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# HATCHING SUCCESS OF EGGS OF *HEXAGENIA BILINEATA* (EPHEMEROPTERA) EXPOSED TO BRIEF THERMAL SHOCK

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**Abstract**—1. Hatching of *Hexagenia bilineata* eggs was significantly reduced after brief exposure (5–15 min) to above-ambient temperature (≥33°C) during oviposition.

2. Comparison of eggs shocked during oviposition with eggs shocked 2 h afterwards indicates that the gametes or some step in the fertilization process may be affected.

3. Survival of nymphs hatching from eggs exposed to 43°C for 10 min during oviposition was significantly lowered, pointing to a latent effect of increased temperature.

## INTRODUCTION

UPPER limits of thermal tolerance for aquatic insects are based mainly upon relatively long-term, constant temperature tests using larvae or nymphs (see Talmage & Coutant, 1978, 1979, 1980, for recent reviews), although a few studies have used eggs (Friesen *et al.*, 1979; Rivard *et al.*, 1975; Sawchyn & Gillott, 1974; Tennessen & Miller, 1978; Trpis *et al.*, 1973; Tsui & Peters, 1974). The wide range of upper tolerance values undoubtedly reflects the variation in the thermal environments from which the test species were taken. Because the different life stages of a single species may differ in sensitivity to temperature, the most sensitive stage must be determined in order to establish protective limits in areas of waste-heat discharge.

One of the aims in our study of the burrowing mayfly *Hexagenia bilineata* (Say) was to determine whether brief exposure to above-ambient temperatures, similar to what eggs would experience during oviposition in a thermal plume and subsequent sinking to an ambient lake bottom, would affect hatching success. Because fertilization of insect eggs apparently occurs during oviposition (Chapman, 1971), sperm may also be exposed to temperature changes. Therefore, eggs exposed to heated water during oviposition were compared with eggs exposed 2 h after oviposition. The latter treatment ensured that the eggs were fertilized and that sperm were not directly exposed. In addition, sperm were examined for motility following various temperature–time exposures. Finally, nymphs hatching from the temperature treatments were maintained in small laboratory cultures for observation of possible post-treatment mortality.

## MATERIALS AND METHODS

### *Eggs*

Adult females of *H. bilineata* were collected between 2100 and 2200 h central daylight time on June 30, 1980, as they were attracted to a u.v. light placed along the shore of Second Creek embayment in Waterloo, Lauderdale County, Alabama. This area does not receive heated-water discharge; water temperature was 28°C.

For thermal shock during oviposition (DO), approx. 100 females were simultaneously placed in each of four insulated glass dishes (19 cm diam. × 10 cm deep) containing dechlorinated tapwater at 28 (ambient), 33, 38 and 43°C (ΔTs of 0, 5, 10 and 15°C). The floating females were removed in 3 min, as a sufficient number of eggs had been collected. Eggs were pipetted to smaller dishes containing ambient water after three timed exposures of 5, 10 and 15 min. This procedure comprised 12 treatments (4 temperatures × 3 durations). The dishes were then transported to the laboratory where eggs were transferred from each treatment to Plexiglass, flow-through containers. Approximately 1000–2000 eggs were placed in each of six containers per treatment (total of 72 containers). The containers were then placed in aquaria maintained at 28 ± 0.5°C in incubators; photoperiod was 14L:10D. On the following day, when the eggs had adhered to the bottoms of the containers, the aquaria were lightly aerated. During the 12-day incubation period, the positions of the containers within each aquarium were interchanged to minimize possible local differences in temperature, DO and light intensity.

For thermal shock after oviposition (AO), 300–400 females were placed in a large glass dish containing dechlorinated tapwater at 28°C. After oviposition, the females were discarded and the eggs were transported to the laboratory. Two hours later, the eggs were exposed to treatments identical to those in the above DO procedure, after which 72 replicates were set up and handled in the same manner. The 3-factor design was 2 shock times × 4 temperatures × 3 durations = 24 treatments; 6 replicates per treatment totalled 144 replicates.

On the 12th day after treatment, the number of eggs hatched in a subsample of 500 eggs per replicate was counted and transformed to a percentage. The data were analysed by a 3-factor analysis of variance.

Because parthenogenesis could mask effects in the above experiment, eggs were removed from subimagoes and subjected to: (1) mild heat (35°C for 10 min); (2) cold (10°C for 10 min); and (3) stirring (28°C for 3 min). The eggs were held at 26–28°C for 2 weeks, after which the percentage of hatching was determined by subsampling 500 eggs in each of 16 replicates.

