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Capacity for Extended Egg Diapause in Six *Isogenoides* Klapalek Species (Plecoptera: Perlodidae)

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ABSTRACT

The eggs of six *Isogenoides* species have been incubated at a single, approximately simulated, San Miguel River, Colorado, seasonal temperature regime for 2–5 years. Eggs were collected from reared, laboratory-mated females of *I. colubrinus, I. doratus, I. elongatus, I. frontalis, I. varians*, and *I. zionensis*. Eggs were held in 8 ml containers and visually examined usually weekly for development and hatch.

Only some *I. zionensis* eggs from a combined first and second mass of a Leopard Creek, Colorado population hatched directly within two weeks, continuing until late September, and resuming mainly May–June the following year; one and two eggs hatched in 2002 and 2004, respectively. *I. doratus* and *I. varians* experienced hatch only after an over-summer, 3-4 month diapause, and *I. varians* again in late August–September during the second year. Populations of *I. colubrinus, I. elongatus, I. frontalis* and a San Miguel River, Colorado population of *I. zionensis* began hatching only in the year following deposition, after a 10–12 month diapause. Some hatching of these four species occurred at spring-summer temperatures in subsequent years (2^{nd}, 3^{rd}, 3^{rd}, and 4^{th} years, respectively), with no intervening hatch at winter temperatures. The study confirms extended, usually asynchronous diapause and hatching for from 3 months to 4 years, probably genetically controlled, in the six species, and a great degree of adaptive capacity for diapause in the genus *Isogenoides*.

INTRODUCTION

The eggs of some homodynamic stonefly species hatch directly within a few weeks after oviposition (Harper 1973, Hynes 1976, Stewart and Stark 2002, Zwick and Hohmann 2003), or may extend or delay development and hatching over short or long number of days by dormancy, depending on temperature experienced during incubation (Brittain 1977, Lillehammer 1986, 1987, Elliott 1989, Frutiger 1996, Zwick 1996a, b, 2002, Stewart and Stark 2002). Eggs of the few heterodynamic species that have been studied have the capacity for extended, and sometimes asynchronous diapause with hatching from 5–7 months up to three years or possibly longer (Khoo 1968a, Marten 1991, Oberndorfer and Stewart 1977, Snellen and Stewart 1979a, b). The strategies of dormancy or diapause allow “waves of hatch” (Hynes 1976), cohort splitting (Butler 1984, Humpesch and Elliott 2003) or asynchronous hatch over several years, sometimes missing a season or even year(s). Such extended egg development is presumably an adaptation, probably genetically programmed, to enhance survival through unpredictable or adverse temperatures, water flow or other environmental conditions. Either dormancy or diapause of eggs may be in different phases of development (with no apparent eyespot appearance) or as an arrested development of protonymphs within the egg (apparent eyespot appearance), but this has not been delineated in most previous studies.

The strategy of sibling eggs from a single batch or female, or seasonal adult population, breaking diapause and hatching at different times, results in nymph recruitment over extended periods. This enhances the probability of favorable conditions for survival of at least some of the progeny, fulfilling at least in part the presence of r–adaptiveness in insects that produce large numbers of eggs. Zwick (1996a, 2002) has proposed that dormant or diapausing eggs in streams represent a "seed bank" from which recruitment of nymphs may be made over an extended time. Giesel (1976) used the phrase "relict seeds" for eggs with delayed and sometimes asynchronous development that allows parents to effectively prolong their reproductive life span. Humpesch and Elliott (1987, 2003) proposed that delayed egg development with a long hatching period is one mechanism for life cycle partitioning and enhanced nymph survival in Plecoptera and Ephemeroptera.

Our knowledge of stonefly egg development must still be considered meager from the standpoint of the few detailed experimental incubation studies that represent few genera and species in the families **Chloroperlidae** (Lillehammer 1987, Harper 1973), **Leuctridae** (Lillehammer 1985, Elliott 1987, Snellen and Stewart 1979a), **Nemouridae** (Elliott 1986, Brittain and Lillehammer 1987, Harper 1973), **Perlidae** (Harper 1973, Snellen and Stewart 1979b, Lillehammer 1986, 1987, Elliott 1991a, Marten 1991, Frutiger 1996, Moreira and Peckarsky 1994, Zwick 1996a, b, 2002), **Perlodidae** (Khoo 1968a, Harper 1973, Oberndorfer and Stewart 1977, Elliot 1991b, Dewalt and Stewart 1995, Sandberg and Szczytko 1997) and **Taeniopterygidae** (Khoo 1968b, Brittain 1977, Zwick and Hohmann 2003).

The North American stonefly genus *Isogenoides* currently contains 9 species. The eggs of all except *I. colubrinus, I. elongatus* and *I. zionensis* were studied and figured with SEM by Kondratieff (2004), who found no distinguishing differences in species. Egg development and hatching are unknown except for the Dewalt and Stewart (1995) study establishing that the eggs of *I. zionensis* from the Rio Conejos of Colorado diapaused at least over their first summer and winter. Eggs laid by June-emerging females hatched in March–April the following year. This, and the study by Oberndorfer and Stewart (1977), proving that eggs of the perlodid *Hydroperla crosbyi* in Texas undergo diapause for approximately eight months, from February-emerging females until about October, led us to be interested in determining the prevalence of diapause in the genus *Isogenoides*, and the capacity of eggs of various species for extended and asynchronous diapause. Our study of the systematics and drumming of *Isogenoides* (unpublished), that began in 1999, afforded the opportunity to collect and rear nymphs, mate virgin adults, and obtain eggs for incubation experiments to address these questions for six species.

