

# Aquatic vertebrate predation threats to the Platte River caddisfly (Trichoptera: Limnephilidae)

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## Abstract

The Platte River caddisfly, *Ironoquia plattensis* Alexander & Whiles (Trichoptera: Limnephilidae), was once the most abundant component of the benthic macroinvertebrate community in Platte River backwater sloughs, attaining larval densities of approximately 1,000 individuals per m<sup>2</sup> and accounting for approximately 40% of the emerging secondary production. Surveys for the species conducted between 1999 and 2004 found 6 sites with *I. plattensis*, and recent sampling has found 29 additional sites with the caddisfly; 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 vertebrates which could potentially threaten the species' persistence. This project tested the ability of seven fish species and a tadpole to consume *I. plattensis* larvae. Replicated experiments presented vertebrates with 3 early instar *I. plattensis* larvae in 9.5 liter aquaria. Based on Kruskal-Wallis one-way ANOVA ( $P = 0.05$ ), significant predation was observed only with brook stickleback, *Culaea inconstans* Kirtland (Gasterosteiformes: Gasterosteidae), feeding trials. *C. inconstans* consumed a mean of 0.49 *I. plattensis* larvae per 24 h. Our results suggest *I. plattensis* populations may be reduced by the presence of brook stickleback in backwater sloughs. Alterations to the Platte River may increase the chances for *I. plattensis* and *C. inconstans* habitat overlap from greater river connectivity.

Key Words: brook stickleback; fish behavior; invertebrate conservation; benthic macroinvertebrate; Nebraska USA

## Resumen

El frigánea del Río Platte, *Ironoquia plattensis* Alexander & Whiles (2000) (Trichoptera: Limnephilidae), es un limnefilido que forma un estuche mineral y es endémica de la parte central de Nebraska. En la localidad tipo, *I. plattensis* fue una vez el componente más abundante de la comunidad de macroinvertebrados bentónicos, alcanzando una densidad de larvas de aproximadamente 1,000 individuos por m<sup>2</sup> y representó aproximadamente el 40% de los insectos acuáticos emergentes del hábitat. En un sondeo de esta especie realizado entre el 1999 y el 2004, se encontraron 6 parcelas con *I. plattensis*, y un muestreo recién ha encontrado 29 sitios adicionales con las frigáneas; sin embargo, sólo una población tiene una densidad comparable a las encontradas en la localidad tipo. Los pantanos de agua estancada donde ocurren *I. plattensis* proveen un hábitat para una gran variedad de vertebrados acuáticos que potencialmente podrían amenazar la persistencia de la especie. Este proyecto puso a prueba la capacidad de 7 especies de peces y un renacuajo para consumir larvas de *I. plattensis*. Los experimentos replicados presentaron los vertebrados con 3 larvas de los primeros estadios de *I. plattensis* en un acuario de 9.5 litros. Basado sobre la ANOVA de una vía de Kruskal-Wallis ANOVA ( $P = 0.05$ ), se observó una depredación significativa sólo con el pez espinoso del arroyo, *Culaea inconstans* (Kirtland, 1840) (Gasterosteiformes: Gasterosteidae), en las pruebas de alimentación. *C. inconstans* consumieron un promedio de 0.49 larvas de *I. plattensis* por 24 horas. Nuestros resultados sugieren que las poblaciones de *I. plattensis* pueden ser reducidas por la presencia del pez espinoso del arroyo en los pantanos de agua estancada. Las alteraciones en el Río Platte pueden aumentar la posibilidad de superposición del hábitat de los *I. plattensis* y *C. inconstans* por la mayor conectividad del río.

Palabras Clave: comportamiento de pez espinoso del arroyo; conservación de invertebrados; macroinvertebrados bentónicos; Río Platte, Nebraska EEUU

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Trophic interactions can shape aquatic macroinvertebrate assemblages and alter food webs depending on habitat type and complexity (Wellborn et al. 1996). In permanent freshwater aquatic systems, fish are the dominant predators of aquatic invertebrates (Wissinger et al. 1999). Intermittent and ephemeral habitats with regular disturbance support a variety of invertebrate predators, including dragonflies (Batzler & Wissinger 1996), hemipterans, beetle larvae, and amphibians such as 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 (Chapman & Warburton 2006; Hodges & Magoulick 2011). This hydrologic variability is often displayed in a braided river system such as the Platte River (Eschner et al. 1981; Whiles & Goldowitz 2005).

