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LIFE CYCLE OF *ISOPERLA LATA* (PLECOPTERA: PERLODIDAE) IN A CENTRAL WISCONSIN TROUT STREAM

John B. Sandberg¹ and Stanley W. Szczytko²

ABSTRACT

Monthly qualitative samples of *Isoperla lata* Frison were made from January 1992 to June 1993 in Ripley Creek, a small second order trout stream in Lincoln County Wisconsin. Additional collecting and an in-stream hatching experiment were conducted in 1994. This species exhibited an S1 (slow) univoltine life cycle. Emergence was synchronous and occurred in late April through early May when stream temperatures in the field were approximately 9–14°C and laboratory stream temperatures were 7–17°C. Laboratory longevity was 2–25 (x = 18.2 ± 4.51) days for males and 7–39 (x = 21.7 ± 5.35) days for females. Mean fecundity of dissected females was 322 ± 122 eggs/female. Females did not deposit egg masses in the laboratory until being held together with males inside modified screened plastic containers. Field-collected females did not have eggs. The egg shape was ovoid and circular in cross section. Mature eggs were light brown and measured 371.7 ± 12.6 mm and 260.7 ± 10.2 mm in length and width respectively. Eggs required a 40–46 day in-stream incubation period and first instar nymphs hatched synchronously over a two day period when stream temperature reached 20°C. Nymphal growth was nearly exponential from June to January and then declined until emergence. The greatest growth increment occurred between June and October and the average maximum size attained occurred in February. Males and females had approximately 18 and 19 instars respectively. Nymphs were primarily carnivorous throughout development and fed on larval Chironomidae, Ephemeroptera, and Plecoptera.

Plecoptera are hemimetabolous with aquatic nymphs that occur primarily in lotic habitats. They are considered to be indicators of good water quality because they usually inhabit clean, cold streams and have low tolerance to organic and inorganic pollution. Fifty seven species of stoneflies have been recorded in Wisconsin (Hilsenhoff 1995) of the ca. 600 species that occur in North America (Stark et al. 1986). The genus *Isoperla* occurs commonly in Wisconsin in unpolluted streams where nymphs cling to substrate or debris (Hilsenhoff 1995). Hilsenhoff and Billmyer (1973) studied the taxonomy and distribution of Wisconsin Perlodidae and provided a regional species key. General knowledge of important life history features of Wisconsin *Isoperla* is limited.

*Isoperla lata* Frison is widely distributed over the eastern and north central United States and Canada (Stark et al. 1986). This species appar-
ently inhabits cold, lower order stream systems with good water quality and
does not appear to be abundant throughout its range compared to other
congeners. In Wisconsin it is one of the first *Isoperla* to emerge and appears
to be limited to the northeastern half of the state (Hilsenhoff and Billmyer
1973). Frison (1942) described the adults and nymphs of *I. lata* and Har­
den and Mickel (1952) provided a key to the Minnesota *Isoperla* species
including *I. lata*. Harper and Magnin (1969) studied some aspects of the life
cycle of this species in Quebec and reported a slow growth univoltine
life cycle with emergence occurring in early May and oviposition in early
July.

SITE DESCRIPTION

The study was conducted on the lower reach of Ripley Creek, above and
below Wisconsin state highway 107 located in the Lincoln County Park,
Camp Newwood. The stream within the study reach is a second order sys­
tem, which supports a resident brook trout, *Salvelinus fontinalis* (Mitchill)
population. The perceived water quality of this stream was excellent and it
supported a remarkable number of other stonefly species and large beds of
aquatic plants. The headwaters are located in shallow tamarack (*Larix laric­
tina* (Du Roi) K. Koch.) wetlands with the stream flowing southeast into the
Wisconsin River. Ripley Creek is 5.3 km long and has an overall change in el­
evation of 57.9 m. There is a small permanent spring fed tributary entering
194 m upstream from the bridge. Sampling was conducted from the mouth to
400 m upstream (Fig. 1).