MATERIALS AND METHODS

Species and collection sites

This study was begun in 1999, and still in progress in fall 2004, with eggs of the various species and populations having been in incubation from approximately 1.5 to 5 years. Virgin adults of six species were reared from mature nymphs collected as follows: (1)
I. colubrinus (Hagen), Yampa River at 12 Mile Gulch and Cross Canyon, 7.1 miles NW of Elk Springs, Moffat Co., Colorado, 28 May 2004; (2) *I. doratus* (Frison), Rock River at Lakewood Corner on Hwy 75, 7 miles South of Rock Rapids, Lyon Co., Iowa, 09 February 2002; (3) *I. elongatus* (Hagen), Colorado River at Riffle city park, Garfield Co., Colorado, 12 March 2000; (4) *I. frontalis* (Newman), Rocky Run Creek, 1.5 miles North of Brule, Douglas Co., Wisconsin, 07 April 2000; (5) *I. frontalis* (Newman), Confluence of Fish and Pine Rivers, Intersection of Fish Cr. Rd. and Old US 2, Bayfield Co., Wisconsin, 09 April 2000; (6) *I. varians* (Walsh), Big Otter River, Bridge on Hwy 24 @ CR 711 and 709 intersections, 2 miles West of Campbell County line, Bedford Co., Virginia, 26 February 2004; (7) *I. zionensis* Hanson, San Miguel River @ Placerville, San Miguel Co., Colorado, June 1999; (8) *I. zionensis*, San Miguel River @ Specie Cr., 5 miles West of Placerville, San Miguel Co., 14 June 2000; (9) *I. zionensis*, Leopard Creek, Hwy 62, 4 miles North of Placerville, San Miguel Co., 29 June 2000.

Rearing

Pre-emergent nymphs from field collections were held and reared individually in perforated Styrofoam cups containing stream substrate, suspended in a Living StreamÆ. The stream was adjusted to a single simulated seasonal temperature regime determined by submerging a Ryan Model 100 thermo-recorder in the San Miguel River, Colorado, for the period July 1999 to July 2000. During that year, the laboratory stream was adjusted to periodic San Miguel River temperatures obtained from a thermometer during trips from Texas to Colorado for an associated study of *I. zionensis* life history (Fig. 1). In subsequent years, the laboratory stream temperature regime was kept as close as possible to the simulated annual regime, read from the Ryan Charts, with some usually minor fluctuations due to functional variations of the Living StreamÆ temperature regulator (Fig. 1). Photoperiod was adjusted every year from a minimum of 9.5 hr of fluorescent room light (January 1–15) to a maximum of 14.5 hr (June).

Emergent adults were transferred to individual Styrofoam cups and labeled with species, sex and emergence date. They were paired beginning after 1–7 days, and allowed to mate by transferring a male into the female's container.

Collection and incubation of eggs

When an egg mass was produced by a mated female, it was placed in stream water in a small, approximately 8 ml glass container, covered with 100 μm mesh NitexÆ, labeled by date and female, kept in larger glass container, and incubated in the laboratory stream at its annual temperature regimes (Fig. 1) for the duration of the study.

Fungal growth inside incubation containers was controlled in 2000 and 2001 using stabilized chlorine oxides in the commercial tropical aquarium MarOxyÆ

(Mardel Laboratories, Inc.) and an aqueous solution of Methylene Blue. These treatments were added to egg incubation containers monthly, during these years, when large amounts fungal hyphae were observed. After 2001, no more treatments were needed.

Egg inspection and recording of hatch

Incubation containers were visually inspected, usually weekly over the long period, for hatch by removing them from the larger containers, and gently shaking them while looking for movement or suspension of hatchlings. If presence of young larvae was detected, then that container was opened and its contents scanned under either a Wild M5D or Zeiss Stemi SV6 dissecting microscope at 0.8–50X. A 2.5 cc B-D GlaspakÆ syringe was used to siphon up the live or dead hatchlings and any exuviae, their numbers recorded, and remaining eggs were returned to the large glass containers containing stream water, and to the laboratory stream within 0.5–3.0 hr. to ameliorate as much as possible any thermal stress. Each incubation container was emptied into a petri dish for removal of dead eggs and post-hatch eggshells at approximate 6-month intervals. Dead or damaged eggs were black or clear inside. Only eggs that were near hatching had eyespots of the developing protonymph visible inside the chorion. These, and eggs with solid white contents, whether or not covered with fungus or algae, were considered viable and were returned to the laboratory stream within 0.5–3.0 hr. First instars, older hatchlings and exuviae were counted and preserved in 80% EtOH and held in genitalia vials in larger 4-dram vials labeled by date, egg batch and female origin. Final totals of eggs/mass were determined from accumulated counts over the variable incubation period for each species.