The Platte River caddisfly, *Ironoquia plattensis* Alexander & Whiles (Trichoptera: Limnephilidae), is a benthic macroinvertebrate adapted to the intermittent hydrology of backwater sloughs in central Nebraska and is likely endemic to the state as well (Whiles et al. 1999). Unlike most other caddisflies, members of the *Ironoquia*

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genus migrate to land as fifth instars (Williams & Williams 1975; Whiles et al. 1999). The transition to its terrestrial life stage coincides with an increase in water temperatures and is associated with seasonal drying (Whiles et al. 1999). In 2004, *I. plattensis* numbers had declined substantially at known sites including the type locality for the species (Alexander & Whiles 2000; Goldowitz 2004). Subsequent surveys in 2007 found *I. plattensis* to be absent from two previously occupied sites (Vivian et al. 2013). It was recently considered for protection under the Endangered Species Act (USFWS 2012). Currently, *I. plattensis* occurs in disjunct, fragmented populations along the Platte, Loup, and Elkhorn river systems at historically low densities (Vivian et al. 2013).

Temporary aquatic habitats, such as backwater areas and intermittent wetlands, serve as important nursery grounds for fish species (Sheaffer & Nickum 1986), and backwater sloughs that support various fish species are common along the major river systems in the Great Plains. The dynamic hydrology within the Great Plains is responsible for more distinct, less diverse aquatic communities when compared with more permanent systems (Dodds et al. 2004). Several species of fish were found at the type locality of *I. plattensis* including 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 native fish species in sloughs with extant *I. plattensis* populations. These species included fathead minnow, *Pimephales promelas* Rafinesque, black bullhead, *Ameiurus melas* Rafinesque, Iowa darter, *Etheostoma exile* Girard, and green sunfish, *Lepomis cyanelus* Rafinesque, and introduced western mosquitofish, *Gambusia affinis* Baird & Girard (Vivian 2010). In addition to fish, Whiles et al. (1999) also found adult and larval plains leopard frogs, *Lithobates blairi* Mechem, Littlejohn, Oldham, & Brown, western chorus frogs, *Pseudacris triseriata* Wied-Neuwied, and Woodhouse's toads, *Anaxyrus woodhousii* Girard at the *I. plattensis* type locality. Recent *I. plattensis* surveys have found American bullfrog, *Lithobates catesbeianus* Shaw, tadpoles and adults at occupied sloughs (Cavallaro, personal observation).

The reasons for the limited distribution and range of population sizes of *I. plattensis* have not been fully identified. The alterations to the river systems in Nebraska, specifically the Platte River, influence the connectivity of *I. plattensis* habitat to the main river channels. These changes indirectly facilitate the colonization and abundance of fish and amphibians, which otherwise may not occur. The goal of the present research was to evaluate the potential for vertebrate predation on early *I. plattensis* instars by direct observation under laboratory conditions.

## Materials and Methods

To quantify fish and larval amphibian predation on *I. plattensis*, we conducted a series of laboratory experiments at the University of Nebraska at Kearney ecology laboratory. Experiments were conducted using second and third instar *I. plattensis* to provide a more accurate representation to predation vulnerability. During this development stage, larvae are active, above the benthic substrate, and more prone to predation. *Ironoquia plattensis* were obtained from a backwater area along the Platte River near Gibbon, Nebraska. 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, Nebraska. Bullfrog tadpoles were purchased from Carolina Biological Inc. (Burlington, North Carolina). Plains topminnows were collected from a production pond located at Sacramento-Wilcox Wildlife Management Area near Holdrege, Nebraska.