*Sperm*

Adult males were collected July 24, 1980, at Shoal Creek embayment, Lauderdale County, Alabama. In the laboratory, the seminal vesicles were removed and placed on a glass slide. A cover slip was pressed down to liberate the spermatozoa, and a drop of dechlorinated tapwater was added to the edge of the cover slip. Each slide was examined immediately under × 200 magnification for motile spermatozoa. If spermatozoa were swimming, the slide was placed on a slide warmer set at either 28, 33, 38 or 43°C and left for 5, 10, 15, 20 or 25 min. After these timed exposures, the spermatozoa were again examined for motility. This procedure was repeated on three individuals at each temperature–time combination.

*Newly-hatched nymphs*

Nymphs hatching from eggs subjected to four of the treatment combinations during oviposition (28°C–15 min; 33°C–15 min; 38°C–15 min; and 43°C–10 min) were placed in glass dishes measuring 9 cm diam. × 5 cm deep and containing approx. 0.7 cm of mud and 3 cm of water. There were 25 nymphs per dish and 10 dishes per treatment. There were too few hatchlings in the 43°C–15 min treatment to facilitate their use. Approximately 0.01 g of powdered food consisting of 90% hog chow plus 10% liver was added to the dishes 2 days prior to introducing the nymphs. All dishes were covered but were equipped with small holes for gas exchange. Although no forced aeration was provided, dissolved oxygen concentrations did not fall below 7 ppm throughout the 30-day growth period.

After 30 days the surviving nymphs were removed and counted, and percentage of survival calculated; head capsule widths were measured as an indicator of growth.

## RESULTS

*Egg hatching*

Percentage of hatching decreased as experimental temperature and duration of exposure increased (Table 1). The main effect of temperature was a slight decrease in hatching up to 38°C and a very sudden, large decrease at 43°C. As shock duration increased, the percentage of hatching decreased only slightly overall. Eggs exposed during oviposition hatched at a significantly lower rate than eggs exposed 2 h after oviposition. The interaction between temperature and time of shock showed a dramatic difference in hatching success as temperature increased (Fig. 1). This result indicates that above-ambient temperature affected the gametes or some step in the fertilization process. A closer look at the mean percentages at 43°C (Table 1) shows a marked decrease in the DO treatments but not the AO treatments with increased duration. These results show that longer exposures to above-ambient temperatures during oviposition added to the detrimental effect of temperature and that eggs were more resistant to heat after the fertilization process was complete.

The sinking rate of eggs in still water in a large graduated cylinder was determined to vary from 0.36 to 0.43 cm $s^{-1}$ at 30°C. This rate is much slower than the 25 cm $s^{-1}$ reported by Fremling (1973). Using the former rate, we estimated that eggs could sink to a depth of 3 m in 11.5–14 min. However, wave action, current and turbidity probably keep eggs suspended longer, increasing the time they would be exposed to a thermal plume. Eggs laid in thermal plumes confined to the upper 1 m would be exposed to warm water for 4–5 min.

*Parthenogenesis*

If *H. bilineata* were parthenogenetic, the percentages of hatching in Table 1 could be a composite from fertilized and unfertilized eggs, making differences due to effects on sperm indiscernible.

Although Fremling (1967) was unsuccessful in attempts to hatch eggs of *H. bilineata* parthenogenetically, our experiment yielded a low mean percentage of hatching in unfertilized eggs [(1) mild heat: mean 0.41% (SE 0.08); (2) cold: 0.31% (SE 0.06); (3)

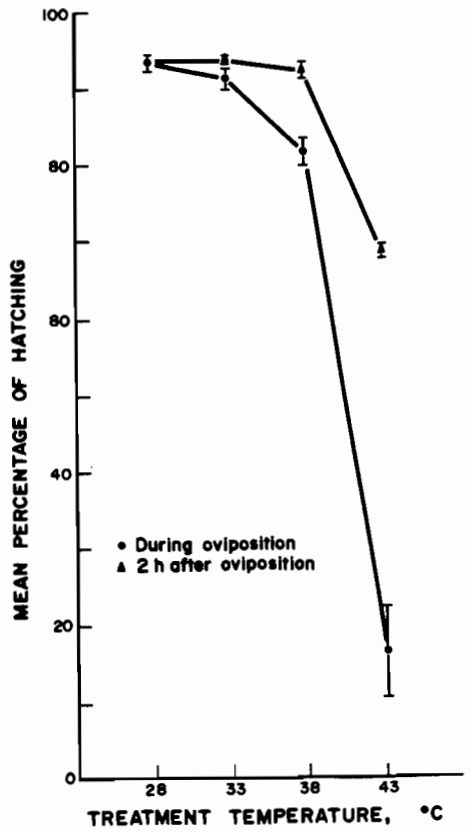


Fig. 1. Effect of brief thermal shock during and after oviposition on hatching success of eggs of *H. bilineata*. Vertical lines indicate 95% confidence limits.