RESULTS

Viable eggs from all six *Isogenoides* species were successfully obtained and incubated, with an overall 24–92% hatch over the variable periods of approximately 14 days to 5 years (Table 1).

I. colubrinus

Four egg masses from each of four females were placed into the laboratory stream environment between 03–18 June, 2000, when simulated regime temperature ranged from 12–17∫C (Fig. 1). Hatch was not direct in the year of deposition, and eggs diapaused approximately 10 months until some began hatching in April 2001 (Fig. 2) when stream temperature was 10∫C, and they continued hatching until late June. The remaining eggs continued diapause through approximately 11 months (July 2001 to June 2002) of their second year. The last eggs to hatch from all female masses occurred between 12 June and 20 July, 2002, when temperature was 13.5∫C. None hatched in 2003, and on 24 February, 2004, the remaining 50 eggs had no eyespots but appeared to be viable. Through that date, then, a 37–79% hatch had been obtained for the four egg batches, with a confirmed asynchronous extended diapause and hatch extending over at least two years; average total number of eggs/batch was 632.5 (Table 1, Fig. 2).

I. doratus

One egg mass from one female, containing 996 eggs, was placed into the laboratory stream environment on 16 April, 2002, when simulated regime temperature was 6∫C (Fig. 1). Hatch was not direct, and eggs diapaused approximately 3–5 months over the summer, until 34% hatched between 03 August and 21 September, 2002 (Fig. 3). On 24 February, 2004, the remaining 616 eggs were examined under the dissection microscope; none had eyespots and they appeared to remain alive (Table 1). Through that date, then, only an over-summer diapause has been confirmed for this species.

I. elongatus

Six egg masses from each of six females were placed into the laboratory stream environment between 01–11 May 2000, when simulated regime temperature was12∫C (Fig. 1). Hatch was not direct in the year of deposition and eggs diapaused approximately 11.5 months until some began hatching 14 April, 2001, (Fig. 4) when stream temperature was 10∫C. Hatching continued until 30 June, 2001. The remaining eggs continued diapause through approximately 11 additional months until a small hatch resumed again in June, 2002, and another very small hatch of six eggs occurred April–June, 2003 (Fig. 4). On 24 February, 2004, the remaining 425 eggs appeared to remain alive in incubation (Table 1), and six of these had eyespots suggesting further expected hatching with increasing temperature. Through that date, then, a 24–92% hatch had been obtained for the six egg batches, with a confirmed asynchronous egg diapause and hatch extending over at least three years; average total number of eggs/batch was 708.2 (Table 1, Fig. 4).

I. frontalis

Six egg masses from each of six females were placed into the laboratory stream environment between 24–26 May 2000, when simulated regime temperature was12∫C (Fig. 1). Hatch was not direct in the year of deposition, and eggs diapaused through approximately 10.5 months until some began hatching on 14 April, 2001, (Fig. 5) when stream temperature was 10∫C. Coincidentally, this is the same date that *I. elongatus* began to hatch. Hatching continued until 16 June, 2001, and the remaining egg continued diapause though approximately 12 additional months until hatching resumed again in June and early July, 2002. Another very small hatch of six eggs occurred April–June, 2003, after approximately 10–12 additional months of diapause. On 24 February, 2004, the remaining 210 eggs (Table 1) were examined under the dissection microscope; they appeared to remain alive and only one had eyespots. Through that date, then, a 33–88% hatch had been obtained for the six egg batches, with a confirmed asynchronous egg diapause and extended hatching over at least three years; average total eggs/batch was 415 (Table 1, Fig. 5).

I. varians

One egg mass from one female, containing 94 eggs, was placed into the laboratory stream environment on 14 April, 2001, when simulated regime temperature was 6∫C (Fig. 1). Hatch was not direct, and eggs diapaused through approximately 4 months, over summer, until 92% of them hatched over a 13-day period from 18 August to 01 September, when stream temperature was 16∫C (Fig.

6). Another very small hatch of seven eggs began on 24 August, 2002, after approximately 11.5 additional months of diapause and continued until 31 August. The remaining five eggs on 24 February, 2004, (Table 1) were consumed by fungal infection and removed from the laboratory stream. Therefore, 92% of the 94 eggs hatched, with a confirmed asynchronous egg diapause and hatch extending over at least one year.

I. zionensis

On 26 June, 1999, a single egg mass, containing 368 eggs, from one San Miguel River, Colorado, female was collected and temporarily held in Quartz Creek, Colorado, at temperatures ranging from 10–12ʃC; and then later placed into the laboratory stream on 16 July, 1999, at 11ʃC (Fig. 1). A second egg batch, containing 733 eggs, collected from a second San Miguel River female was placed into the laboratory stream on 14 June, 2000, when stream temperature was 17ʃC (Fig. 1). Two egg masses, containing 1092 eggs, from one Leopard Creek, Colorado, female were collected and placed in a single container on 01 and 06 June, 2000, when laboratory stream temperature was 12ʃC (Fig. 1). These two masses have been treated as one in this study.

