LIFE HISTORY OF THE STONEFLY ISOGENOIDES ZIONENSIS (PLECOPTERA: PERLODIDAE) FROM THE SAN MIGUEL RIVER, COLORADO

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ABSTRACT

The detailed life history of a San Miguel River, Colorado, population of Isogenoides zionensis is reported. Adult emergence, determined from exuvial counts, and adult presence were synchronized over a 20-day period from late June to early July 1999. Sex ratio for 1999 adults was 1.85 male:1 female. Observed oviposition behavior involved undulating flights over the river, with females descending to the water surface to release eggs. Females emerge with eggs nearly mature and produce up to three egg masses with an average of 499, 176, and 50 eggs, respectively. Captive-reared males and females lived an average of 7 and 8 days respectively. The approximate 8-month or longer egg diapause and growth of nymphs in the field, beginning in May the following year, resulted in a semivoltine life cycle.

Keywords: Plecoptera, Perlodidae, Isogenoides, life history

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INTRODUCTION

The Perlodidae is a large and important family of stoneflies in North American stream ecosystems. It contains 30 genera and 122 species in the two subfamilies Isoperlinae and Perlodinae, and is unique taxonomically, from the perspective that half of its genera are monospecific. In terms of species numbers, the Isoperlinae genus Isoperla has experienced the greatest radiation, with well more than 60 Nearctic species (Stark 2001, S.W. Szczycyko, personal communication), and Isogenoides is the largest Nearctic Perlodinae genus with eight currently recognized species (revision by J.B. Sandberg and K.W. Stewart In Preparation). Except for Isoperla, adults are largely nocturnal, cryptic during the day, and generally little is known of their behavior and longevity other than anecdotal observations of times and places of discovery by collectors.

Of the 61 Nearctic Perlodinae species, detailed studies of life histories have been reported for only six (10%): Diploperla robusta Stark and Gaufin (McCaskill and Prins 1968), Hydroperla crosbyi (Needham and Claassen) (Oberndorfer and Stewart 1977), Kogotus modestus (Banks) (Stewart and Sandberg 2003), Malirekus hastatus (Banks) (Huryn and Wallace 1987), Megarcys signata (Hagen) (Taylor et al. 1998, Gath and Gaufin 1975), and Skwala americana (Klapálek) (Short and Ward 1980, Hassage and Stewart 1990, DeWalt and Stewart 1995). Life histories of the remaining 90% of species for this subfamily are unknown or the knowledge limited to adult presence reports or notes on certain aspects of their biology such as food habits or nymphal growth; the life histories of representatives of 10 genera are totally unknown (Stewart and Stark 2002). Isogenoides is widely distributed, with five eastern species distributed from Atlantic Canada and Minnesota south to Mississippi and South Carolina,
and three western species distributed in the western cordilleran and Pacific Northwest from Alaska to Saskatchewan and northern California southward to New Mexico. Populations of *I. frontalis* (Newman) from the east and *I. colubrinus* (Hagen) from the west form a transcontinental distribution across Canada. The species are usually found in small mountain rivers and their tributaries, having good water quality, but populations of some western species are able to inhabit large silty rivers with unstable substrates. In western streams they can be the lone large perlodid, or often they coexist with *Diura knoultoni* (Frison) or *Skwala americana* (Klapálek). Relatively little is known about *Isogenoides* life histories and ecology, with only one published study of nymphal growth for the eastern *I. olivaceus* (Walker) (Haro et al. 1994), an unpublished thesis on life history of a Utah population of *I. zionensis* Hanson (Shakarjian 1997), and a partial life history of *I. zionensis* reported from the Rio Conejos of Colorado by DeWalt and Stewart (1995).

*Isogenoides zionensis* is a widely distributed western species, with only two known collection localities as far north as British Columbia and the Northwest Territories. The remainder of its usual distribution is southwestern in the “four-corner” states of Arizona, Colorado, New Mexico and Utah. Stark et al. (1986) reported Alaska as part of the distribution, but no known museum, personal holding, or other published records can be located to support this. Large populations of *I. zionensis* have been found in the following localities in Colorado: Blue River-Summit County, Rio Conejos-Conejos County, and the San Miguel River-San Miguel County. A fourth location investigated during this study, the Uncompahgre River, Montrose County, supports a smaller population of *Isogenoides*, but until males are reared the species identity remains tentative. Shakarjian (1997) determined that *I. zionensis* had a univoltine life cycle and that egg development was direct for a Virgin River, Utah population. DeWalt and Stewart (1995) reported a 9–10 month egg diapause and a semivoltine life cycle for a Rio Conejos population. The purpose of this study was to complete an ecological investigation of *I. zionensis* from a known large population in the San Miguel River, to provide more information about its egg diapause, nymphal growth, emergence, adult riparian resting habitat, egg mass details, and oviposition. Eggs collected during this 1999-2000 study were incubated over an extended four years and adults reared from nymphs were utilized in drumming experiments. Results of incubation experimental protocols are reported separately in Sandberg and Stewart (2004).

