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Effect of Fluctuating Temperature on the Duration of Embryonic Development in Two *Ecdyonurus* spp. and *Rhithrogena cf. hybrida* (Ephemeroptera) from Austrian Streams

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Summary. Eggs of *Ecdyonurus picteti* from the Herrnalmbach and Seebach, and *E. venosus* and *Rhithrogena cf. hybrida* from the Seebach were fertilized artificially and kept at fluctuating temperatures (range 2.8°–18.1° C) in the laboratory. The percentage of eggs that hatched at each sinusoidal temperature cycle ranged from 0 to 49% and values were similar to those obtained for eggs reared under constant temperature conditions. The hatching time (days after fertilization for 10%, 50% and 90% of the eggs to hatch) decreased with increasing temperature and the relationship between the two variables was well described by a power-law within the range 2.8°–18.1° C for *E. venosus*. A similar relationship has been found for the effect of constant temperature on the hatching time of eggs of *E. venosus*. It appears that the effect of temperature on the rate of change in the hatching time and the rate of development is approximately similar for both constant and fluctuating temperatures.

Introduction

The duration of embryonic development of Ephemeroptera appears to be affected significantly only by temperature (Elliott and Humpesch 1980). The relationship between hatching time (days after oviposition or fertilization) and constant water temperature for *Ecdyonurus picteti* Meyer-Dür, *E. venosus* (Fabr.) and *Rhithrogena cf. hybrida* Etn. was well described by a power-law over the range of natural temperatures that eggs are likely to encounter in the course of their development (Humpesch 1980; Humpesch and Elliott 1980). As the temperatures that eggs encounter in the field are mostly fluctuating and rarely constant, estimates of hatching time from experiments at different constant temperatures may not be applicable when temperatures fluctuate (Humpesch 1980; Humpesch and Elliott 1980). Although there are several studies on the duration of development of poikilothermic organisms under conditions of fluctuating temperature (references in Keen and Parker 1979, Hoffmann 1980, Remmert 1980a, b), there is a paucity of similar information in aquatic exopterygote insects such as Ephemeroptera.

The purpose of the present study was therefore to examine the effect of fluctuating temperatures on the hatching time and to test whether the estimated values for the duration of the embryonic development at different constant temperatures are also applicable at fluctuating temperatures. The aim is to provide predictive relationships that can be applied to field populations, where cohorts cannot be easily distinguished and it is therefore difficult or impossible to obtain life-table information.

Methods

Eggs of *Ecdyonurus* spp. and *R. cf. hybrida* were obtained from females reared from mature larvae, then fertilized artificially. The laboratory experiments were performed in incubators with temperature patterns that simulated those occurring naturally in the field (e.g. Fig. 1). The arithmetic mean temperatures were 5.7° C (range 2.8°–8.5° C), 9.4° C (range 6.4°–11.8° C), 10.6° C (range 8.1°–13.1° C), 11.3° C (8.8°–13.8° C) and 14.3° C (10.5°–18.1° C) (e.g. Fig. 1).

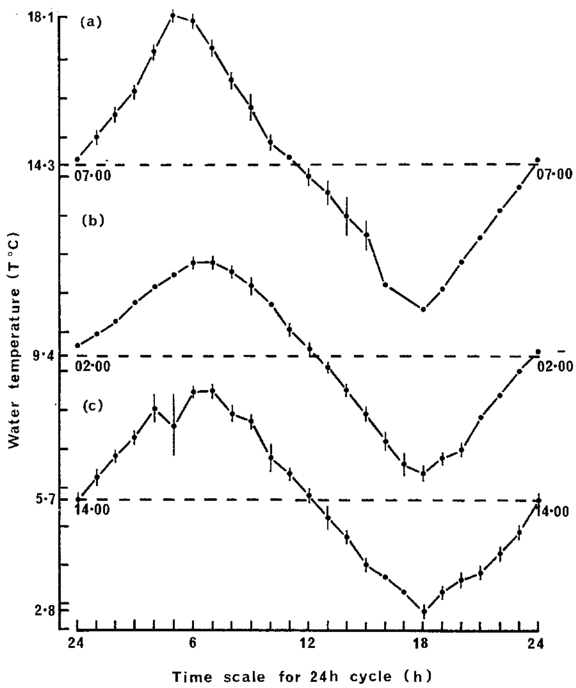

Fig. 1. Examples of the 24-h sinusoidal temperature cycles (T° C) in the laboratory: (*a*) 10.5°–18.1° C (average 14.3° C), (*b*) 6.4°–11.8° C (average 9.4° C), (*c*) 2.8°–8.5° C (average 5.7° C); each *point* is the arithmetic mean and 95% confidence limits for the mean temperature; *broken line* indicates the arithmetic mean temperature with the starting time of each temperature cycle