Vertebrate predators were fed freeze-dried bloodworms and veggie rounds (Omega One®, Ferndale, New York) ad libitum for 72 h before each trial. For each trial, fifteen 9.5 liter (2.5 gal) aquaria were placed in a Percival® environmental chamber (Percival Scientific, Inc., Perry, Iowa). To simulate spring conditions when larvae are early instars, chambers were set to a 14:10 h L:D, at 50% RH, and a constant temperature of  $10.0 \pm 1.0$  °C.

Before the beginning of each trial, all fish and tadpoles were measured to the nearest 1.0 mm, and larval cases were measured to the nearest 0.1 mm using a digital caliper. Large predators (e.g., green sunfish and black bullhead) used were no greater than 10 cm to mimic the size of naturally occurring populations surveyed at sites occupied by *I. plattensis*. All measured larvae were placed in a 250 mL beaker. For each laboratory trial, 5.6 L (1.5 gal) of water was placed into 9.5 liter (2.5 gal) aquaria. Three randomly selected 2nd and 3rd instars were placed into each tank along with a single vertebrate predator. Fifteen replicates were performed for each predator species. For all trials, an additional control aquarium contained 3 larvae and no predator. Additional tests were performed with brook stickleback by providing larvae with approximately 3 cm of leaf detritus added to the aquaria as refuge.

During the 72 h test period only caddisfly larvae were offered as a source of food. The number of living caddisflies was enumerated after intervals of 24, 48, and 72 h for each replicate, and daily consumption rates were calculated for each vertebrate species. These data were not normally distributed and thus, the number of consumed 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, California USA). An IACUC permit was issued by the University of Nebraska at Kearney which approved the methodology used for this study.

## Results

There was a significant difference in predation of early instar *I. plattensis* among predators tested ( $P < 0.05$ ). Average daily consumption rates per vertebrate ranged between 0.0 larvae consumed by Iowa darter and 0.49 larvae consumed by brook stickleback (Table 1). Significant predation by brook stickleback prompted additional tests which compared the effects of habitat complexity by adding detritus. There was no significant difference ( $P = 0.487$ ) between predation rate when larvae were given detritus as a refuge (Table 2).

Across trials conducted with brook stickleback, 53 of 135 (39.25%) larvae were consumed during the 72 h test period; of the 53 larvae consumed, 35 were removed from their cases (66%). The mean case lengths were larger for surviving larvae after the 72 h test period indicating that smaller larvae were consumed, however, this difference in case sizes was not significant (t-test,  $P = 0.16$ ).

A Spearman rank order correlation was used to determine if there were any significant correlations ( $P < 0.05$ ) between fish length, larval case length, and the number of larvae consumed. There was a negative correlation ( $P = -0.345$ ) between the number of larvae consumed after 72 h and fish length, and this correlation was significant ( $P < 0.05$ ). This indicates fish length and number consumed are not related. Size of fish does not determine how many larvae were consumed for this test.

Among the vertebrate species that consumed greater than a single larva over the 72 h test period, brook stickleback, fathead minnow, and black bullhead displayed considerable aptitude for consuming cased larvae. Each fed on 50% of their total larvae consumed within the first 24 h of the experiment. Western mosquitofish and Plains topminnow consumed the majority of the larvae offered during the final 24 h segment (Fig. 1).

**Table 1.** Total number of *Ironoquia plattensis* larvae consumed by each predator species tested.

Species	Common name	Daily feeding rates	Total larvae consumed	Removed from cases (percent removed from cases)	Mean larval case length before (cm)	Mean larval case length after (cm)
<i>Culaea inconstans</i>	Brook stickleback	0.49*	22*	17 (77)*	0.59	0.64
<i>Lepomis cyanellus</i>	Green sunfish	0.24	11	3 (27)	0.67	0.45
<i>Ameiurus melas</i>	Black bullhead	0.20	9	0 (0)	0.55	0.44
<i>Pimephales promelas</i>	Fathead minnow	0.11	6	2 (33)	0.49	0.53
<i>Gambusia affinis</i>	Western mosquitofish	0.08	4	1 (25)	0.58	0.60
<i>Fundulus sciadicus</i>	Plains topminnow	0.06	3	0 (0)	0.52	0.54
<i>Lithobates catesbeianus</i>	Bullfrog	0.02	1	0 (0)	0.48	0.51
<i>Etheostoma exile</i>	Iowa darter	0.00	0	0 (0)	0.53	0.53