**STUDY STREAM**

This study was conducted on the middle reach of the San Miguel River, at and below the Placerville bridge on Hwy 145, San Miguel County, Colorado. The San Miguel River is approximately 129 km long and originates from high elevation streams on Wasatch Mountain and Telluride Peak in the Uncompaghre National Forest and the alpine basins, Ophir and Trout lakes. It flows northwest through Montrose County, merging with the Dolores River, 11.3 km west of the historic mining town, Uravan. San Miguel River study sites were located at the Placerville bridge (2,286 m elevation) and approximately 100 m downstream, the San Miguel River Camp Site, and at the confluence with Specie Creek at M44 Rd. intersection on Hwy 145, ca. 9.7 km west of Placerville (Fig. 1). The substrate consists mostly of cobble and gravel riffles with larger rock-rubble to boulder substrates intermixed. The riparian forest is dominated by Cottonwood (*Populus* sp.), Willow (*Salix* sp.) and Alder (*Alnus* sp.), with scattered Gambel Oak (*Quercus gambeli*), Utah Juniper (*Sabina utahensis*), Engelman Spruce (*Picea engelmannii*) and Oregon Grape / Grape Holly (*Mahoula fremontii*).
Fig. 1. Maps showing the Dolores River drainage system and the San Miguel River study sites, San Miguel County, CO.

Adult Emergence: Emergence period and frequency were determined from last instar exuviae collected from the Placerville bridge abutments (Fig. 1). A single, sample of residual exuviae, representing an estimate of the entire 1998 adult population, was collected on 11 June 1999, 10 days before the 1999 emergence began. Fresh exuviae collections were then conducted from daily to three-day intervals, beginning June 20th and ending July 9th when no more exuviae were found. This yielded a precise time-line of emergence for 1999. At the same times, a count of male and female adults on the abutments was conducted to estimate adult presence. A third, single sample of residual exuviae, representing an estimate of the 2000 adult population, was collected
on July 8th, 2000, after no emergence activity and no more nymphs could be detected. This final sample is smaller than previous years because of decreased flow that year, and the majority of exuviae were located on the now-exposed and dry cobble-rock-rubble river substrate under the bridge. On July 1st and 2nd, 1999, nymphal transformation and adult resting habitat were documented at the Specie Creek study area using a video camera (Sony®) (Fig. 1). A sex specific line chart was constructed for both exuviae and adults, collected and observed from Placerville bridge abutments.

Oviposition, Fecundity, Longevity and Egg Incubation: Mature *I. zionensis* nymphs from the study site and adjacent tributary Leopard Creek (Fig. 1) were field-reared in two 54 Quart Igloo® coolers partially submerged and anchored by placing rocks inside and around them in Quartz Creek, Pitkin, Colorado. Inside coolers were sheets of Styrofoam with circular holes that held Styrofoam cups partially filled with gravel substrate. Cups were covered with labeled plastic lids and examined daily for adults. Virgin adults were transferred individually to dry cups containing pieces of slightly wetted paper towel for determination of longevity and obtaining eggs for incubation experiments and for an associated study of drumming behavior.

Mating was observed but not videotaped. After a mated female produced its first egg mass, it and associated males were removed. Egg masses were either preserved in 75% ethanol, or contained inside small glass vials with Nitex® mesh held over openings with modified lids for incubation (Fig. 2). Two egg masses deposited in June by reared and mated *I. zionensis* females from the San Miguel River were incubated in 1999 and 2000 through February 2004. The first year’s results of these are reported here. Egg incubation vials were initially placed into
field-rearing chambers and kept in Quartz Creek, Colorado, where temperature was recorded daily. At the end of the 1999 field season, the eggs, that were thought to be diapausing, were transported to the University of North Texas and placed into a Living Stream. During the 1999 study year, Living Stream temperature was adjusted to near San Miguel River field conditions estimated from periodic measurements at the study site. Beginning in late July 2000, Living Stream temperature was adjusted weekly to average temperatures recorded the previous year by the Ryan RL-100. Monthly light/dark cycles for the Living Stream were obtained from the Internet at [sunrisesunset.com]. First instar nymphs, obtained from hatching experiments above, were preserved in 75% ethanol, for later description.