Table 1. Mean percentages of hatching (±SE) in eggs of *H. bilineata* exposed to brief thermal shocks during (DO) and after (AO) oviposition

| Treatment temperature (°C) | Corresponding $\Delta T$ | Duration (min) | Mean percentage of hatching | |
|---|---|---|---|---|
| | | | DO | AO |
| 28 (ambient) | 0 | 5 | 93.3 ± 0.51 | 93.6 ± 0.61 |
| | | 10 | 93.8 ± 0.85 | 93.5 ± 0.97 |
| | | 15 | 93.0 ± 0.63 | 93.7 ± 0.24 |
| 33 | 5 | 5 | 94.1 ± 0.59 | 93.7 ± 0.38 |
| | | 10 | 91.3 ± 0.91 | 93.6 ± 0.33 |
| | | 15 | 88.2 ± 0.56 | 94.0 ± 0.20 |
| 38 | 10 | 5 | 83.5 ± 0.83 | 94.3 ± 0.38 |
| | | 10 | 82.0 ± 0.91 | 92.6 ± 0.43 |
| | | 15 | 79.6 ± 2.00 | 89.8 ± 0.43 |
| 43 | 15 | 5 | 32.0 ± 0.65 | 68.3 ± 0.41 |
| | | 10 | 10.3 ± 1.03 | 68.4 ± 1.04 |
| | | 15 | 6.7 ± 1.17 | 69.1 ± 0.82 |

| Main effect | Mean percentage* |
|---|---|
| Temperature (°C) | |
| 28 | 93.5 ± 0.33a |
| 33 | 92.5 ± 0.33b |
| 38 | 87.0 ± 0.33c |
| 43 | 42.5 ± 0.33d |
| Duration (min) | |
| 5 | 81.6 ± 0.29a |
| 10 | 78.2 ± 0.29b |
| 15 | 76.8 ± 0.29c |
| Shock time | |
| DO | 70.7 ± 0.24a |
| AO | 87.0 ± 0.24b |

* Means followed by different letters are significantly different (95% least significant difference test).

stirring: 0.68% (SE 0.09)]. Hatching percentage may increase with longer development times (Paul H. Carlson, personal communication), but parthenogenetic development probably contributed an insignificant amount to the hatching percentages reported in Table 1.

*Sperm motility*

Sperm survived temperatures up to 38°C for 20 min, beyond which motility was greatly or totally impaired (Table 2). Some sperm were motile after 5–10 min at 43°C, but no movement was observed in exposures longer than 10 min. The trend of these data showing reduction in response with increasing exposure coincides with the hatching success of eggs given the same temperature–time treatment combinations during oviposition. However, a significant reduction in hatching percentage occurred at the 38°C treatments (Fig. 1), but sperm were still motile after 5–15 min at 38°C. Motile sperm in two out of three trials at 43°C for 5 min does not coincide with the low percentage of hatching (32%) in the 43°C–5-min treat-

Table 2. Number of trials out of three in which sperm of *H. bilineata* were motile (M) or non-motile (N) after exposure to various temperature treatments

| Time (min) | Temperature (°C) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 28 | | 33 | | 38 | | 43 | |
| | M | N | M | N | M | N | M | N |
| 5 | 3 | | 3 | | 3 | | 2 | 1 |
| 10 | 3 | | 3 | | 3 | | 1 | 2 |
| 15 | 3 | | 3 | | 3 | | | 3 |
| 20 | 3 | | 3 | | 2 | 1 | | 3 |
| 25 | 3 | | 3 | | | 3 | | |

Table 3. Percentage of *H. bilineata* nymphs surviving 30 days after hatching from eggs subjected to brief thermal shocks during oviposition (incubation temperature was 28 ± 1°C)

| | Treatment combination in egg stage | | | |
|---|---|---|---|---|
| | 28°C–15 min | 33°C–15 min | 38°C–15 min | 43°C–10 min |
| Mean* | 70.8a | 80.4a | 66.5a | 8.0b |
| SE | 5.06 | 2.76 | 7.85 | 0.84 |
| Range | 36–92 | 72–92 | 36–96 | 4–12 |
| *N* | 10 | 7† | 8† | 10 |

* Means followed by different letter are significantly different based on Scheffe test.

† Several replicate dishes were lost in laboratory handling.

