritus component in some backwater lotic systems (Shaftel et al., 2011). *I. plattensis* populations have been identified in sloughs surrounded by grasslands

(Vivian, 2010). The highest p-values in this study for wet weight before and after were observed in the prairie cordgrass and reed canarygrass treatments. Grasses are viewed as low priority to the detritus stock because of their low nutritional quality. During the prairie cordgrass and reed canarygrass treatments, larvae had a mean case length value which decreased after each trial. This behavior has been documented by *I. plattensis* larvae under hypoxic conditions and is attributed as a stressful behavior. These results suggest *I. plattensis* larvae display a relatively flexible diet but habitats dominated by grasses may not sustain high densities of *I. plattensis* because of low nutrient content and stress induce behaviors.

The food-choice assays showed a general preference for native plant species. It has been well documented in the literature that aquatic insects select plant material low in structural carbon (Moline and Poff, 2008). Of the four species in the food-choice assay, *E. angustifolia* was consumed least by *I. plattensis* larvae. *E. angustifolia* has a high percentage of cellulose and lignin content which is consistent with higher structural carbon (Canhoto and Graca, 1995). Other Trichoptera larvae have exhibited remarkable flexibility in their feeding. Contrary to several studies on macroinvertebrate consumption of conifer leaf litter (Grafius and Anderson, 1980; Friberg and Jacobsen, 1994, 1999), Campos and Gonzalez (2009) found *Sericostoma vittatum* larvae consumed and efficiently utilized needles from *Pinus sylvestris* litter.

Terrestrial larvae did not feed on any of the vegetation provided, unlike other semi-terrestrial caddisfly larvae (Erman, 1981). *Desmona bethula* exhibits temporal diel

movements regulated by temperature where larvae migrate to land from water where they feed on semiaquatic plants (Erman, 1981).

Increasing use and anthropic pressures on Platte River hydrology disrupts the natural flooding and drying which are important to the *I. plattensis* life cycle (Whiles et al., 1999). Hostile environments facilitated by changes in hydrology induce stressful conditions for macroinvertebrates (Nebeker et al., 1996; Bjelke, 2005; van der Geest, 2007). During this study, a stress induced behavior by *I. plattensis* larvae—case chewing was observed. The decrease in total case length in some treatments was result of larvae chewing and removing pieces of their posterior case or differential survival of smaller larvae. Even with sufficient food resources chewing continued; this also may be a result of stress on larvae to the controlled conditions. Similar behaviors were documented in a hypoxia tolerance study, where larvae continued to chew on their cases under severely oxygen-limited conditions, 0.3 mg/L (Cavallaro, unpublished data). Decreases in larval case length were observed in the *Phalaris arundinacea* and *Spartina pectinata* treatments, shortening their cases 1.06 and 0.66 cm respectively. In addition, these treatments showed the least increase in head capsule width and wet weight gain (Table 4.2).

Physical breakdown of detritus facilitated by *I. plattensis* is substantial in the ephemeral systems they occupy (Whiles et al., 1999; Meyer and Whiles, 2008). Wetlands and side river channels along the Platte River are dominated by macroinvertebrates which occupy the collector-gather functional feeding group (Darrow and Holland, 1989; Meyer and Whiles, 2008). Higher shredder abundance and biomass was directly correlated to the

occupation of *I. plattensis* at tested sites (Meyer and Whiles, 2008). Shredder biomass was higher in natural wetlands when compared to restored wetlands; the limited distribution of the *I. plattensis* accounts for their absence in restored wetlands (Meyer and Whiles, 2008).

Prairie wetland restoration along the Platte River has required extensive habitat management through mechanical and chemical removal of exotic vegetation. Surveys of the *I. plattensis* on the Platte River show that their distribution is concentrated within the Big Bend region of the Platte River valley. Future studies should examine the effects of the manipulation of the plant communities on *I. plattensis* densities and emergence success.