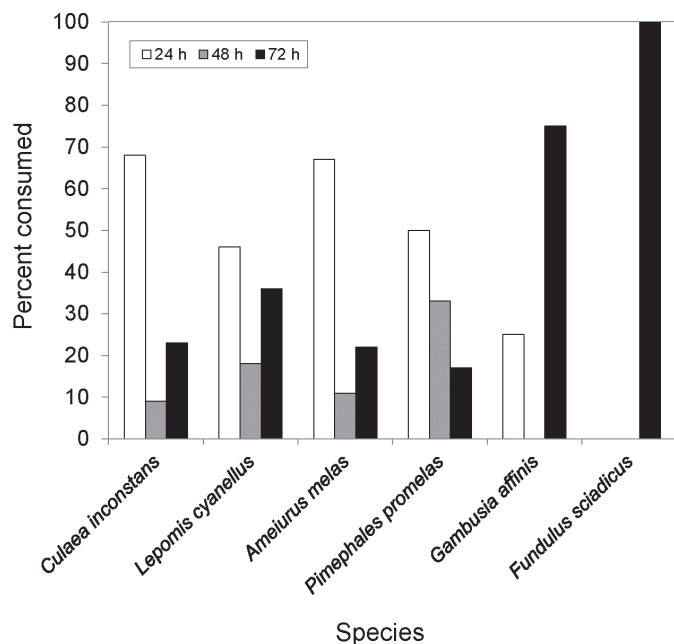
\*Significant Difference ( $P < 0.05$ )

**Table 2.** Total number of *Ironoquia plattensis* 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

## Discussion

An artificial scenario was used to evaluate the potential effects vertebrate predators may have on early *I. plattensis* larvae. This test provided no alternative prey items, no substrate in most trials, and no additional food for 72 h. All predators tested consumed at least one *I. plattensis* larva with the exception of the Iowa darter, *E. exile*. Iowa darters have been documented in all major Nebraska river systems since 1894 (Meek 1894), and a study conducted on their diet habits found them to primarily consume copepods and cladocerans (Balesic 1971).



**Fig. 1.** Percent of total *Ironoquia plattensis* larvae consumed over the 72 h test period for each vertebrate species tested.

Several predators used in this experiment have been documented consuming trichopteran larvae in previous studies, including black bullhead (Leunda et al. 2008), green sunfish (Mancini et al., 1979), and fathead minnow (Duffy 1998). 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 lepto-cerid caddisfly genus *Nectopsyche*, which builds a composite case from leaf and mineral material (Wiggins 1996), have been extracted intact from the stomachs of several predatory fish (e.g., channel catfish, *Ictalurus punctatus* Rafinesque) in lentic systems in Nebraska (Caval-laro, personal observation). Green sunfish are described as having the most diverse diet of the *Lepomis* 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 black bullhead and green sunfish were observed targeting larger *I. plattensis* larvae. The mean larval case length measured before and after the black bullhead and green sunfish trials decreased 0.11 cm and 0.22 cm, respectively. *I. plattensis* larvae achieve their greatest size as aquatic fifth instars, which coincides with their migration to land to aestivate. An increase in the abundance of predators that select for larger prey during the time *I. plattensis* migrate may exploit the available food source. This could leave populations with low densities vulnerable to stochastic events. Future studies should include larger instars in feeding trials.

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 & Roh (1996) found them to be most abundant when pools of backwater areas were available. Fatheads have been documented consuming zooplankton, mayfly (Ephemeroptera) larvae, caddisfly (Trichoptera) larvae, and chironomid midge larvae with zooplankton being the largest component of their invertebrate diet (Held & Peterka 1974; Duffy 1998). However, low predation rates on *I. plat-tensis* were observed during this study.