The Leopard Creek batches (squares in figure) began hatching directly on 14 June, 2000, when stream temperature was 17ʃC, with the first 19 hatchlings only requiring from 8–14 days of development (Fig. 7). Hatching continued for 3 months and ended on 22 September, when stream temperature had decreased to 9ʃC. The remaining eggs continued diapause until some began hatching on 10 March, 2001, and continued through late June; only one nymph emerged later on 1 September, 2001 (Fig 7.). Over the three remaining years of development, only one egg hatched on 12 June, 2002, none hatched in 2003, and two hatched early on 23 February, 2004, presumably because our laboratory stream malfunctioned and temperature increased to 15ʃC for an unknown period of possibly 48 to 72 hours. On 24 February, 2004, the remaining 404 eggs (Table 1) appeared to remain alive; three eggs had eyespots suggesting further expected hatching as temperature increases. Through that date, then, a 38% hatch had been obtained with a confirmed direct and asynchronous extended diapause and hatch over at least four years.

The 1999 San Miguel River batch (diamonds in figure) did not develop directly in the year of deposition, and began hatching on 6 March, 2000, when stream temperature was 6ʃC, after approximately eight months of diapause. Hatching continued until 05 July, 2000. The remaining eggs continued diapause until some resumed hatching on 14 April and 12 May, 2001, when stream temperature was 10–11ʃC. No additional hatching occurred, of the 13 eggs that have appeared to remain alive through 24 February, 2004; none had eyespots on that date. Through that date, then, a 36% hatch had been obtained with a confirmed asynchronous extended diapause and hatch extending over at least two years.

The 2000 San Miguel River egg mass (circles in figure) was deposited on 14 June, 2000, and experienced a nine-month diapause until the first hatching on 11 March, 2001, when stream temperature was 6ʃC. Hatching continued until 23 June, when stream temperature was 12ʃC. Over the remaining three years, 10 eggs hatched on 12 June 2002, 4 hatched on 26 April 2003, and 2 hatched on 23 February, 2004, presumably due to a temporary increase in the laboratory stream temperature. On 24 February, 2004, 190 apparently live eggs remained and three had eyespots suggesting further expected hatching. Through that date, then, a 38% hatch had been obtained with a confirmed asynchronous diapause and hatch extended over at least four years.

DISCUSSION

The results show a great capacity in the genus *Isogenoides* for extended, sometimes asynchronous, egg diapause and hatching, and that diapause in all species studied occurs early in development with no apparent eyespot appearance until just before hatching. The incubation of eggs of the six species populations from widely separated latitudes and longitudes of North America, at a single seasonal temperature regime, suggest that this capacity in each species is intrinsically, probably genetically, controlled. The simulated San Miguel River, Colorado, seasonal temperature regime yielded good cumulated 24–92% egg mass hatch percentages (Table 1) despite the fact that it probably did not closely correspond with the natural seasonal regimes of the origin streams of most tested populations, particularly of Iowa (*I. doratus*), Wisconsin (*I. frontalis*), or Virginia (*I. varians*). The declining and small hatches after one year of diapause (*I. colubrinus* Fig. 2, *I. elongatus* Fig. 4, *I. frontalis* Fig. 5) could be a reflection of the unnatural experimental temperature regime, other conditions not met in the laboratory stream, or an intrinsic, genetically programmed decline over extended years.

The number of presumably live eggs remaining for various species in February, 2004 (Table 1), leaves open the possibility of an additional year or more of extended hatch, beyond that recorded for some species. The study should be followed up by incubating eggs of each species at their origin-stream seasonal temperatures over extend years, to more precisely determine the details of their diapause adaptation. An attempt was made in January-February, 2004, to obtain eggs for incubation, of the southernmost known population of *I. varians* of Westville Creek, in Simpson County, Mississippi. Three males and eight females were reared and paired, as described above for other species, but copulation was not observed and females extruded no egg masses.

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Table 1. Emergence, mating, egg mass deposition dates, and numeric egg hatch summary data (February 2004) for six species of *Isogenoides*. Species ID are species initials except for *I. zionensis* where locations (San Miguel River and Leopard Creek) are indicated.