ACKNOWLEDGEMENTS

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FIGURES
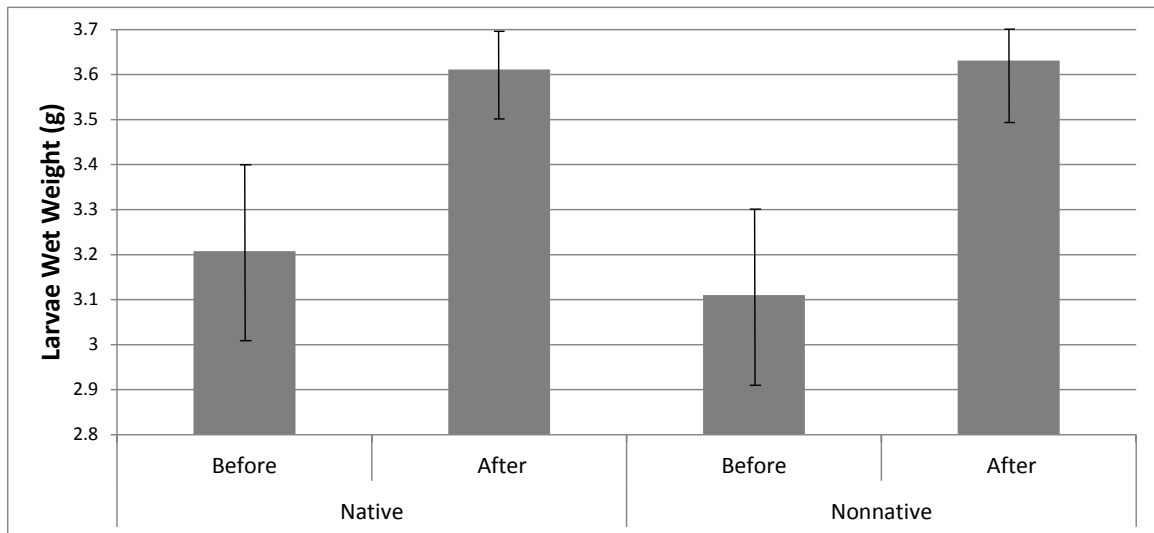
Figure 4.1 Mean of all before and after wet weights (± 1 S.E.) in native and nonnative treatments. No significant difference in wet weight of *Ironoquia plattensis* larvae before and after in both native (p = 0.28) and nonnative (p = 0.15) trials.
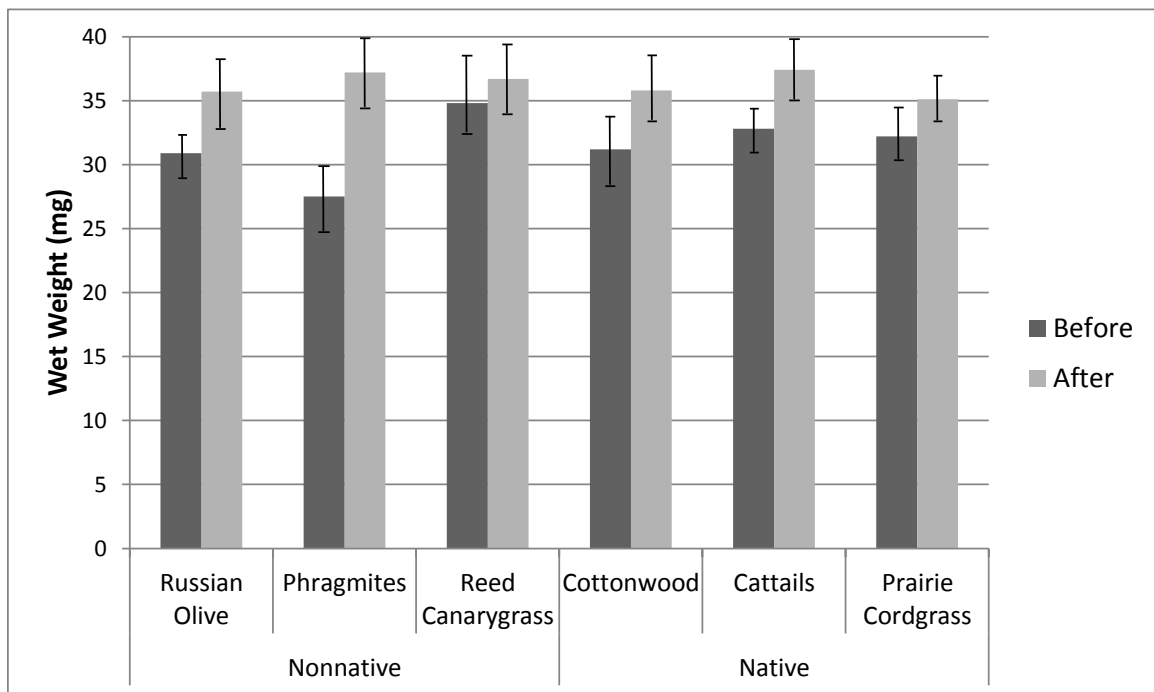
Figure 4.2 Mean of all before and after wet weights (± 1 S.E.) in each treatment. No significant difference in wet weight of *Ironoquia plattensis* larvae before and after among individual treatments.
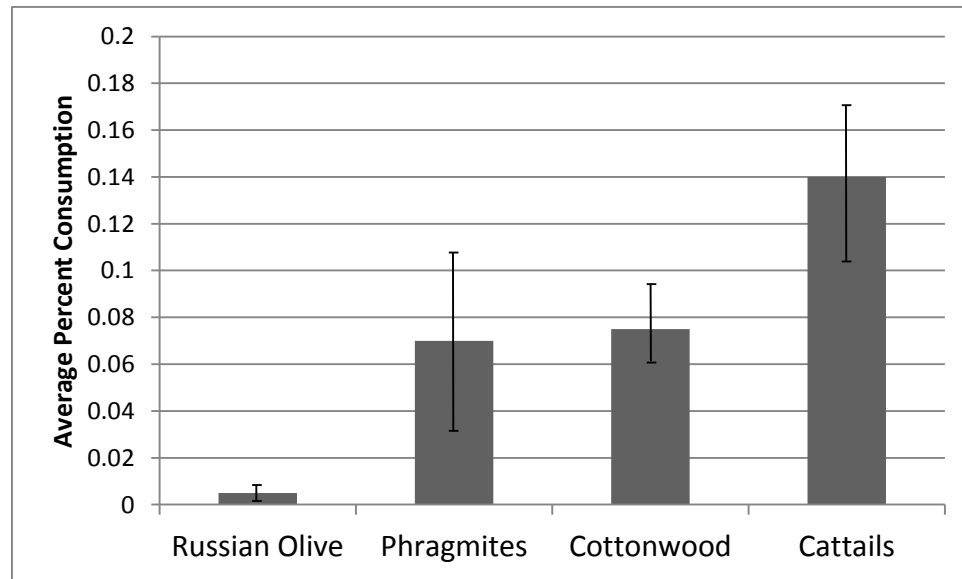
Figure 4.3 Mean (%) consumption by aquatic fifth instars. Choice-tests revealed no significant differences in plant species preference by *Ironoquia plattensis* larvae (p = 0.17).