Typical substrates in the study reach included gravel and cobble which
formed riffles interrupted by either small cataracts or pools. In August
1992, decreased flow caused two pools to become isolated from the main
flow of the stream. Many boulder and cobble substrates within the study
reach were covered with leafy liverworts (*Chiloscyphus* sp.) and water moss
(*Fontinalis* sp.) which provided cover and resting sites for emerging insects.
The banks of the stream above the bridge were primarily undercut, with
well-developed riparian vegetation consisting of grasses, sedges and white
pine forest.

Other stoneflies collected within the study area included *Acroneuria* sp.,
*Allocapnia* sp., *Amphinemura delosa* (Ricker), *Clioperla cilio* (Newman), *Hap­
loperla brevis* (Banks), *Isoperla transmarina* (Newman), *Isoperla cotta*
Ricker, *Isoperla slossonae* (Banks), *Isoperla signata* (Banks), *Leuctra tenuis*
(Pictet), *Paracapnia angulata* Hanson, *Prostoia completa* (Walker),
*Strophopteryx fasciata* (Burmeister) and *Taeniopteryx nivalis* (Fitch). Aquatic
plants common at study sites included: leafy liverworts (*Chiloscyphus* sp.),
water moss (*Fontinalis* sp.), and water cress (*Nasturtium* sp.).

METHODS AND MATERIALS

Nymphs were qualitatively collected monthly with a 600 µm mesh rec­
tangular frame kick net from January 1992 to June 1993 unless shelf ice pre­
vented access to the stream. A minimum sample size of 100 nymphs (com­
bined from all kick samples) was arbitrarily used to define a complete sample
for a particular sampling date. Sampling periods varied in duration from one
to four days. Pre-study sampling from a variety of stream microhabitats indi­
cated that *I. lata* nymphs were primarily collected from undercut banks with
gravel and cobble substrates. Sampling efforts were concentrated, but not
limited to these habitats throughout the study. Nymphs were picked from debris and preserved in 80% isopropyl alcohol for later measuring in the laboratory. Live late instar nymphs were also collected when pre-emergent nymphs (dark wing pads) were first observed in the stream. These nymphs were transported back to the laboratory in Styrofoam containers with stream
water for rearing experiments. A modified 230 µm mesh second stage bag was attached to the kick net behind the 600 µm mesh bag to facilitate collection of early instars. Hyporheic habitats were sampled to a depth of 30 cm below the substrate surface by digging into the stream bed with a spade and collecting nymphs in the two stage net.

Growth was charted from interocular distance (IOD-shortest distance between the eyes) and body length (BL-exclusive of antennae and cerci) measured with a calibrated ocular micrometer fitted to a stereo-dissection microscope. Nymphs were sexed by the presence or absence of a gap in the posterior setal row of the eighth sternum (Stewart and Stark 1988). Middle instar (ca. 0.69 IOD) female nymphs exhibited this gap and males did not. Early instar nymphs were not sexed due to the incomplete development of this posterior setal row. Sex specific growth histograms were constructed using 0.03 mm size classes and instars were interpreted from individual peaks on the histograms. The methods of Janetschek (1967), Dyar (1890), and Harper (1973a) were used to interpret growth and instar development. Stomach and foregut contents of monthly field collected nymphs were removed by dissection and identified to the lowest practical taxonomic level.

Adult emergence frequency histograms and sex ratio were determined by collecting exuviae from trees and other emergence structures. Attempts to collect adults with pitfall traps (10–15 cm dia. coffee cans filled with 1–2 cm 100% kerosene), a malaise trap set over stream margins, sticky-trap applied to trees, and sweep-netting failed to trap sufficient numbers. Exuviae were collected daily and preserved in 80% isopropyl alcohol. Because of their low numbers and elusive behavior, adult presence in the field was estimated from the exuviae collections. Mating, longevity, fecundity, additional observations of emergence, and sex ratio were documented in the laboratory from reared individuals.