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Table 1. Summary of the following information on experiments with fertilized eggs of *Ecdyonurus* spp. and *R. cf. hybrida* in the laboratory: species and locality where collected, if experiments were successful (some eggs hatched), number of experiments (*n*), arithmetic mean temperature (T °C) at which the experiments were performed

Species	Locality	Experiments	*n*	Mean T °C (and *n* if >1)
E. picteti	Herrn-almbach	Unsuccessful	3	5.7 (2), 9.4
		Successful	3	9.4 (2), 14.3
	Seebach	Unsuccessful	3	5.7, 9.4, 11.3
		Successful	6	5.7 (2), 9.4, 10.6, 11.3 (2)
E. venosus	Seebach	Unsuccessful	12	5.7 (2), 9.4 (4), 10.6, 11.3 (4), 14.3
		Successful	15	5.7 (6), 9.4 (2), 10.6 (2), 11.3 (2), 14.3 (3)
R. cf. hybrida	Seebach	Unsuccessful	6	5.7 (2), 9.4, 10.6, 14.3 (2)
		Successful	3	5.7, 9.4, 10.6

Eggs from one female were either all used in one experiment or divided into batches, each of which was placed in a different incubator. Therefore the number of eggs per experiment given in the Appendix table does not represent the number of eggs per female.

The experimental techniques for rearing eggs are described in detail by Humpesch (1980). A number of experiments were unsuccessful with no eggs hatching (see Table 1), and details of the successful experiments are given in the Appendix table. Information on the localities is given by Humpesch (1980) and Humpesch and Elliott (1980).

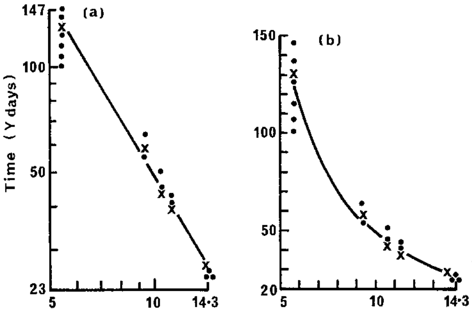

Fig. 2a, b. Relationship between the time required (Y days) for 10% of the eggs of *Ecdyonurus venosus* to hatch and the arithmetic mean of fluctuating temperature (T °C) in the laboratory. **a** On log/log scale with linear regression line. **b** On arithmetic scale with curvilinear regression line. *Crosses:* hatching time for eggs at constant temperatures (from Humpesch 1980)

Results

The number of eggs used at each temperature varied considerably in the range 206–4,583 (see Appendix table). The percentage of eggs that hatched at each temperature ranged from 0 to 49% and this range was similar to that obtained for eggs reared under constant temperature conditions (cf. values in Humpesch 1980; Humpesch and Elliott 1980). As only a few experiments were successful for *E. picteti* and *R. cf. hybrida* (Table 1), these species were not used for the subsequent analyses. Although there were only a few results for *E. venosus,* an attempt was made to analyse the data as described by Humpesch (1980). The relationship between time required (Y days after the start of the experiment) for 10%, 50% and 90% of the eggs to hatch and water temperature (T °C) over arithmetic mean temperatures in the range 5.7°–14.3° C (overall range 2.8°–18.1° C) was found to be curvilinear on an arithmetic scale and linear on a logarithmic scale (Fig. 2). Therefore the relationship between hatching time (Y days) and arithmetic mean water temperature (T °C) was given by the regression equation

Table 2. Comparison of days required for 10%, 50% and 90% of eggs to hatch: values for eggs kept at fluctuating temperatures are either estimated (as 95% confiderence limits for mean) from the equation given for *E. venosus*, or single values for other species; values for eggs kept at constant temperatures are estimated (as 95% confidence limits for mean) from equations in Humpesch (1980) and Humpesch and Elliott (1980); T °C is the arithmetic mean of the fluctuating temperature

