

## OXYGEN CONSUMPTION BY DEVELOPING AND DIAPAUSING EGGS OF *EUPHOLIDOPTERA SMYRNENSIS* (ORTHOPTERA: TETTIGONIIDAE)

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**Abstract**—Oxygen consumption by eggs of the Mediterranean bush-cricket *Eupholidoptera smyrnensis* was manometrically measured at 18, 24, 30 and 33°C. The aim was to study changes in oxygen consumption during embryonic development, and to compare a facultative initial and a facultative penultimate diapause with subitaneous developing eggs and an obligatory final diapause. In developing eggs, oxygen consumption increased as the embryo grew, but remained constant during katatrepsis. A comparison of the different diapauses revealed that in the initial diapause, oxygen consumption was lower than that of the corresponding embryonic stage in subitaneous eggs; the temperature dependence of oxygen consumption was also low. In contrast, in penultimate and in final diapause, oxygen consumption remained at the same high level as in developing embryos of the same size, and the temperature dependence of oxygen consumption was high. The results suggest that it is energetically profitable to the embryo to spend the hot season in a facultative initial and/or penultimate diapause.

**Key Word Index:** Oxygen consumption, embryogenesis, diapause, *Eupholidoptera smyrnensis*, Tettigoniidae

### INTRODUCTION

Most of the European Tettigoniidae have complex life cycles, due to a facultative or obligatory dormancy sequence in the egg stage. This sequence basically consists of an initial diapause after blastoderm formation, and a final diapause in the fully grown embryo. Central European species make use of the dormancy sequence for hibernations in successive years, while Mediterranean species, e.g. the Rhodian population of *Eupholidoptera smyrnensis*, can use it for aestivation and hibernation in an univoltine life cycle (Ingrisch, 1986b). On Rhodos, *E. smyrnensis* becomes adult in May. An initial diapause is induced either after oviposition at a photophase shorter than 14 h 10 min or when the eggs are incubated at 30°C. It is terminated either by aestivation (4 weeks at 30°C) or by hibernation. The most suitable temperature for early embryonic development is 24°C. At 24°C, it can proceed until final diapause. However, in about half of the embryos, it stops at the three-quarter stage, and further development requires temperatures below 20°C (Ingrisch, 1986a).

Comprehensive studies suggest that the complex life cycles of the European Tettigoniidae, including facultative summer diapauses, serve to synchronise embryogenesis with the seasons, and to prevent late embryonic stages (e.g. the final diapause stage) from occurring during the heat of summer. Therefore it was supposed that it is unfavourable to the embryo to stay during the summer in a late embryonic stage that is almost ready for hatching (Ingrisch, 1986a). The reason might be a too-high energy demand. Measurements of the oxygen consumption could help to solve this question. The present study investigates the changes of oxygen consumption during the course

of embryogenesis. Stress is laid on a comparison of the different diapauses to find out if it is energetically profitable to the embryo to spend the summer in early or median embryonic stages.

### MATERIALS AND METHODS

#### *Maintenance of the eggs*

The experiments were done with eggs of the eastern Mediterranean species *Eupholidoptera smyrnensis* from Rhodes. The insects were bred from the egg, either at light–dark 12:12 or 16:8. Thus, the females laid eggs with or without initial diapause, respectively. The eggs laid in sand were sieved out twice a week, and incubated in Petri dishes (93 mm dia) on moist filter paper at 24°C. Two different aspects were studied.

*Oxygen consumption of non-diapausing eggs.* Eggs developing without initial and without penultimate diapause need at least 11 weeks at 24°C to reach the final diapause (Ingrisch, 1986a). Thus, oxygen consumption was measured in weekly intervals up to 12 weeks after oviposition, using the same eggs at different temperatures, and partly the same eggs at different phases. A possible influence of the experimental conditions on development should have been very small, since temperature deviated only 5 (in some experiments 7.5) hours per week from the standard conditions of incubation (24°C), the mean temperature of the deviations being 24°C. Thus, the course and speed of development of the eggs used for the experiments did not deviate from that of eggs incubated at 24°C constantly.

The embryonic development of the Tettigoniidae can be divided into 25 stages, grouped into 7 phases

(Warne, 1972; Ingrisch, 1984). During the 12 weeks of the experiments, embryonic development proceeded as follows (see Ingrisch, 1986a):

- week 0–2: formation of blastoderm and embryonic primordium (embryonic stages [e.st.] 1–4),
- week 2–4: differentiation of embryonic primordium, anatrepsis, and early mesentrepes (e.st. 5–13),
- week 4–6: late mesentrepes (e.st. 13–14),
- week 6–8: katatrepsis (e.st. 15–18),
- week 8–10: early dorsal closure (e.st. 19–20),
- week 10–12: late dorsal closure, completion and final diapause (e.st. 21–23/24).