Figure 4.4 Field evidence of the physical breakdown of leaf litter by *Ironoquia plattensis* larvae.
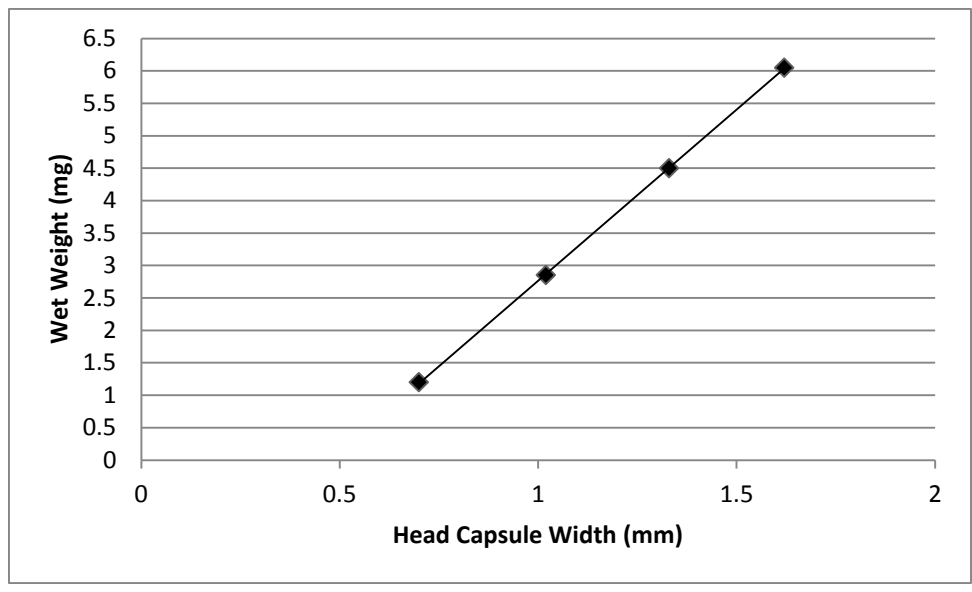
Figure 4.5 Points indicating mean head capsule width for larvae instars II-V.
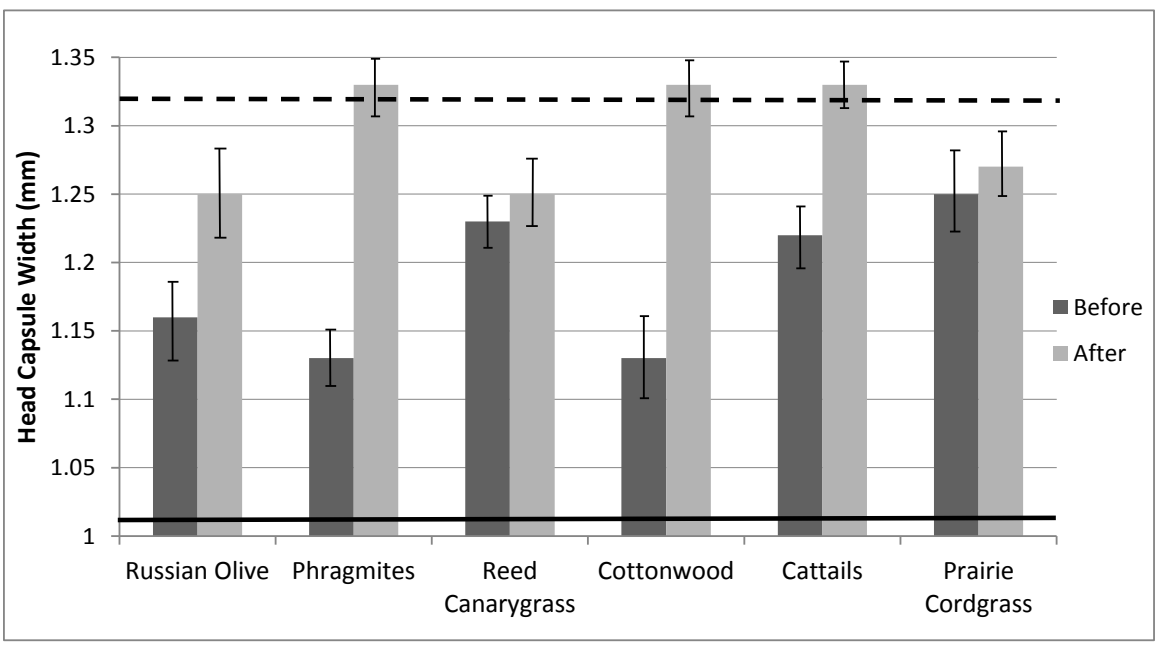
Figure 4.6 Mean head capsule width (± 1 S.E.) increase in all treatments relative to subsequent instars. Head capsule size of instar III is represented by a solid line and instar IV by a dashed line.

TABLES

Table 4.1 Larvae biometric Student's t-tests comparisons before and after 30 day trials.

	Total Length (mm)			Head Capsule Width (mm)			Wet Weight (g)		
	Before	After	p value	Before	After	p value	Before	After	p value
Native	9.69	9.81	0.56	1.19*	1.32*	0.02*	0.032083	0.036114	0.28
Nonnative	9.91	9.75	0.71	1.17	1.26	0.18	0.031103	0.036314	0.15

* Significant Difference ($p < 0.05$)

Table 4.2 Larvae biometric t-test comparisons before and after individual plant species treatments.

|	Total Length (mm)			Head Capsule Width (mm)			Wet Weight (mg)		
	Before	After	p	Before	After	p	Before	After	p
Russian Olive	9.53	9.77	0.73	1.16	1.25	0.33	30.9	35.7	0.42
Phragmites	9.38	9.57	0.62	1.13	1.33	0.27	27.5	37.2	0.40
Reed Canarygrass	10.81	9.75	0.31	1.23	1.25	0.89	34.8	36.7	0.72
Cottonwood	8.74	9.5	0.11	1.13*	1.33*	0.03*	31.2	35.8	0.32
Cattails	10.06	10.31	0.69	1.22	1.33	0.13	32.8	37.4	0.36
Prairie Cordgrass	10.26	9.6	0.39	1.25	1.27	0.89	32.2	35.1	0.56

* Significant Difference ($p < 0.05$)

Table 4.3 Cumulative number of surviving larvae after the trials of each treatment.