Species	Locality	T °C	Days required for hatching					
			(a) at fluctuating temperatures			(b) at constant temperatures		
			10%	50%	90%	10%	50%	90%
E. picteti	Herrnalmbach	9.4	60	62	65	56–62	58–65	63–71
		14.3	28	29	34	30–34	31–35	33–39
	Seebach	5.7	80	98	125	86–93	91–98	94–104
		9.4	45	46	47	42–45	44–46	47–49
		10.6	38	40	45	35–37	37–39	39–42
		11.3	34	35	55	32–34	33–35	35–38
E. venosus	Seebach	6.0	103–123	126–146	134–156	115–127	123–135	132–152
		10.0	46–52	51–57	56–63	51–54	53–56	58–64
		14.0	25–31	27–32	30–36	29–31	30–32	33–37
R. cf. hybrida	Seebach	5.7	79	81	98	79–91	83–93	83–107
		9.4	46	47	49	41–45	43–47	46–55
		10.6	39	40	43	35–38	37–40	40–47

Table 3. Comparison of days (given as a range) required for 10%, 50% and 90% of eggs hatching in single experiments in the field and estimated values (given as 95% confidence limits) obtained from equations in Humpesch (1980) and Humpesch and Elliott (1980); T °C is the arithmetic mean ±S.E. and range of the water temperature in the field

Species	Locality	Month	T °C		Days required for hatching					
			Mean±S.E.	Range	(a) in field			(b) in laboratory (at constant temperature)		
					10%	50%	90%	10%	50%	90%
E. picteti	Seebach	May	8.2±0.02	6.2−10.2	52–53	54–55	56–57	51–55	53–57	56–60
R. cf. hybrida	Seebach	June	8.3±0.02	6.6−10.5	51–53	55–56	57–58	48–53	51–56	54–65

$$Y = a\,T^{-b} \qquad (1\,a)$$

or in logarithmic form

$$\log_e Y = \log_e a - b \log_e T \qquad (1\,b)$$

where a and b are constants. The values of the constants (with 95% confidence limits) were $a = 2{,}093.13 \times 1.44$ and $b = 1.63 \pm 0.17$ for 10% hatched; $a = 3{,}491.67 \times 1.39$ and $b = 1.81 \pm 0.15$ for 50% hatched; and $a = 3{,}292.13 \times 1.41$ and $b = 1.75 \pm 0.16$ for 90% hatched. The regressions were a good fit to the data and F-values from the variance ratio were highly significant ($P < 0.01$). The proportion (r^2) of the variance of Y due to the regression of Y on T was always $\geqq 0.97$. Therefore, at least 97% of the variability in the time required for hatching was accounted for by variations in temperature, which was clearly the major factor affecting the time required for hatching in the laboratory. Therefore the hatching time was apparently unaffected by variations in the time of year fertilization occurred.

Estimates were made of the actual number of days required for 10%, 50% and 90% of the eggs to hatch at 6°, 10° and 14° C (Table 2) and these show clearly the difference in the hatching times in the range 6°–14° C.

Discussion

Various attempts have been made to determine the effect of fluctuating temperatures on the duration of the embryonic development of insects (Hoffmann 1974; Neumann and Heimbach 1975; Welbers 1975; references in Howe 1967), but this information is not available for aquatic exopterygote insects such as Ephemeroptera.

In this study, the laboratory experiments were deliberately performed only in the same temperature range as that used in all the experiments at constant temperatures (Humpesch 1980; Humpesch and Elliott 1980), and with the temperature amplitude which eggs might encounter in the field (Humpesch 1978, 1979; Malicky 1978).

Less than 50% of eggs hatched in each experiment. This low hatching success was also obtained from eggs that hatched at constant temperatures in the laboratory (Humpesch 1980; Humpesch and Elliott 1980) and from eggs that hatched at fluctuating temperatures in the stream (Humpesch and Elliott 1980). Therefore hatching success was apparently unaffected by variations in temperature. It was originally suggested (Hunt 1951; Humpesch 1980) that the low hatching success values may be due to the artificial fertilization of eggs in the laboratory, but Humpesch and Elliott (1980) showed that hatching success values were similar for eggs fertilized naturally and artificially. Therefore, the low hatching success in these species probably also occurs in the field and must be taken into account in the interpretation of their life-cycle and population dynamics.