Species ID	Date Emerged	Date Mated	Date Deposition	Total # Masses/ # Eggs	# Eggs Hatched (% hatch)	# Eggs Dead-Damaged	Remain-#Eggs Incuba-ting
IC-01	02-VI-2000	02-VI-2000	03-VI-2000	1/801	566 (71)	227	8
IC-02	02-VI-2000	04-VI-2000	05-VI-2000	1/906	679 (75)	219	8
IC-03	11-VI-2000	11-VI-2000	12-VI-2000	1/572	453 (79)	107	12
IC-04	15-VI-2000	16-VI-2000	18-VI-2000	1/251	92 (37)	137	22
ID-01	09-IV-2002	13-IV-2002	16-IV-2002	1/996	340 (34)	40	616
IE-01	01-VI-2000	01-VI-2000	01-VI-2000	1/531	306 (58)	177	48
IE-02	04-VI-2000	04-VI-2000	05-VI-2000	1/436	104 (24)	309	23
IE-03	06-VI-2000	06-VI-2000	06-VI-2000	1/859	444 (52)	242	173
IE-04	07-VI-2000	07-VI-2000	07-VI-2000	1/967	629 (65)	318	20
IE-05	11-VI-2000	11-VI-2000	11-VI-2000	1/848	558 (65)	132	158
IE-06	11-VI-2000	11-VI-2000	11-VI-2000	1/608	558 (92)	47	3
IF-01	22-V-2000	23-V-2000	24-V-2000	1/532	184 (35)	283	65
IF-02	24-V-2000	24-V-2000	24-V-2000	1/614	202 (33)	404	8
IF-03	22-V-2000	23-V-2000	24-V-2000	1/395	325 (82)	25	45
IF-04	23-V-2000	24-V-2000	26-V-2000	1/364	322 (88)	25	17
IF-05	21-V-2000	25-V-2000	26-V-2000	1/383	307 (80)	25	51
IF-06	25-V-2000	25-V-2000	26-V-2000	1/200	95 (47)	81	24
IV-01	05-IV-2001	12-IV-2001	14-IV-2001	1/94	86 (92)	8	0
SMR-01	24-VI-1999	25-VI-1999	26-VI-1999	1/368	134 (36)	221	13
SMR-02	02-VI-2000	14-VI-2000	14-VI-2000	1/733	283 (38)	260	190
LC-01	29-V-2000	31-V-2000	1 & 6-VI-2000	2/1092	419 (38)	269	404

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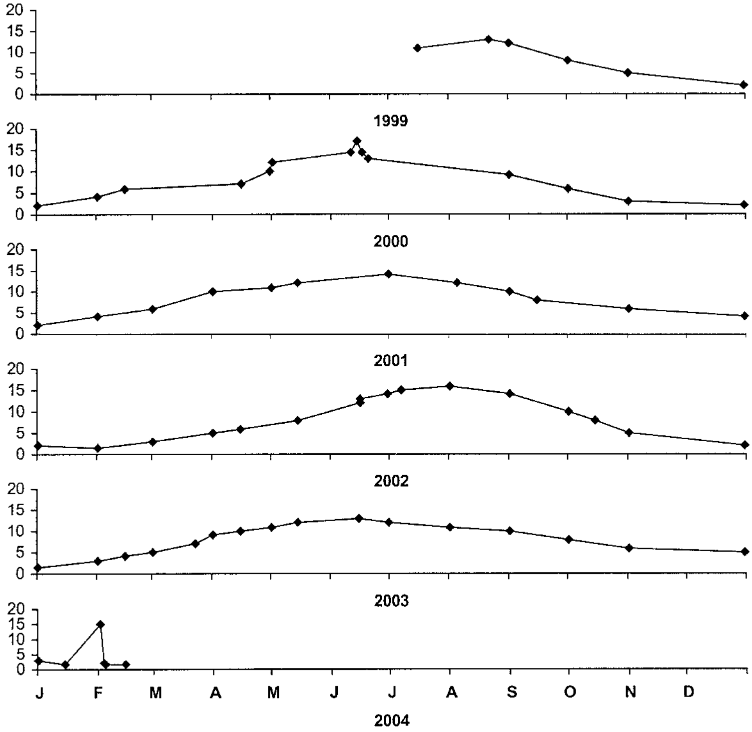

FIG. 1. Simulated laboratory incubation temperature of San Miguel River for years 1999–2000. First egg masses entered Living Stream on 16 July 1999. Maximum and minimum temperatures were 17 and 1.5°C. Temporary coolant malfunction occurred during the week of 01 February 2004 when temperature reached 15∞C and was slowly cooled down to 1.5°C.