	Before	After
Russian Olive	20	18
Phragmites	20	8
Reed Canarygrass	20	16
Cottonwood	20	16
Cattails	20	20
Prairie Cordgrass	20	20

Table 4.4 Percent consumption after 24 hour food choice assay (n = 20).

	Average Percent 10 mm^2 Square Consumed
Russian Olive	0.005
Phragmites	0.07
Cottonwood	0.075
Cattails	0.14

CHAPTER FIVE: AQUATIC VERTEBRATE PREDATORS OF THE PLATTE RIVER CADDISFLY (*IRONOQUIA PLATTENSIS*) IN A LABORATORY SETTING

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ABSTRACT

The Platte River caddisfly, *Ironoquia plattensis* Alexander and Whiles, is a mineral case forming limnephilid that is endemic to central Nebraska. At the type locality, *I. plattensis* was the most abundant component of the benthic macroinvertebrate community, attaining larval densities of approximately 1,000 individuals per m^2, and accounting for approximately 40% of insect emergence from the slough. Surveys for the species conducted between 1999 and 2004 found six sites with *I. plattensis*, and recent sampling has found 26 additional locations; however, only one population has densities comparable to those found at the type locality. Backwater sloughs where *I. plattensis* occur provide habitat for a variety of aquatic vertebrate predators which could threaten the species' persistence, particularly at sites with low larval densities. This project tested the ability of seven fish species and a larval amphibian to consume *I. plattensis* larvae. Replicated experiments presented vertebrates with three early instar *I. plattensis* larvae in 9.5L aquaria. Based on Kruskal-Wallis one-way ANOVA (p=0.05), significant predation was observed only with trials conducted using brook stickleback, *Culaea inconstans* which consumed a mean of 0.39 *I. plattensis* larvae per 24 h. The results showed minimal predation from the other predators tested: plains topminnow, *Fundulus sciadicus*, mosquitofish, *Gambusia affinis*, fathead minnow, *Pimephales promelas,* black bullhead, *Ameiurus melas,* Iowa darter, *Etheostoma exile*, green sunfish, *Lepomis cyanellus*, and larval bullfrogs, *Lithobates catesbeianus*. Our results suggest *I. plattensis* populations may be reduced by the presence of brook stickleback in backwater habitats.

KEYWORDS: *Ironoquia*, invertebrate conservation, fish predation, *Culaea inconstans*, benthic

INTRODUCTION

Trophic interactions often shape aquatic invertebrate assemblages and food webs depending on complexity and habitat (Hornung and Foote, 2006). In permanent aquatic systems, fish are the dominant predators of invertebrates (Wissinger et al., 1999). Intermittent and ephemeral habitats with regular disturbance include a variety of predators of invertebrates, such as large aquatic insects—e.g. dragonflies (Batzer and Wissinger, 1996), beetle larvae and hempiterans (Schumann et al., 2012), or amphibians e.g. frogs (Wellborn et al., 1996) and salamanders (Wissinger et al., 1999). Fish may also seasonally invade temporary waters especially when connections to a permanent water body occur via hydrologic events (Labbe and Fausch, 2000; Chapman and Warburton, 2006; Hodges and Magoulick, 2011).

The Platte River caddisfly, *Ironoquia plattensis*, is an endemic, benthic macroinvertebrate that is adapted to the hydrology of the flooding and drying of backwater sloughs in central Nebraska. Unlike most other caddisflies, members of the *Ironoquia* genus migrate to land as fifth instars (Whiles et al., 1999). The transition to its terrestrial life stage coincides with increasing water temperatures associated with seasonal drying (Whiles et al., 1999). In 2000, *I. plattensis* numbers had declined substantially at known sites including the type locality and is currently being considered for protection under the Endangered Species Act (USFWS, 2009). Currently, *I. plattensis*

occurs in disjunct, fragmented populations in the Platte, Loup, and Elkhorn river systems (Vivian, 2010).

Temporary aquatics habitats, such as backwater areas and intermittent wetlands, serve as important nursery grounds for fish species (Sheaffer and Nickum, 1986), and backwater sloughs that support various fish species are common along the major river systems in Nebraska. These habitats occur frequently in aquatic Great Plains ecosystems. The dynamic hydrology within the Great Plains is responsible for more distinct, less diverse aquatic communities when compared to more permanent systems (Whiles et al., 1999). Whiles et al. (1999) reports several species of fish found at the type locality of *I. plattensis*. These included the plains topminnow, *Fundulus sciadicus* Cope, common carp, *Cyprinus carpio* L., and brassy minnow, *Hybognathus hankinsoni* Hubbs (Whiles et al., 1999). Fish surveys in 2007-2012 found several additional native fish species in sloughs with extant *I. plattensis* populations including fathead minnow, *Pimephales promelas,* black bullhead, *Ameiurus melas,* Iowa darter, *Etheostoma exile*, and green sunfish, *Lepomis cyanellus* (Vivian, 2010). In addition to fish species, Goldowitz and Whiles (1999) found adult and larval plains leopard frogs (*Lithobates blairi)*, western chorus frogs (*Pseudacris triseriata)*, and Woodhouse's toads (*Anaxyrus woodhousii)* at the *I. plattensis* type locality, Mormon Island. Recent *I. plattensis* surveys have found adult and tadpoles of the American bullfrog, *Lithobates catesbeianus*, at extant *I. plattensis* sites (Cavallaro, personal observation).