In 1993 and 1994 2-quart plastic holding containers modified with 230 or 600 µm plastic mesh windows were used to hold males and females together. Egg batches obtained from gravid females were put into 230 µm mesh sewn envelopes and placed with actual stream substrate into PVC pipe egg hatching containers. These PVC containers were 14 cm long by 3.8 cm wide (ID) and numerous small holes (3–7 mm) were drilled through the pipe to provide for constant exchange of stream water. In 1993 these containers were placed in an artificial stream (Living stream, Frigid Units, Inc., model LS 700) adjusted to field temperature and photoperiod. In 1994 the egg containers were placed in the study creek and anchored in habitats suitable for hatching experiments. Ova were inspected at least every other day with a dissecting microscope during the suspected hatching period until first instars hatched.

Ova were dissected from preserved gravid females and prepared for scanning electron microscope study using methods described by Szczytko and Stewart (1979) and Stark and Szczytko (1982). Scanning electron micrographs of ova were made with a JOEL JSM-5400 scanning electron microscope at the University of Wisconsin-Stevens Point.

Depth, velocity, temperature and dissolved oxygen were measured at sample collection sites for each sampling date. Velocity was measured to the nearest 0.01 ft/sec with a Marsh-McBirney model 201D portable electronic current meter that was calibrated at the beginning of the project. Velocity measurements were taken at sites where net samples were obtained (within the water column) and were within 7.62–15.24 cm of actual nymph habitats. The depth to substrate was measured with a 1.83 stainless steel Price wading rod calibrated in 3.05 cm units. Dissolved oxygen was measured using a
Hach field kit (Model AL-368) and temperature was measured with a standard handheld thermometer.

RESULTS AND DISCUSSION

Emergence occurred in the evening beginning at 1800 hr and continued sporadically until 0230 hr. Nymphs crawled up to 1 m from the stream margin and climbed trees where ecdysis occurred from a few centimeters high to heights reaching 3 m. This observation is consistent with findings reported by Harper and Magnin (1969). Ecdysis required 20–30 min. in the laboratory and wings were soft and held vertically over the abdomen for approximately 1 hr, after which wing color and veination changed from an obscure white-gray to normal translucent black. In the field, after ecdysis, several teneral adults crawled up trees, presumably seeking cover in the tree canopy as has been reported for other stonefly species (Hynes 1976, Jop and Szczynko 1984, Stewart and Stark 1988). Non-teneral adults were difficult to find near the stream.

Extruded egg masses were not collected from six females that made flights back to the stream from the riparian canopy on 8 and 9 June, 1994. This observation occurred coincident with I. cotta flights and was nearly one month after I. lata had completed emergence. These flights were observed at 1930 hr and occurred one hour after the main pulse of the I. cotta flight period. Whether the egg masses were dislodged during handling, or the adults had previously oviposited is unknown. Minshall and Minshall (1966) observed Clioperla cilia making glides down to the stream when oviposition did not occur. The flight pattern for the six females was downstream which is opposite to the upstream flight pattern reported for Isoperla signata (Jop and Szczynko 1984; and personal observation), and the order Plecoptera in general (Hynes 1976). Consistent with previous oviposition observations, the females landed on the stream surface and fluttered their wings while entrained in the current.

Field exuviae collections suggested that adults had a relatively synchronized emergence pattern. Exuviae were first observed and collected on 30 April, 1993 and 1994 when stream temperature was 12°C for both years (Figs. 2 & 3). The sex ratio (male:female) in 1993 was 0.74:1 and 1.09:1 in 1994, which was determined from 519 exuviae collected each year. The emergence period for 1993 and 1994 lasted 6 and 10 days respectively. Emergence presumably ended on 5 May 1993 and 9 May, 1994 when no additional exuviae or nymphs were collected.

Emergence (exuviae collection) was slightly protandrous in 1994 and nearly 50% of males and females emerged on 1 May and 2 May respectively. Emergence frequencies were skewed slightly negative in 1993 and 1994, and fit the definition of a short, synchronous emergence which is concentrated in the beginning of the period (Harper and Pilon 1970). Protandry was not observed in 1993 when nearly 80% of both males and females emerged by 1 May. A short period of high water and increased stream flow may have interrupted emergence before 30 April 1993.