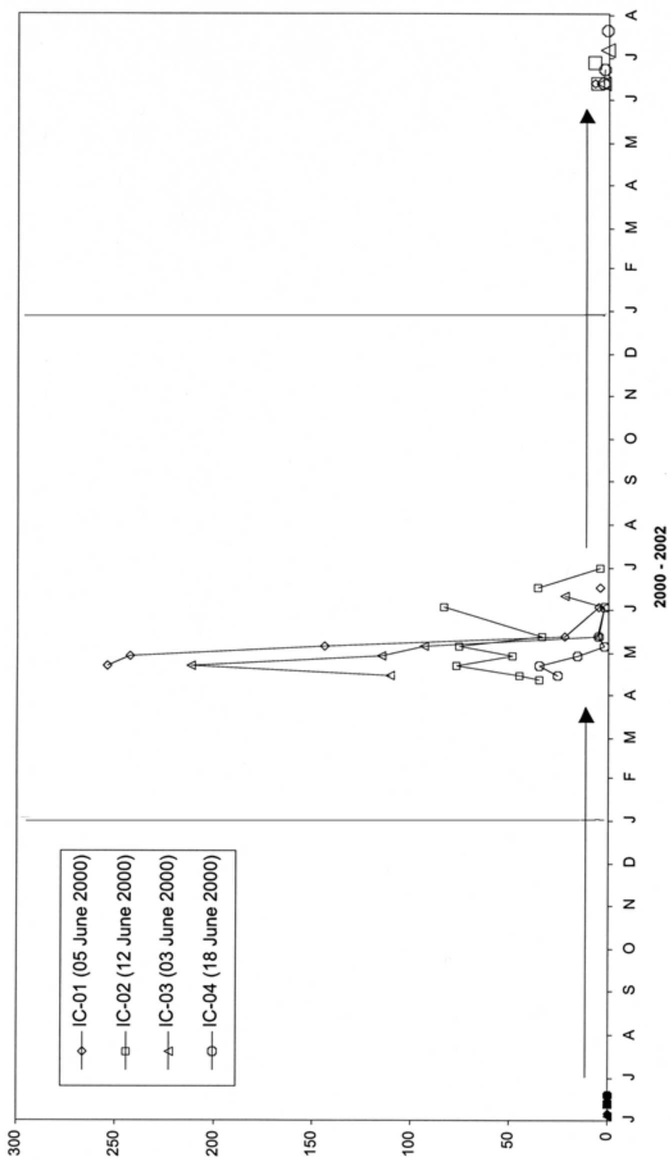

FIG. 2. Hatching of four *I. colubrinus* egg masses from 4 females collected from Yampa River, CO. Egg mass totals before hatching were IC-01 (N=801), IC-02 (N=906), IC-03 (N=572), and IC-04 (N=251). Egg mass depositions indicated by filled symbols (2000), diapause indicated by horizontal arrows.

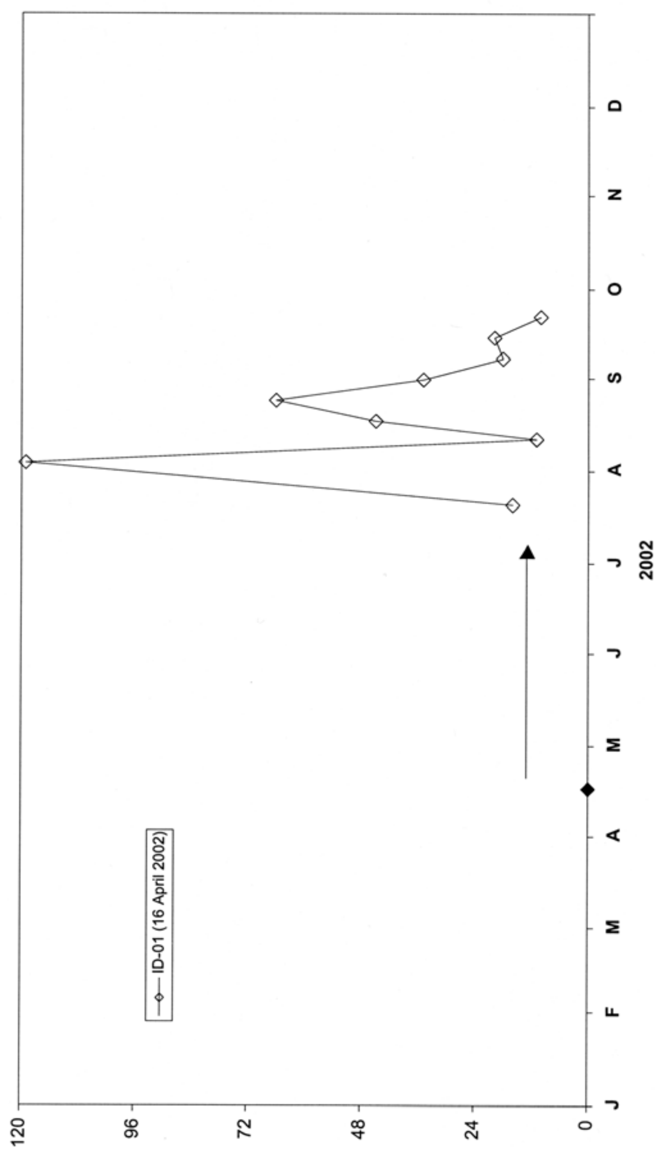

FIG. 3. Hatching for one *I. doratus* egg mass from female collected at Rock River, IA. Egg mass total before hatching was ID-01 (N=996). Egg mass deposition (16 April 2002) indicated by filled symbol, diapause indicated by horizontal arrows.