Fcundity was estimated from number of egg batches deposited and number of eggs per batch. No attempt was made to enumerate the remaining eggs inside the ovarioles. Mated females were held as described above, but placed inside an empty cooler near Quartz Creek until all egg batches were collected and females expired.

**Nymphal Growth:** Seasonal growth was determined from qualitative nymph samples taken from May 1999 through July 2000. Sample collection ranged from monthly during warm months to tri-monthly during winter, and total monthly numbers collected ranged 8–228. Samples were collected by kicking mineral and organic substrates upstream from a Wildco® coarse (800 x 900 μm) mesh, triangular dip net. Early instars were collected on May 29, 2000, using a modified two-stage kick net with coarse first stage (800 x 900 μm) and variable (200 x 200, 140 x 200, and 60 x 60 μm), fine mesh second stage. A terminal and removable Wisconsin Plankton Bucket (63 μm mesh) was attached to the second stage to facilitate sample removal. The two-stage net was anchored in shallow, slow current with steel rods and upstream substrates were agitated and removed with a sharp-shooter spade to a depth of approximately 30 cm. Preserved samples taken back to the laboratory were floated in strong saltwater solution to separate organic from mineral debris and the organic debris was completely examined for early instars at high power (50x) under a Wild M5 dissecting microscope.

Interocular distance (IOD, the minimum distance between the eyes) of nymphs was measured with a calibrated ocular micrometer fitted to the stereo-dissection microscope. Gender of middle to late instar nymphs was determined by the presence of a gap in the posterior setal margin of the eighth sternum of females (Stewart and Stark 2002). A sex specific line chart was constructed by placing male and female nymphs into 0.02 mm size classes and graphing the mean IOD and standard deviation for each month. Additional first instar laboratory hatching data from extended incubation experiments were added to provide greater detail in deciphering growth classification (Sandberg and Stewart 2004).

**RESULTS AND DISCUSSION**

Stream discharge and temperature were variable, depending on depth of snow pack and spring weather. In the study-years 1999 and 2000, peak flow occurred in July and May respectively, and temperature ranged from -2°C in late January 2000 to 13.5°C in early July, 2000 (Fig. 3).


Other dominant insect genera, tribes and families associated with *I. zionensis* in the San Miguel River included: **EPHEMEROPTERA:** *Acentrella* sp., *Baetis* sp., *Cinygmula* sp., *Drunella doddsi* (Needham), *Drunella grandis* (Eaton), *Epeorus* sp., *Rithrogena* sp.; **COLEOPTERA:** *Brachius* sp., *Naropus concolor* (LeConte), *Helichus striatus* LeConte, *Optioserus castanipennis* (Fall), Coleoptera: Tribe Hydroporini, *Zaitzevia parvula* Horn; **TRICHOPTERA:** *Aptania* sp., *Arctopsyche grandis* (Banks), *Brachycentrus occidentalis* Banks, *Glossosoma* sp., *Lepidostoma* sp., *Rhacophila* sp.; **DIPTERA:** *Antocha* sp., *Atherix pachypus* Bigot, *Chironomidae, Diranota* sp., *Dolichopodidae,

Fig. 3. Average temperature (°C) and stream flow (CFS) of San Miguel River, San Miguel County, Placerville, CO, July 15, 1999 – July 9, 2000. Stream flow data from USGS station: 09172500, near Placerville.
Hexatoma sp., Simulium sp., and Agathon sp.

Adults: The *I. zionensis* emergence was well synchronized, occurring over a 20 day period, from late June to early July, 1999, (Fig. 4). The two vertical bridge abutments near Placerville were primary transformation sites for the river population during spring flows, and the periodic field exuviae collections gave a precise time-line for emergence of the local population. Exuviae were first observed and collected on June 20, 1999, when river temperature was 12°C at noon and 15°C at 16:00 (Fig. 4). The actual sex ratio (male:female) for 1999 adults was 1.85:1 (N=1331). Total exuviae sex ratios, collected on 11 June, 1999, for the estimated 1998 adults was 1.21:1 (N=736), and July, 8, 1999 collection for the estimated year-2000 adults 0.71:1 (N=246). Emergence in 1999 was not protrandrous with both sexes of adults and exuviae simultaneously observed at the beginning. Maximum numbers of exuviae were observed four days apart, with males first on June 27, followed by females on July 1, 1999. The peak numbers of female exuviae and adult presence coincide with oviposition flights observed two and four days later. Adult presence was estimated using counts of live adults found on the Placerville bridge abutments at the same time that exuviae counts were made (Fig. 4).