The relationship between the time required for hatching and the arithmetic mean of the fluctuating temperature has been examined for the first time in the present study, and has been well described by a power-law within the range of about 5.7°–14.3° C for *E. venosus*. A similar relationship has been found for the effect of constant temperature on the hatching time of eggs of *E. venosus*. A comparison of the values of constant a and b from the regression equations for 10%, 50% and 90% of eggs hatched at constant and at fluctuating temperatures shows that while the values of a and b from the regression equations for 10% and the values of a for 50% and 90% of eggs hatched at constant temperatures were not significantly different ($P > 0.05$) from those obtained at fluctuating temperatures, their b values for 50% and 90% of eggs hatching were just significantly different ($P < 0.05$). Despite these slight differences, confidence limits for estimates from the two equations overlapped considerably and were not significantly different (cf. values for *E. venosus* in Table 2). There were, however, some slight discrepancies for the species for which only single values were available (*E. picteti* and *R. cf. hybrida* in Table 2). Agreement was also found between the hatching times of eggs at fluctuating temperatures in the field and at constant temperatures in the laboratory (Table 3). Therefore, the regression equations calculated from the results at constant temperatures can probably be used to estimate the hatching time at fluctuating temperatures in both the laboratory and the field. Thus, the effect of temperature on the rate of change in the hatching time and the rate of development appears to be approximately similar for both constant and the fluctuating temperatures.

If predictions about hatching time in the natural habitat are to be made from laboratory experiments, two questions concerning temperature have to be considered: (a) how the pattern of fluctuating temperature should be summarized to evaluate its effect on the hatching time; and (b) whether or not fluctuating temperatures stimulate or inhibit the rate of development. The latter question cannot be answered unless the constant temperature equivalent of the fluctuating temperature can be determined. Both questions have recently been discussed in detail by Keen and Parker (1979). When their method of data analysis was applied to the data of this study a good agreement was found between these results and those presented in the earlier analysis. The latter results can be used to identify and separate cohorts in the field and are therefore an essential part of quantitative studies on the life cycle of Ephemeroptera.

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Appendix (successful experiments only)

Source of eggs (species and locality where collected) and experimental conditions for hatching in *Ecdyonurus* spp. and *Rhithrogena cf. hybrida:* water temperature (*T* °C), photoperiod (LL = continuous light, DD = continuous dark), number of experiments (*n*), number of eggs and percentage of eggs that hatched at each temperature, the period over which the eggs hatched (a dash indicates that no data are available)

Species	Locality	Month	*T* °C Mean	(range)	Photo-period	*n*	Number of eggs Mean	(range)	Hatched eggs Mean%	(range%)	Hatching period days)
E. picteti	Herrnalm-bach	Aug. 1976	9.4	(6.4–11.8)	LL	1	3,737	–	2.1	–	59–70
		Sept. 1977	9.4	(6.4–11.8)	LL	1	1,253	–	6.3	–	61–69
		Aug. 1976	14.3	(10.5–18.1)	LL	1	4,388	–	21.5	–	27–57
	Seebach	May 1977	5.7	(2.8–8.5)	LL	1	3,571	–	1.4	–	80–204
		July 1977	5.7	(2.8–8.5)	LL	1	–	–	–	–	110–202
		May 1977	9.4	(6.4–11.8)	LL	1	4,583	–	48.9	–	45–64
		June 1976	10.6	(8.1–13.1)	DD	1	4,303	–	9.5	–	38–55
		June 1975	11.3	(8.8–13.8)	LL	2	3,987	3,810–4,163	22.7	0.6–44.8	33–59
E. venosus	Seebach	June 1977	5.7	(2.8–8.5)	LL	2	2,293	1,940–2,646	7.7	1.7–13.6	96–180
		July 1977	5.7	(2.8–8.5)	LL	2	275	–	6.2	–	110–180
		Aug. 1977	5.7	(2.8–8.5)	LL	1	–	–	–	–	125–170
		Sept. 1977	5.7	(2.8–8.5)	LL	1	365	–	8.8	–	125–180
		Aug. 1977	9.4	(6.4–11.8)	LL	1	–	–	–	–	54–65
		Sept. 1977	9.4	(6.4–11.8)	LL	1	202	–	19.8	–	63–70
		July 1976	10.6	(8.1–13.1)	LL	2	2,030	1,377–2,682	15.2	4.4–25.9	45–66
		July 1975	11.3	(8.8–13.8)	LL	2	827	588–1,065	14.0	12.8–15.1	41–66
		July 1976	14.3	(10.5–18.1)	LL	2	1,611	1,363–1,858	0.8	0.6–1.0	26–36
		Sept. 1976	14.3	(10.5–18.1)	LL	1	1,276	–	11.1	–	24–34
R. cf. hybrida	Seebach	May 1977	5.7	(2.8–8.5)	LL	1	1,532	–	0.8	–	79–107
		May 1977	9.4	(6.4–11.8)	LL	1	1,938	–	9.9	–	45–58
		May 1976	10.6	(8.1–13.1)	LL	1	2,061	–	37.7	–	38–53

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