Table 4. Mean head widths of newly-hatched and 30-day-old nymphs of *H. bilineata* hatching from eggs subjected to four temperature–time treatment combinations during oviposition

| | Temperature–time treatment during oviposition | | | |
|---|---|---|---|---|
| | 28°C–15 min | 33°C–15 min | 38°C–15 min | 43°C–10 min |
| | | Newly hatched | | |
| Mean (mm) | 0.1380 | 0.1377 | 0.1376 | 0.1371 |
| SE | 0.0004 | 0.0003 | 0.0003 | 0.0004 |
| *N* | 300 | 300 | 300 | 300 |
| Range (mm) | 0.123–0.160 | 0.123–0.160 | 0.123–0.160 | 0.116–0.160 |
| | | 30 Days | | |
| Grand mean (mm)* | 0.3225a | 0.3350a | 0.3166a | 0.4357b |
| SE | 0.0080 | 0.0114 | 0.0137 | 0.0233 |
| 95% confidence limits | 0.304–0.341 | 0.307–0.363 | 0.284–0.349 | 0.383–0.488 |
| *N* | 177 | 147 | 133 | 20 |

* Means followed by different letter are significantly different based on Scheffe test.

ment. There may be a wide range of variability among males in sperm motility requiring greater replication than used here. It is also possible that motility is not a good indicator of sperm viability or its ability to penetrate and fertilize eggs.

*Post-treatment survival and growth of nymphs*

Applying the Scheffe test (Wilcox *et al.*, 1979) to the mean percentages of nymphs surviving the 30-day post-treatment period (Table 3) showed that the mean for the 43°C–10-min treatment was significantly different ($P < 0.05$) from the other three means. Therefore, even though embryos developed and hatched, the brief exposure to this high temperature during oviposition must have impaired some function or pathway vital for early nymphal life.

Mean head capsule widths of newly hatched nymphs (Table 4) did not differ significantly among the four treatments ($P = 0.2$). However, at the end of the 30-day post-treatment period, the relatively few surviving nymphs in the 43°C–10-min post-treatment dishes were significantly larger (Scheffe test) on the average than those from the other post-treatments (Table 4).

## DISCUSSION

The reduction in hatching of eggs exposed to very brief thermal shock during oviposition demonstrates a greater temperature sensitivity than is apparent from tolerance tests on nymphs (Tennessen & Miller, 1982). Our data indicate that a 2–5% reduction in hatching can be expected at a water temperature of 33°C, depending on exposure time (water depth). Above 33°C, more drastic reductions may be expected; interpolating from Fig. 1, a 50% reduction in mean hatching would occur at 40.5°C.

The duration of exposure for eggs laid in a thermal plume depends on the depth to which the plume extends, and can be estimated using the egg-sinking rate. Increased brief exposure, temperature-wise and/or time-wise, will decrease hatching success. If the thermal plume extends to the lake bottom, hatching success can be predicted from constant temperature experiments, although different populations may vary slightly in response (Tennessen & Miller, 1978; Wright *et al.*, in preparation).

Sperm may not be as tolerant as the test on motility indicates (Table 2); the lower hatching rates of eggs exposed to ≥33°C could be the result of fertilization failure or impairment of zygote development. Another possibility is that above-ambient temperature causes some change in the egg which interferes with fertilization. Embryological studies are needed to determine the point at which the effect is manifested. Eggs are more tolerant of temperature increases after fertilization is completed. Therefore, eggs laid in

ambient water may drift into thermal plumes as warm as 38°C for brief periods without a significant reduction in hatching (Table 1, AO).

The latent effect of brief shock in the egg stage on subsequent nymph survival was not significant below the extreme pretreatment of 43°C. Thermal discharges in the Tennessee River–Reservoir system were not found to exceed 35°C at the water surface during the warmest part of the year (Tennessen & Miller, 1978, 1982). Therefore, nymphs hatching from eggs sinking through such thermal plumes probably do not experience significant latent mortality due to heat.

The greater size of the few surviving nymphs from the 43°C pre-treatment is probably due to lowered density, although slower-growing, smaller individuals may have been selected against by the heat. The experimental design was not adequate to separate the effects of the two variables, pre-treatment temperature and ultimate density. Further studies on nymphs should look for increased tolerance due to predisposition to heat in the egg stage. If increased tolerance is found, changes in protein synthesis may be detectable using electrophoretic techniques (Vincent & Tanguay, 1979). Results may be useful in understanding the dynamics of large mayfly populations found in some thermal discharge areas.

This study was an attempt to simulate the exposure of newly-oviposited eggs to surface thermal discharges and has shown that delayed lethal effects can occur from brief exposure to temperature increases.

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*Key Word Index*—Egg; Ephemeroptera; Ephemeridae; insect; heat shock; *Hexagenia bilineata*; latent effect; mayfly; nymph; parthenogenesis; sperm; thermal tolerance.