The reasons behind the observed decline of the *I. plattensis* have not been fully identified. Past studies have defined how detrimental seemingly benign trophic

interactions can be to the structure of benthic communities (Gilinsky, 1984; Diehl, 1992; Feld and Hering, 2007). Therefore, in this study I assessed the potential for vertebrate predation on early aquatic instars of the *I. plattensis* and the effect habitat complexity has on foraging behaviors from dominant *I. plattensis* predators.

MATERIALS AND METHODS

To quantify fish and larval amphibian predation on the *I. plattensis*, a series of laboratory experiments were conducted at the University of Nebraska at Kearney ecology laboratory. Experiments were conducted using second and third instar *I. plattensis* obtained from a backwater area of the South Channel along the Platte River near Gibbon, NE. Brook stickleback, western mosquitofish, Iowa darter, black bullhead, fathead minnow, and green sunfish were collected by seining from a backwater slough on the Platte River near Kearney, NE. Bullfrog tadpoles were purchased from Carolina Biological Inc. (Burlington, NC). Plains topminnow were collected from a production pond located at Sacramento-Wilcox Wildlife Management Area near Holdrege, NE.

Vertebrate predators were fed freezed-dried bloodworms and veggie rounds (Omega One®, Ferndale, NY) ad libitum 72 hours prior to each trial. For each trial, fifteen 9.5 L aquaria were 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 water temperature remained constant at $10.0 \pm 1.0°$ C.

Fish and tadpoles were measured to the nearest 1.0 mm and *I. plattensis* larval cases were measured to the nearest 0.1 mm using a Neiko Digital Caliper. All measured *I. plattensis* larvae were placed in a 250 mL beaker. For each laboratory trial, 5.6 L (1.5

gallons) of water was placed into 9.5 L (2.5 gallon) aquaria. Three randomly selected 2nd

and 3rd instars were placed into each trial tank along with a single vertebrate predator.

Fifteen replicates were performed for each predator species. For all trials, an additional

control tank had only three caddisfly larvae and no threat of predation. Additional tests

were performed with brook stickleback by providing *I. plattensis* larvae with

approximately 3 cm of leafy detritus as refuge.

The total number of caddisflies alive was enumerated after intervals of 24, 48, and

72 hours for each individual aquarium; daily consumption rates were calculated for each

vertebrate species. These data were not normally distributed and thus, the number of

consumed *I. plattensis* larvae was compared among species using a Kruskal-Wallis one-

way ANOVA followed by a Dunn's test (Sigma Plot, Systat Software, Inc., San Jose,

CA).

RESULTS

There was a significant difference in predation of early instar *I. plattensis* among

predators tested ($p < 0.05$). Daily consumption rates of larvae per individual ranged from

0.0 consumed by Iowa darter and 0.49 consumed by brook stickleback (Table 5.1).

Significant predation by brook stickleback defined them as a dominant predator. There

was no significant difference ($p = 0.487$) between predation rate when *I. plattensis* larvae

were given detritus as refuge (Table 5.2).

Across trials conducted with brook stickleback, 53 of 135 (39.25%) of *I.*

plattensis larvae were consumed during the 72h test period; of the 53 *I. plattensis* larvae

consumed by brook stickleback, 35 were removed from their cases (66%). The mean case

lengths were larger for surviving *I. plattensis* after the 72h test period indicating that smaller *I. plattensis* larvae were consumed, however, this difference in case sizes was not significant (p = 0.16). Predation by brook stickleback prompted addition tests which compared habitat complexity (detritus vs. no detritus). Predation rates were similar (p = 0.49) when *I. plattensis* larvae were given detritus as a refuge (Table 5.2).

DISCUSSION

This study demonstrates that *I. plattensis* faces potentially high predation rates from brook stickleback during the early aquatic phase of its lifecycle. Our trials were conducted at 10ºC based on seasonal temperatures when these predators would encounter 2nd and 3rd instars. Brook stickleback have been found to alter both pelagic and benthic invertebrate communities; and are generally described as a moderate-level omnivore (Hornung and Foote, 2006; Stewart et al., 2007). Hornung and Foote (2006) documented brook stickleback to have a direct negative effect on macroinvertebrate biomass in 24 wetlands in the Western Boreal Forest. Predation mostly affected early larval stages of invertebrates, resulting in depletion of potential prey for predaceous invertebrates; causing the biomass of predaceous invertebrate biomass decreased (Hornung and Foote, 2006). Miler et al. (2008) reported brook stickleback predation to affect sex ratios in populations of a benthic water moth, *Acentria ephemeralla*.

Previous accounts of brook stickleback consuming trichopteran larvae are documented in Stewart et al. (2007). Shifts in brook stickleback diets in the Northwest Territories of Canada found trichopteran larvae in stomach samples in late-summer to early-autumn (Stewart et al., 2007). Tompkins and Gee (1983) describe brook stickleback

as possessing flexible foraging behavior depending on available prey items based on prey abundance. Observations made during the 72h test period found brook stickleback persistently targeting the larvae within the case; most of the larvae consumed by brook stickleback were removed from their cases, suggesting gape limitations or inability to digest case material efficiently. Other limnephilid species, which construct a gravel based case, have been observed in the stomachs of brook trout, *Salvelinus fontinalis*, in Nebraska lotic systems (Brust, personal communication.).