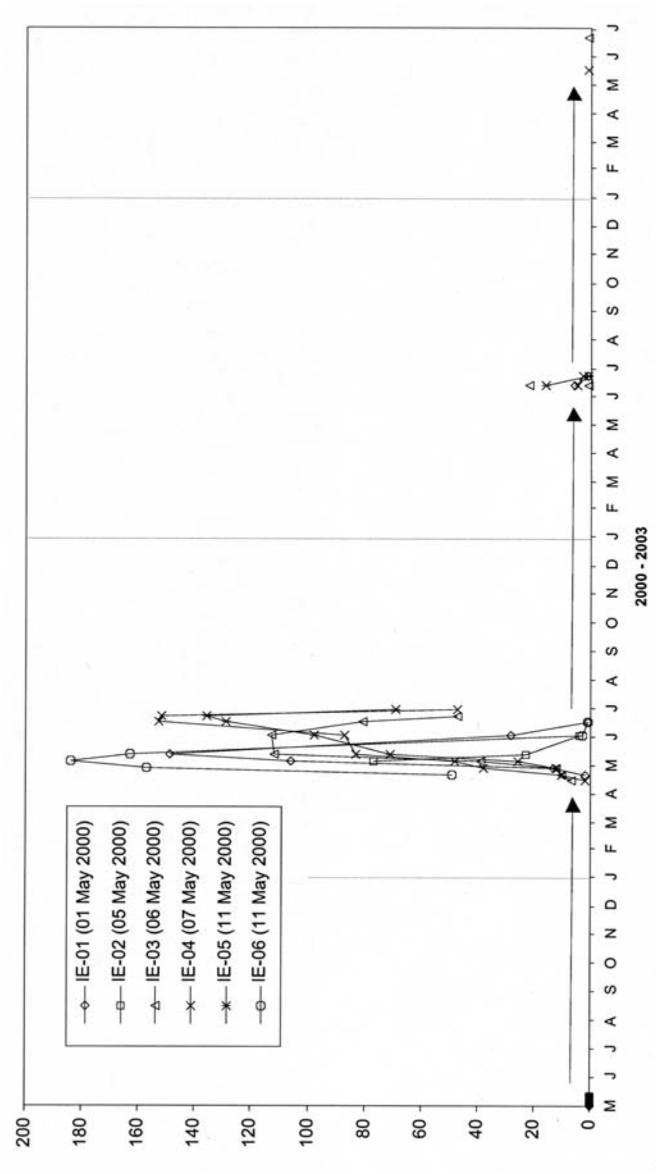

FIG. 4. Hatching for six *I. elongatus* egg masses from 6 females collected from Colorado River, CO. Egg mass total before hatching were IE-01 (N=531), IE-02 (N=436), IE-03 (N=859), IE-04 (N=967), IE-05 (N=848), and IE-06 (N=608). Egg mass depositions indicated by filled symbols (2000), diapause indicated by horizontal arrows.

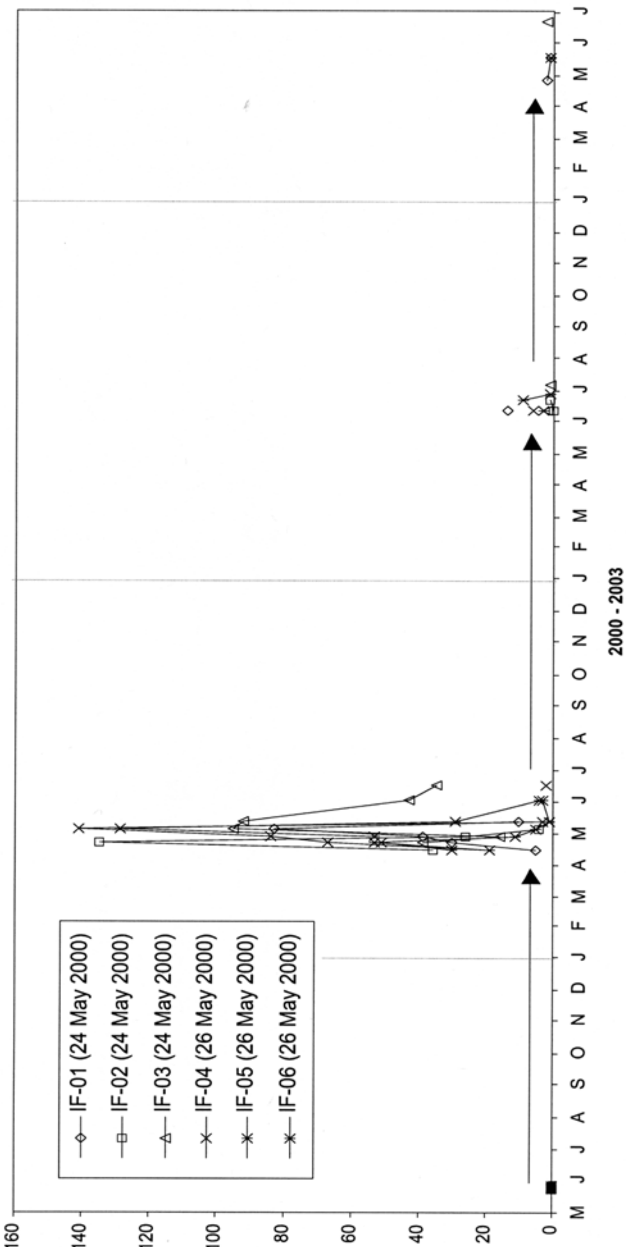

FIG. 5. Hatching for six *I. frontalis* egg masses from 3 females collected at Rocky Run Cr. and 3 females collected from Fish Cr. WI. Egg mass totals before hatching were IF-01 (N=532), IF-02 (N=614), IF-03 (N=395), IF-04 (N=364), IF-05 (N=383), and IF-06 (N=200). Egg mass depositions indicated by filled symbols (2000), diapause indicated by horizontal arrows.