Late instar nymphs were collected from late March to early April 1992, and began emerging under laboratory conditions on 27 April, when stream temperature increased to 7°C, and ended on 7 May, when stream temperature reached 17°C (Figs. 2 & 4). Dissolved oxygen in the stream was usually above 70% of saturation for the entire study period and was not considered to be a limiting factor (Fig. 2). These laboratory and field results might suggest an emergence cue of ca. 7–17°C for laboratory emergence and 12–14°C for
Figure 2. Temperature (circles & solid line) and dissolved oxygen (squares & dotted line) profiles for Ripley Creek, Lincoln Co. Wisconsin for 1992 and 1993.

Figure 3. *Isoperla lata* emergence patterns in 1993 and 1994 determined from 519 exuviae collected from Ripley Creek, Lincoln Co. Wisconsin in each year—male (dashed line) and female (solid line).
field emergence, however more detailed thermal and emergence data are needed to support this hypothesis. By 29 April, and 1 May, fifty percent of the males and females emerged respectively. Mating occurred soon after emergence and continued throughout the early laboratory life span. From the 98 laboratory reared adults, the male:female ratio was 1.5:1 and longevity was 2–25 days ($\bar{x} = 18.2 \pm 4.51$) for males and 7–39 days ($\bar{x} = 21.7 \pm 5.35$) for females. Mean longevity increased for males and decreased slightly over time for females. The mean fecundity of 71 dissected females was $322 \pm 121$ eggs. Average egg counts increased for females during the midpoint of emergence, and then declined.

Forewing and body lengths of adults that emerged early in the 1992 laboratory emergence period were slightly larger than those emerging later. Size of adult stoneflies generally decreases in the latter part of the emergence period (Khoo, 1964, 1968). It is thought that the stimulus to emerge causes smaller individuals to "catch-up" and emerge before attaining their full size. Average adult female (N=88) body length (10.61 ± 0.63 mm) and forewing length (11.75 ± 0.55 mm) were larger than males (N=103), (9.03 ± 0.47 mm) and (9.69 ± 0.34 mm) respectively. Laboratory rearing efforts continued in 1993 and 1994, and were modified to ensure collection of egg masses. Males and females were held in 2-quart screened plastic holding containers and mated frequently and produced egg masses. Laboratory emergence patterns from 1993 and 1994 were slightly protandrous and synchronous (Fig. 4). In 1993, the sex ratio (male:female) of adults was 1:1.1 (N=75) and 0.7:1 in 1994 (N=213). Fifty percent of laboratory adults had emerged by 28 April, 1993 and 20 April, 1994. Laboratory reared females emerged with eggs partially developed in 1993-1994, and mated frequently during their first day of life. This finding suggests that eggs begin to develop in late instar nymphs. The mean number of eggs per egg mass and the number of egg masses decreased through time in 1994.

The general egg shape was ovoid and the cross section circular. Mature eggs were light brown and average length and width were $371.7 \pm 12.6$ and $260.7 \pm 10.2 \mu m$ respectively. The collar was well developed, and offset from the egg body by a low shoulder (Figs. 5 & 6). The collar was stalked and irregularly incised with low longitudinal carinae and apically flanged rim. The shoulder was low with elevated carinae. The chorionic surface was covered with irregular hexagonal follicle cell impressions (FCI's); FCI walls were raised and thickened and the floor was flat with numerous shallow punctations. The eclosion line was well developed near the posterior pole, elevated and thickened (Figs. 6 & 7). Micropyles were positioned singularly on top of the FCI ridges along an irregular posterior row above the eclosion line. Two sticky gelatinous matrices were observed expanding from hydrated eggs, one surrounding the egg body and the second forming an umbrella-shaped anchoring over the collar.

In 1992, reared females did not produce extruded egg masses when held singularly or in combination with males in Styrofoam cups, however 22,916 eggs were dissected from 71 adult females of which 12,692 mature eggs were used for petri dish hatching experiments. An unidentified fungus infected the eggs and hatching failed.