During trials with detritus, the majority of brook stickleback tested oriented their body sideways appearing to lie down, burying themselves under the detritus. Degraeve (1970) discussed three burrowing behaviors displayed by brook stickleback; during his observations he noted how individuals could submerge and emerge with relative ease from silt substrate and loose detritus. During the study it was discovered the stickleback had difficulties manipulating sandy substrates which deterred these behaviors. *I. plattensis* larvae during their early instars readily move from benthos and sandy substrates as they develop and construct larger cases (Cavallaro, personal observation).

Different stream substrates and accumulation of leaf litter detritus affect benthic macroinvertebrate community composition as a result of microhabitat availability (Culp and Davies, 1985). Stream substrates may be altered by the construction of dams, irrigation canals, and water diversions—which have occurred in the Platte River system (Eschner et al., 1981). The presence of detritus, in which the caddisflies could burrow, did not increase survival when exposed to brook stickleback (Table 5.2).

Molecular phylogeographic evidence subdivides brook stickleback into two primary lineages which are genetically distinct from one another. This division has been traced back to the Pliocene era (3.6-4.8 mya) (Ward and McLennan, 2009). Behavioral studies conducted with brook stickleback from different regions of North America have revealed varying aggressiveness associated with food (McLennan and Ward, 2007). Nine behaviors were identified and observations were made on individuals from 3 locations (Algonquin Park, Ontario; Tooley Creek, Ontario; Sutherland Creek, Nebraska). Brook stickleback collected from Nebraska displayed the most aggressive behaviors when compared to stickleback collected from other areas (McLennan and Ward, 2007). The current *I. plattensis* distribution shows an active site in Sutherland, Nebraska, the most western site in their known range (Vivian, 2010). Brook stickleback have been collected upstream and downstream of the central Platte River since 1942.

Although brook stickleback are considered native to Nebraska (Fischer and Paukert, 2008) the range appears to have expanded recently to include the central Platte River (Chadwick et al., 1997; McAllister et al., 2010). The method of fish collection in Whiles et al. (1999) was not clearly stated; the cryptic, benthic nature of brook stickleback may have eluded documentation at the type locality. These fish are common in *I. plattensis* habitat and have been surveyed from several active sites (Cavallaro and Vivian, personal observation). Collection data provided by the Nebraska Game and Parks seemingly reveals brook stickleback expansion throughout the Platte River since 1970 (Figure 5.2). More intense sampling efforts of the fisheries associated with Nebraska river systems has increased substantially over the past 30 years facilitated by threatened

river and stream fish species (e. g. Pallid sturgeon, *Scaphirhynchus albus*, Plains topminnow, *Fundulus sciadicus,* etc). Concluding this apparent expansion is likely because of increase surveying and monitoring efforts—not range expansion; *I. plattensis* and brook stickleback have likely always occupied similar habitats.

Other native predators tested consumed at least one *I. plattensis* larva with the exception of the Iowa darter, *Etheostoma exile*. Iowa darters are documented in all major Nebraska river systems since 1894 (Meek, 1894), more recently in 1942 (Jones, 1963), and a study conducted on their diet habits found them to primarily consume copepods and cladocerans (Balesic, 1971).

Several predators which have been documented consuming Trichoptera larvae in previous studies including black bullhead (Leunda et al., 2008), green sunfish (Mancini et al., 1979), and fathead minnow (Duffy, 1998) consumed between 10 and 25% of larvae. Black bullheads utilize most freshwater habitats including slow moving lotic systems (Leunda et al., 2008). Dietary analysis conducted by Leunda et al. (2008) classified them as generalists that exhibit benthophagous feeding behavior. Dominant components of their diet included microcrustaceans, caddisfly larvae, and Oligochaeta in lotic systems. Every larvae consumed by a black bullhead in our study was ingested with its case. Members of the genera *Nectopsyche*, which builds a composite case from leaf and mineral material, have been extracted intact from the stomachs of several predator fish (channel catfish, *Ictalurus punctatus*) in lentic systems in Nebraska (Lungren, personal communication). Green sunfish have been described as having the most diverse diet of the sunfish species (Minckley, 1963), and they are commonly found in all Nebraska river

systems (Jones, 1963). In addition, they have been a model species for past studies

pertaining to macroinvertebrate predation (Mancini et al., 1979; Sih et al., 1990). The

only Cyprinid species tested, fathead minnow, are widespread in Nebraska as a result of

their popularity as a bait fish. Sampling conducted by Lynch and Roh (1996) found them

to be most abundant when pools of backwater areas were available. Fatheads have been

documented consuming zooplankton, Emphemroptera larvae, Trichoptera larvae, and

chironomid larvae with zooplankton being the largest component of their invertebrate

diet (Held and Peterka, 1974; Hambright and Hall, 1992; Duffy, 1996).

Invasive species have repeatedly been found to cause declines in native fauna.

Such examples include the western mosquitofish, *Gambusia affinisis,* and bullfrogs,

Lithobates catesbeianus (Lawer, 1999; Lowe et al., 2000). Western mosquitofish have

reduced abundances of fairy shrimp that are typically found in fishless waters (Leyse et

al., 2004). 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 et al., 1972; Crivelli and Boy,

1987; Blanco et al., 2004). Sites with the *I. plattensis* 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; Lynch, 1988;

Haynes, 1993). No studies to date have reported the effects of mosquitofish on

Trichoptera or other benthic dwelling insect orders, including Ephemeroptera. Bullfrogs,

as adults, have been found to out-compete native frog species in some regions of the

United States (Lawler, 1999). Bullfrog tadpoles have not previously been documented to consume caddisflies, and the single predation event observed was likely accidental. The caddisfly larva ingested was <50mm in length.