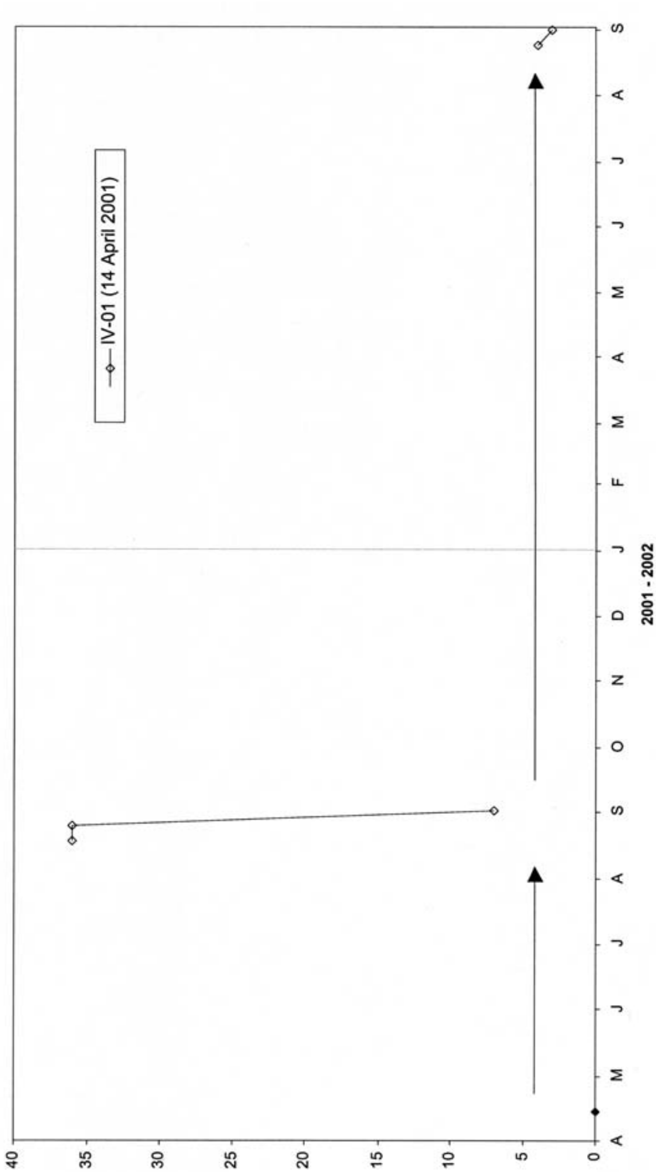

FIG. 6. Hatching for one *I. varians* egg mass from 1 female collected at Big Otter River, VA. Egg mass total before hatching was IV-01 (N=94). Egg mass deposition (14 April 2001) indicated by filled symbol, diapause indicted by horizontal arrows.

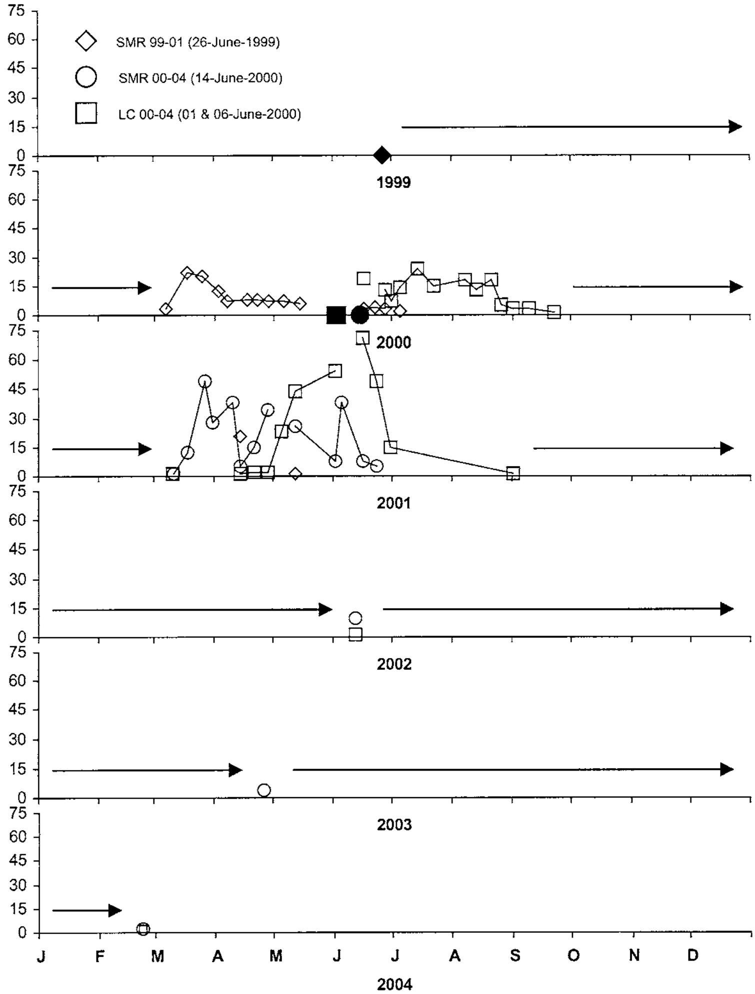

FIG. 7. Hatching for two *I. zionensis* egg masses from 2 females collected from San Miguel River (SMR), and two egg masses (treated as one) from one female collected at Leopard Cr. (LC), CO. Egg mass totals before hatching were SMR 99–01 (N=368), SMR 00–04 (N=733), and LC 00–04 (N=1092). Egg mass depositions indicated by filled symbols, diapause indicated by horizontal arrows.