No eggs hatched from the 1993 laboratory egg hatching experiments. Egg masses collected from females held together with males in 2-quart screened plastic holding containers were placed into 230 μm mesh envelopes and then into PVC pipe hatching chambers with stream substrates. The initial temperature was set at 15° C in an artificial stream containing actual Ripley Sandberg and Szczytko: Life Cycle of *Isoperla Lata* (Plecoptera: Perlodidae) in a...
Figure 4. *Isoperla lata* laboratory emergence patterns from 1992 (N=98), 1993 (N=75) and 1994 (N=213)—male (light bars), female (dark bars).
Creek water and substrates. Temperature and photoperiod were adjusted to approximate stream conditions as time elapsed. The egg hatching experiment began on 9 May and the last egg mass was added on 18 May. Artificial stream temperature was adjusted to a maximum temperature of 19°C by 20 June, however it was apparent at this time that the eggs were infected by a fungus and had turned from medium brown to milky white and the experiment was terminated.

In 1994, egg hatching followed a synchronous, short term development pattern typical of homodynamic species (Mutch and Pritchard 1982, Jop and Szczytko 1984, Vaught and Stewart 1974). Two egg hatching chambers were anchored in Ripley Creek on 30 April, 1994 when stream temperature was 8°C. On 5 May stream temperature was 13°C and three more egg masses were put in envelopes and were placed into a third egg hatching chamber and anchored near the first two. By 12 June no eggs had hatched,
however on 14 June when stream temperature reached 20° C, 92% of the eggs (N=246) had hatched and hatching chambers were removed from the stream. These results suggest a temperature cue of ca. 20° C for egg hatching, however additional detailed field thermal data and hatching experiments are needed to substantiate this hypothesis. Egg development ranged from 40–46 days (x = 43 ± 3). It is apparent from our hatching experiments that we were not successful in emulating the important environmental variables for this species in the laboratory to initiate egg hatching. This suggests that egg hatching of this species is sensitive to subtle, natural in-stream oscillations of temperature, water chemistry and other important environmental variables.

Nymphal growth rates were roughly exponential from June to October. By early September nymphs were half grown and greatest growth occurred from August–October and decreased from November–April (Fig. 8). In 1992 and 1993 stream temperature was less than 2° C from November–March and increased to ca. 6° C by mid April. Slow growth occurred during the coldest months (November–February) and decreased until emergence. These characteristics along with emergence in late April to early May conform to Hyne's (1961) classification of an S1 (slow) univoltine life cycle.
Maximum size was attained in late February and declined until emergence. The decline in growth rate was probably due to small sample size, or that the larger, more developed nymphs moved to different microhabitats, and or, escaped sampling. Several larger, more developed nymphs were collected during this period of decline from within the overhanging bank structure. A large size range throughout the seasonal growth period was observed as previously reported by other workers (Brink 1949, Hynes 1941, 1961, Harper and Magnin 1969, Harper and Pilon 1970, Harper 1973b, Jop and Szczytko 1984, Oberndorfer and Stewart 1977). Differential growth rates, hatching sequence and sexual dimorphism among individuals probably contributed to the observed large size range of nymphs.

First instar nymphs from in-stream incubation studies crawled out of eggs after they pushed open a hinged cap that split along the eclosion line (Degrange 1957, Heiman and Knight 1970, Vaught and Stewart 1974, Oberndorfer and Stewart 1977, Snellen and Stewart 1979). First instars (Fig. 9) were unpigmented, distinctly setose, slightly sclerotized, without ocelli and had three cercal and nine antennal segments. Mean IOD was 0.17 ± 0.01 mm, and body length ranged from 0.58 mm to 1.08 mm. Guts (N=25) contained amorphous unidentifiable material.

Nymphs were primarily collected from undercut banks with predominantly cobble and gravel substrates in current velocities of 0.17-0.61 m/sec and depths of 0.07-0.32 m. The *I. lata* nymphs were not collected from hyporheic habitats, however early instar *P. angulata* nymphs were. The *I. lata* nymphs were first sexed when they were approximately 0.69 mm IOD (8–9 instar). Later instars were dark with dorsal longitudinal yellow bands on the abdomen with distinct yellow patterns on the head and thoracic nota. Nymphs reached an average maximum size of 1.51 ± 0.07 mm (IOD) and 10.73 ± 0.50 mm (BL) for males, and 1.77 ± 0.11 mm (IOD) and 12.90 ± 0.62 mm (BL) for females. Sexual dimorphism in body size is common for Plecoptera and is usually attributed to the greater body size needed by females for egg production.