Among species tested, *I. plattensis* appears to face potentially high predation rates from brook stickleback during the early aquatic phase of its lifecycle. Our trials were conducted at 10 °C 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. Hornung & Foote (2006) documented brook stickleback to have a direct negative effect on macroinvertebrate biomass in 24 wetlands in the Western Boreal Forest in Canada. Predation mostly affected early larval stages of invertebrates, resulting in

depletion of potential prey for predaceous invertebrates; causing the biomass of predaceous invertebrates to decrease (Hornung & Foote 2006). Miller et al. (2008) reported brook stickleback predation to affect sex ratios in populations of a benthic water moth, *Acentria ephemerella* Denis & Schiffermüller (Lepidoptera: Crambidae).

Previous accounts of brook stickleback consuming trichopteran larvae are documented by Stewart et al. (2007), who found trichopteran larvae in stomach samples of brook stickleback diets in the Northwest Territories of Canada in late summer to early autumn. Tompkins & Gee (1983) describe brook stickleback as possessing flexible foraging behavior depending on available prey items based on prey abundance. Observations made during the 72 h 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.

During trials with detritus, the majority of brook stickleback oriented their body sideways appearing to lie down and, burying themselves under the detritus. Degraeve (1970) discussed 3 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 but had difficulties manipulating sandy substrates which deterred these behaviors. During their early instars, *I. plattensis* readily move between detritus 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 & Davies 1985). Stream substrates may be altered by the construction of dams, irrigation canals, and water diversions all of which have occurred in the Platte River system (Eschner et al. 1981). However, in our tests the presence of leaf detritus, in which the caddisflies could burrow, did not increase survival when exposed to brook stickleback (Table 2).

Although brook stickleback are native to eastern Nebraska (Fischer & Paukert 2008), their range appears to have expanded substantially since 1970, and the species is now common throughout the middle Platte River drainage (Chadwick et al. 1997; McAllister et al. 2010). During recent monitoring of known *I. plattensis* populations, including the type locality, brook stickleback have been captured in low numbers (Cavallaro & Vivian, personal observation). Given the cryptic, benthic nature of brook stickleback and inconsistent fish sampling methods employed, it is unclear whether the species eluded previous documentation at extant *I. plattensis* sites or has actively dispersed to occupy similar habitats.

Exotic species, including native species that colonize new habitats, have repeatedly been found to cause declines in native fauna. Exotic species with documented effects include *G. affinis* (Lawler et al. 1999). 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 (Blanco et al. 2004). Sites with the *I. plattensis* now contain western mosquitofish, which was introduced to Nebraska from the southeastern United States (Lynch 1988; Haynes 1993). As surface feeders, mosquitofish are considered generalist predators (Lawler et al. 1999), and they have not been reported causing any negative effects on benthic dwelling arthropods.

Literature on benthic macroinvertebrate community structure stresses the importance of habitat complexity (e.g., emergent vegetation, substrate composition, etc.) for cover from predators (Gilinsky

1984). The different time segments of the 72 h test period showed several species, specifically brook stickleback, fathead minnow, and black bullhead, were equipped to consume larvae within the first 24 h (Fig. 1). Most of these species either take shelter in the detritus or have considerably larger gapes relative to the other test species making *I. plattensis* larvae slightly more at risk. Among the other species tested that consumed greater than a single larvae, Western mosquitofish and Plains topminnow consumed most of the larvae offered within the last 24 h. These species feature a supra-terminal mouth, allowing them to feed on the surface of the water. Benthic in nature, *I. plattensis* larvae do not appear at risk to these species.

Based on some preliminary results, *I. plattensis* larvae may be vulnerable to predation at sites where there is a paucity of adequate sandy substrate under detritus. 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 & Davies 1985). Invasion of temporary waters may sustain fish populations resulting in decreases of *I. plattensis*, especially in systems where *I. plattensis* are a large component of the macroinvertebrate community. Changes in the hydrologic cycle that increase the permanency of water in *I. plattensis* habitat may contribute to a decrease in population size. We recommend future conservation studies concerned with evaluating the predation threats to specific aquatic macroinvertebrate species should explicitly use various benthic substrates in the experimental design.

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