Literature on benthic macroinvertebrate community structures stress on habitat complexity (e.g., emergent vegetation, substrate composition, etc) for cover from predators (Gilinsky, 1984). Based on some preliminary results, *I. plattensis* larvae may be vulnerable to predation at sites where there is a lacking adequate sandy substrate under benthos. Five of the seven species tested displayed means of predation above the substrate, and brook stickleback consumed larvae in the detritus layer. Adequate cover provides larvae with refuge from predators on top of the slough bed substrate (Culp and Davies, 1985). Invasion of temporary waters may sustain fish populations resulting in decreases of *I. plattensis*, especially in systems where *I. plattensis* are the dominant component of the macroinvertebrate community. The permanency of water in *I. plattensis* habitat may directly correlate with a decrease in population size has it becomes more suitable for predaceous vertebrate species. Future work should investigate brook stickleback predation on *I. plattensis* larvae in varying substrates. In addition, further work on brook stickleback burying behaviors could provide a better understanding of their potential effect on benthic macroinvertebrate communities.

ACKNOWLEDGEMENTS

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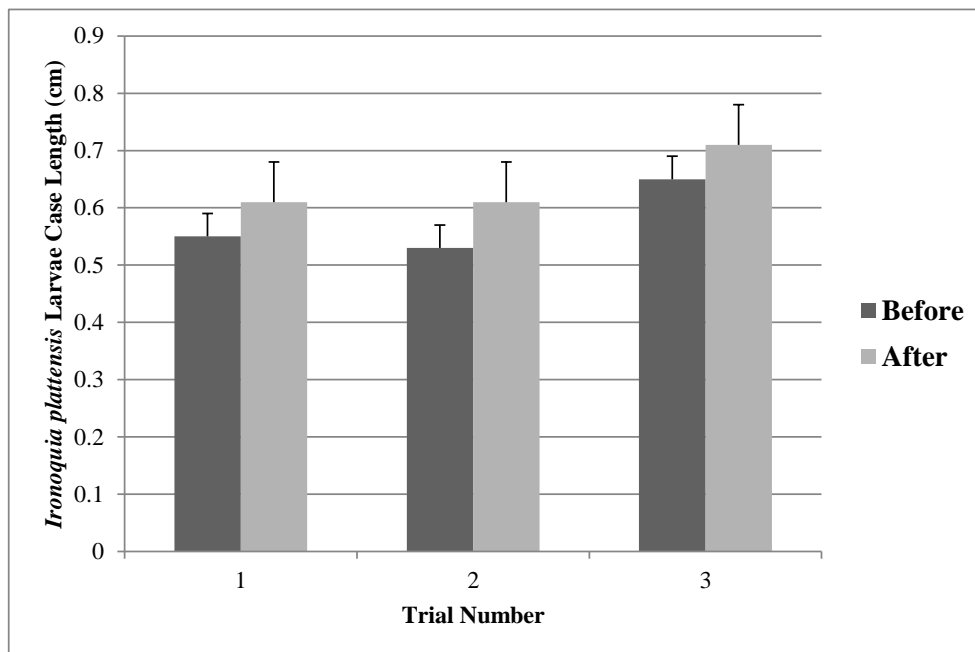
FIGURES

Figure 5.1 Mean case lengths (± 1 S.E.) of *Ironoquia plattensis* larvae before and after each brook stickleback (*Culaea inconstans*) trial.
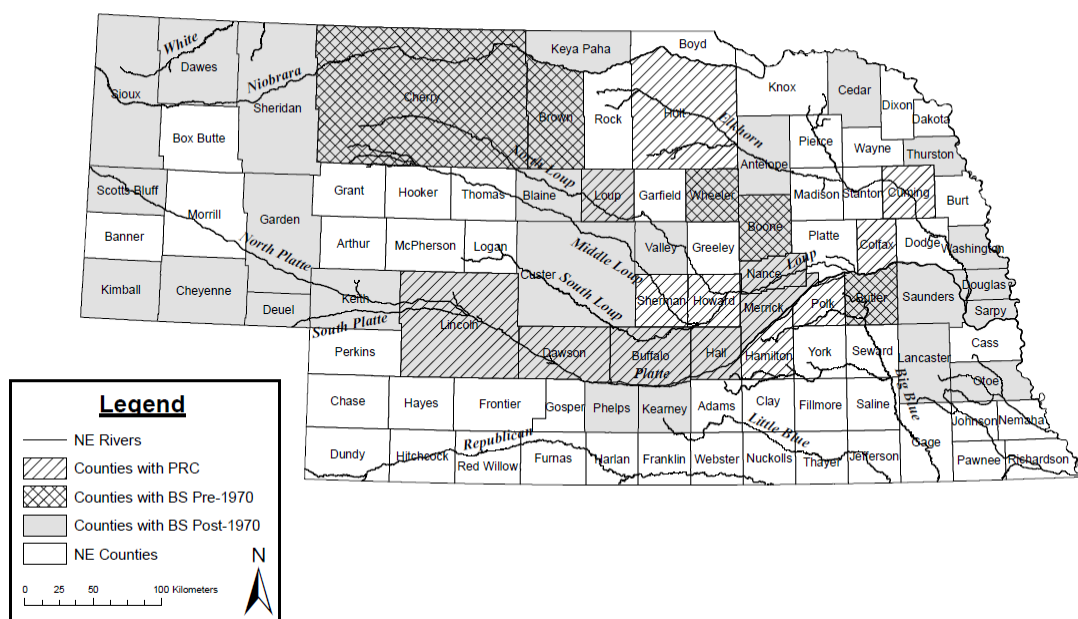

Figure 5.2 Range of *Culaea inconstans* (BS) and *Ironoquia plattensis* (PRC) from point collection data (S. Schainost, personal communication).