The size frequency distributions and Janetschek's (1967) method indicated a combined range of 11–19 total instars for 1047 sexed and 319 unsexed field collected and in-stream hatched nymphs (Figs. 10 & 11). Sixty-four IOD size classes with a 0.03 mm size class interval were used to construct size frequency distributions. The plot of suspected instar number and natural logarithm of IOD for that instar, determined from the size frequency histograms and Janetschek modes, did not conform to Dyar's rule of regular stepwise development (Fig. 12). The growth rate of early instars was slightly greater than later instars. Growth in other univoltine stoneflies has been shown to be concentrated in the wing pads and genitalia during the last instars (Harper 1973a). Early instars did not have a relative constant increment between successive instar points (Fig. 12) which could have been the result of missing IOD observations.

The gaps found between first instar, unsexed, and sexed nymphs may reflect missing instars because nymphs were not field collected in July 1992 (Fig. 8). Three additional instars were intercalated by Harper's (1973a) method. In this study, missing instars were intercalated on a Dyar's law plot after the first instar IOD was plotted in the continuation of a best fit line drawn through observed data points (Fig. 12). A male-female size frequency distribution indicated ca. 18 instars for males and 19 instars for females (including unsexed and intercalated individuals) (Fig. 10).

Harper (1973a) felt these methods were crude and suggested that more attention be given to the underlying theoretical basis of instar number determinations. Future investigations should include vigorous laboratory
Figure 9. *Isoperla lata* first instar nymph—scale = 100:1.
Figure 10. Size frequency histogram of interocular distance from 547 male, 500 female, 209 first instar and 110 unsexed nymphs (clear bars) of Isoperla lata from field collections (1992–1993) and experimentally hatched eggs (1994) from Ripley Creek, Lincoln Co, Wisconsin—numbers above bars approximate instar number.

Figure 11. Instar analysis of 1366 Isoperla lata male, female, unsexed and first instar nymphs from Ripley Creek, Lincoln Co, Wisconsin using the Janetschek method—tics approximate instars.
nymphal rearing as well as field collections to substantiate true or more reliable estimates of larval instars (Fink 1984, Harper 1973a, Jop and Szczytko 1984). Nymphal development described using indirect methods of instar determinations for mayflies and stoneflies has received critical attention (Fink 1980, 1982, 1984; Khoo 1964). The effects of heterogeneous development, temperature, sexual dimorphism within instars, and the plasticity of instar number commonly confound analysis by indirect methods. Hynes (1976) found that stonefly instar number was indeterminate and that laboratory development may not extrapolate to development in natural populations. Most methods for determining instar number are based on linear measurement and describe growth better than development (McClure and Stewart 1976). Butler (1984) stated that the degree of resolution in determining developmental age using instar number was low and new techniques should be employed.

Qualitative examination of 312 foregut and stomach contents suggested that *I. lata* was mostly carnivorous in all size classes except first instars. Chironomidae (35.7%) and Plecoptera (31.8%) formed the majority of food items ingested (Table 1). The highest proportion of empty stomachs (41.7%) was observed just before emergence in late April 1992. Other studies (Frison 1935, Minshall and Minshall 1966) have also reported large numbers of Chironomidae larvae in the foregut of other perlodid species.

Figure 12. Relation between IOD and instar number in *Isoperla lata* nymphs from Ripley Creek, Lincoln Co. Wisconsin—open circle = size of first instar nymphs—vertical lines indicated 3 intercalated instars
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<th>08/20</th>
<th>09/07</th>
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*Percent of total ingested organisms.

*b Represents numbers of specific items found in the gut during each time period.

*c Represents number of foreguts and stomachs in which items occurred, rather than numbers or percentages of organisms.
LITERATURE CITED

Dyar, H. G. 1890. The number of molts of lepidopterous larvae. Psyche 5: 420-422.