The San Miguel River *I. zionensis* population began their 1999 emergence 12 days earlier than a Rio Conejos population in Colorado (DeWalt and Stewart 1995). San Miguel River emergence and adult presence ranged June 20–July 9, 1999. Rio Conejos presence ranged June 8–June 28 (1988–1989); emergence was not protrandrous, and sex ratio was heavily skewed towards males.

Emergence was largely over and nymphs gone on July 15th, 1999, when average stream temperature reached 11.5°C. On July 1, 1999, nymphs began to exit the river at the Specie Creek study area at 21:30, one-half hour after a large, hovering, cloud-like hydro-psychid hatch had occurred. The transformation process for one female lasted 21 minutes; after final exit from the exuvium she rested approximately 40 minutes with wings hanging downwards, while her body hardened. Nymphs continued to leave the river until approximately 00:30. DeWalt and Stewart (1995) observed a similar time for transformation from 20:30 to about 22:00. Teneral adult males were mobile and bright yellow with smoky-gray wings held perpendicularly erect over the body still wrinkled and wet. The majority of observed teneral adults carried minute, orange mites (Hydrachnida) around the wing bases. Most adults, after hardening, moved over rocks and boulders to reach woody vegetation where they crawled upwards.

On July 2nd, 1999, from 09:00 to 12:00 a search of streamside vegetation consisting mostly of young willows and alder was conducted to find the resting habitats of adults that emerged the previous evening. At the emergence site, near the mouth of Specie Creek, many fresh exuviae were still attached to rocks, boulders and only a few young saplings, and a beating sheet was used in an attempt to collect adults from the nearby streamside vegetation. Since none were found, adult searching was moved inland, away from the river. From 12:00 until 14:00, when ambient temperature climbed from 24–29°C, numerous adult males and females were observed from 10–50 m inland, at heights of 1–2 m, under loose bark of mostly alder and cottonwood trees. DeWalt and Stewart (1995) observed emergence occurring <1 m from the ground and found adults in willows either at the bases in the morning or near the tops as the sun rose. Sexes are therefore probably aggregated as “bushtoppers” (Stewart 1994) and males find females through duetting with vibrational communication on woody substrates (Stewart 2001).

Oviposition was observed and videotaped near the Placerville bridge on July 3rd, 1999, from 11:30 to 14:00. Forty-three ascending and descending, “undulating”, flights were observed, either originating from the riparian forest vegetation to the water, or returning from the water back to the forest. Ambient temperature increased from 25°C at 09:00 to 31°C at 12:15, river temperature held constant at 11°C, and the sky was variable throughout the day, from slightly cloudy to clear and sunny. The tallest trees observed in return flights were engelman spruce, estimated to be between 23–30 m in height. Descending *I. zionensis* females were observed landing on the river surface and were quickly swept from view. We have interpreted this behavior to indicate that females release eggs on water...
Fig. 4. Male and female presence and exuviae counts from two bridge abutments on the San Miguel River, Placerville, CO, 1999; and total counts of residual exuviae on the bridge for years 1998 and 2000, collected on 11-VI-1999 and 08-VII-2000, respectively. 1999 oviposition observations indicated by triangles were made on 03 and 06, July, when water temperature was 12°C and air temperature was 26°C.

contact although the flights were of such height, quickness and over the river that females could not be caught before and after touching the water to verify this. DeWalt and Stewart (1995) did observe females fluttering on the surface of the Rio Conejos, Colorado, to release their black egg masses. In interesting contrast during the same San Miguel River observation period, female *Pteronarcella badia* were observed releasing and dropping egg masses from about 2 m above the river.