TABLES

Table 5.1 Total number of larvae consumed by each predator species tested.

Species	Number Of Fish Tested	Larva Available	Daily Feeding Rates	Total Larvae Consumed	Removed from Cases (Percent Removed from Cases)	Mean Larva Case Length Before (cm)	Mean Larva Case Length After (cm)
Brook stickleback	15	45	0.49 *	22 *	17 (77) *	0.59	0.64
Western mosquitofish	15	45	0.08	4	1 (25)	0.58	0.60
Plains topminnow	15	45	0.06	3	0 (0)	0.52	0.54
Iowa darter	15	45	0.00	0	0 (0)	0.53	0.53
Green sunfish	15	45	0.24	11	3 (27)	0.67	0.45
Black bullhead	15	45	0.20	9	0 (0)	0.55	0.44
Bullfrog tadpole	15	45	0.04	2	0 (0)	0.48	0.51
Fathead minnow	15	45	0.11	6	2 (33)	0.49	0.53

* Significant Difference ($p < 0.05$)

Table 5.2 Total larvae consumed by brook stickleback, *Culaea inconstans,* ($n = 15$ per condition) in aquaria with leaf detritus as refuge for larvae and without leaf detritus.

Condition	Total Available Larvae	Total Consumed	Percent Consumed
Detritus	45	14	31
No Detritus	45	17	37

APPENDIX A

Site Name	Dates	Present or Absent	Life stage Surveyed	County; River Basin
Binfield I	Apr-2011	Present	Aquatic Larvae	Hall; Platte
	May-2011	Present	Aquatic/Terrestrial Larvae	
	Jun-2011	Present	Terrestrial Larvae	
	Jul-2011	Present	Terrestrial Larvae	
	Aug-2011	Present	Pupae	
	Sep-2011	Present	Adults	

One of the six historic sites, Binfield I, remains a stable population. Variable hydrology over the past two years may have impacted the number of inhabitants (2012 drought). Fish species collected with a dip net included largemouth bass and western mosquitofish. Plant communities have remained constant since 2009 (Vivian, 2010) which included cottonwoods, green ash, poison ivy, grape vines, and reed canary grass.

Site Name	Dates	Present or Absent	Life stage Surveyed	County; River Basin
Binfield II	Oct-2011	Absent (w/ cases)	Adults	Hall; Platte
	Apr-2012	Present	Aquatic Larvae	

No adults were found in the surveyed areas, but larval cases were found in the northeast portion of the property. Subsequent aquatic sampling Spring 2012 found aquatic larvae. The habitat in the Binfield II slough was similar to the habitat found in the Binfield I slough. No fish species were collected here.

Site Name	Dates	Present or Absent	Life stage Surveyed	County; River Basin
Leaman	Oct-2011	Absent	Adults	Hall; Platte
	Apr-2012	Absent	Aquatic Larvae	

Three sloughs and parts of the north side of the main channel were identified as potential habitat. Both surveying methods did not discover any evidence of PRC presence. The absence of the PRC on the north side of the main channel may be due to the lack of allochthonous leaf litter input.

Site Name	Dates	Present or Absent	Life stage Surveyed	County; River Basin
Cook Dryer	Apr-2012	Absent	Aquatic Larvae	Buffalo; Platte

The slough surveyed on the Cook tract represented an agricultural runoff which severely degraded the habitat quality. Riparian plant community was dominated by reed canary grass. Slough substrate was loaded with fine, silt deposits—unsuitable for PRC larvae. Fish species collected with a dip net included big mouth shiner, black

bullhead, sand shiner, and fathead minnow.

Cottonwood Ranch	Apr-2012	Absent	Aquatic Larvae	
Stall	May-2012	Absent	Aquatic Larvae	Buffalo; Platte
Morse		Absent	Aquatic Larvae	

Cottonwood Ranch, Stall, and Morse were surveyed on two separate occasions for aquatic larvae and were unsuccessful. Slough was dominanted by water quality tolerant macroinvertebrates including water scorpions, backswimmers, giant scavenger beetles, and sinistral snails.

Hostetler	May-2012	Absent	Aquatic Larvae	Buffalo; Platte
Newark		Absent (w/ cases)	Aquatic Larvae	

Although suitable habitat was identified, no evidence of PRC was surveyed at the Hostetler tract. Newark also provided adequate emergent vegetation and sand substrate, but only a single larval case was uncovered during terrestrial surveys. Fish species collected with a dip net included green sunfish, plains killifish, western mosquitofish, largemouth bass, black bullhead, and common carp.

Sherrod		Absent	Adults	Buffalo; Platte
		Absent	Aquatic Larvae	

Three sloughs were identified as potential habitat. Both surveying methods did not discover any evidence of PRC presence. The absence of the PRC may be due to the back water areas being too ephemeral.

	Apr-2011	Present	Aquatic Larvae	
	May-2011	Present	Aquatic/Terrestrial Larvae	
McCormick	Jun-2011	Present	Terrestrial Larvae	Buffalo; Platte
	Jul-2011	Present	Terrestrial Larvae	
	Aug-2011	Present	Pupae	
	Sep-2011	Present	Adults	

Each surveying period of the 0.5 mile slough revealed a higher concentration towards the west end of the slough. These higher densities remained consistent in aquatic and

terrestrial surveys. Fish species collected by single pass backpack shocker included common carp, shortnose gar, largemouth bass, Iowa darter, black bullhead, brook stickleback, western mosquitofish, green sunfish, bluegill, plains killifish, and fathead minnow.