Females emerge with eggs nearly mature. Nine females, ranging in age from several hours to six days, were successfully mated with males held in Styrofoam cups. These experimental females
Fig. 5. Average growth of 250 ♂, 226 ♀ and 375 field collected and laboratory hatched early instar *I. zionensis* nymphs from San Miguel River, CO, 1999-2000. Vertical bars = standard deviation, triangle = emergence (June 20th to July 9th) and vertical arrow = oviposition (3-6 July, 1999). 2001 lab cohort egg mass (N=368) underwent an approximate 8 month diapause (June 26th, 1999 to March 6th, 2000).
produced up to three egg masses with total eggs per female (fecundity) ranging from 549–1093 (Table 1).

Their first and second masses had average number of eggs of 499.2 ± 176.1 and 175.5 ± 78.9 respectively. These first masses took several hours to nine days to be extruded from the genital opening located at the rear of the 8th sternum. Three females, whose ages ranged from several hours to 6 days at the time of mating, produced a third egg mass that had a lower average count of 50.0 ± 22.9 eggs and took an average of 7.7 ± 3.2 days to be produced after mating (Table 1).

These egg mass results compare favorably in number of egg masses produced per female with those reported by DeWalt and Stewart (1995), but averaged fewer per 1st, 2nd, and 3rd mass. Several natural variables such as degree day accumulation and nymphal diet may help to explain the 27–85% difference in fecundity between the San Miguel River and the Rio Conejos (DeWalt and Stewart 1995), Colorado populations.

Longevity was observed for 26 males and 22 females experimentally obtained from Quartz Creek field-rearing chambers. Adults emerged from June 21, to June 26, 1999 and 26 males lived an average 6.8 ± 2.8 days (range 1–12), and 22 females averaged 8.1 ± 3.0 days (range 2–13).

**Egg Development:** The 1999 incubated eggs underwent at least an 8-month diapause (Fig. 5), and protonymphs began eyespot development shortly before hatching (Fig. 6). As these eggs hatched in the year 2000, average interocular distance decreased slightly from a March $\bar{x} = 0.26 ± 0.04$ mm (N=35) to a June $\bar{x} = 0.22 ± 0.18$ mm (N=10). These eggs continued hatching in April-May, 2001 (N=22), after an additional, approximately 22-month diapause.

**Nymphs:** First instars (Fig. 7) were unpigmented, had nine antennal and three cercal segments, and overall mean interocular distance was 0.22 ± 0.2 mm (N=114). Early instar nymphs (N=109) first appeared in samples May 29, 2000 (Fig. 5) when average stream temperature was 8.5°C, and incubation experiments indicated that these were from the previous year eggs. Nymphal growth rates were exponential when transposing the May, 2000 field collected and March–July, 2000 laboratory hatched, early instars onto the 1999 growth curves (Fig. 5). Nymphs were approximately half grown by October to December, and greatest growth occurred from June to December and decreased from March to May (Fig. 5). Fastest growth occurred initially as temperature began to decrease from 13°C in July, to 0.5°C in December 1999. Slowed growth occurred during the coldest months (December–March) then decreased until emergence.

Nymphs reached maximum size on March 12, 2000, when temperature was 3°C. As temperature increased to only 8°C on June 24, growth declined.
until emergence in late June. The decline in growth was possibly due to small sample size, or that the larger, more developed nymphs moved to different microhabitats and escaped sampling or had already emerged. Several larger, more developed nymphs (interocular distance > than 2.6 mm ♂, and 3.2 mm ♀) were collected during this period of declining growth and adults had begun to emerge. A large size range throughout the seasonal growth period was observed as previously reported in other studies (Hynes 1970, Oberndorfer and Stewart 1977). Differential growth rates, extended egg hatching or recruitment periods, and sexual dimorphism among individuals probably contributed to the observed range in size of nymphs.

Nymphs were primarily collected from under cobble-rock-rubble substrates, and from gravel to cobble-sized substrates from under-cut banks. Early instars were collected from shallow hyporheic gravel habitats.

From the Shakarjian (1997) study and our observations of direct hatching in a nearby Leopard Creek population (Fig. 1) to extended diapause in this San Miguel River population and Rio Conejos population (DeWalt and Stewart 1995), it appears that *I. zionensis* has a great capacity for egg development ranging from direct hatching to diapause over several years (Sandberg and Stewart 2004) resulting in either univoltine or variable-length semivoltine life cycles. The currently available data from these studies suggest this range may be genetically determined and expressed in a single egg mass from a female, or that given populations may, due to temperature regimes, or other factors, have adopted a homodynamic, heterodynamic, or combination egg development strategy. The lack of direct hatch of year 1999 and 2000 eggs in this San Miguel River population suggest that it has adopted a semivoltine strategy.

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