*Comparison of the oxygen consumption by the different diapause stages.* The diapause forms investigated occurred in the following embryonic stages: Initial diapause in stage 4 (blastoderm formation completed, embryonic primordium circular), penultimate diapause in stage 20 (the embryo measures 3/4 the length of the egg), final diapause in embryonic stage 23/24 (the embryo is fully grown).

Eggs in initial diapause had been laid at light–dark 12:12, those in penultimate and final diapause at light–dark 16:8. The eggs were incubated at 24°C until they definitely entered one of the above diapause stages. The embryonic stage was controlled by observation through the moistened chorion. Experiments with initial diapause eggs started 4 weeks, those with penultimate and final diapause eggs 13 weeks after oviposition. Oxygen consumption was measured in weekly intervals using partly the same eggs. In the meantime, they were stored at 24°C. Thus, oxygen consumption was measured prior to the effect of diapause-terminating stimuli. The last experiments were done when the eggs became 20-weeks old. Eggs in initial and in final diapause maintained diapause beyond that time. Part of the eggs in penultimate diapause continued to develop, as they also do when

incubated at 24°C constantly. They were excluded from the measurements.

#### *Measurement of oxygen consumption*

Oxygen consumption was manometrically measured by a Warburg-apparatus "V 166" from B. Braun, Melsungen, F.R.G. Samples of about 100 eggs were treated together to improve accuracy. One day before measurement, the eggs were placed on dry filter paper so that the egg weight remained constant during the experiments. Oxygen consumption was successively determined at 18, 24, and 30°C; additionally 33°C was used with young and with diapause eggs. Measurements at 33°C were added to get a better notion about the relations between temperature and oxygen consumption, since it proved that with young eggs at 18°C, oxygen consumption was just measurable. The same eggs were used at the different temperatures, the temperature was changed every 2.5 hours. 200  $\mu$ l of 0.25 M potassium hydroxide were filled in the central insert of each experimental chamber. Adaptation time at the beginning of the experiments and after a change of temperature was 30 min. At every temperature, the manometric differences were read off every 20 min during 2 h. Since oxygen consumption did not decline during the 2 h at the same temperature, an adaptation time of 30 min seemed to be enough in order not to measure the oxygen consumption of a stress phase. Oxygen consumption was calculated in  $\mu$ l per gram fresh weight and hour. All experiments with eggs of the same age were repeated 4–9 times using different eggs.

## RESULTS

### *Change of oxygen consumption with age*

As expected, oxygen consumption increased with

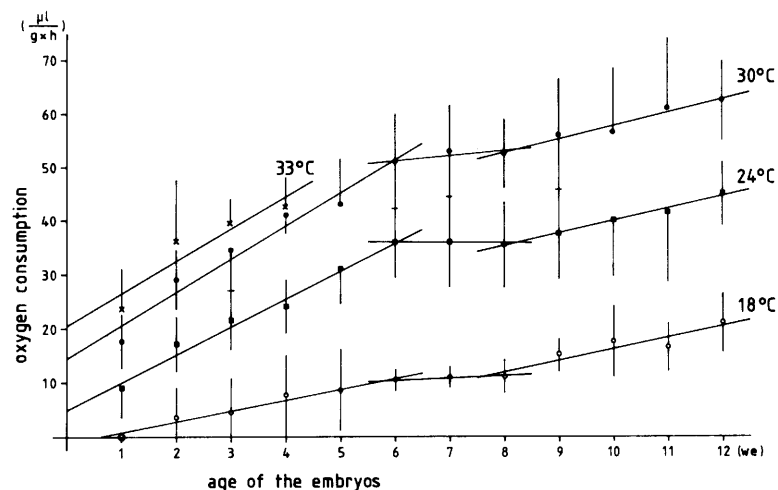


Fig. 1. Oxygen consumption by developing eggs of *Eupholidoptera smyrnensis* from oviposition until final diapause. The eggs were laid at light:dark 16:8 and incubated at 24°C; thus development was without initial diapause. Oxygen consumption was successively measured at 18, 24, 30 and 33°C. Each point represents the mean of 4–9 repetitions; vertical lines = standard deviations (should be read in both directions). Regression lines were calculated to show the relations between oxygen consumption and age (see Table 1). For regression analysis, the eggs were divided into 3 age classes: week 1–6 early development until katatrepsis, week 6–8 katatrepsis, week 8–12 development from katatrepsis until final diapause.

Table 1. Regression analysis of the increase in oxygen consumption with increasing age of the eggs

Age of eggs (weeks)	Temperature (°C)	Regression line $y = Ax + B$		Correlation coefficient ( $r$ )	Significance ( $P$ )	Number of measurements ( $n$ )
		A	B			
1-6	18	1.8	-0.5	0.47	0.01	45
1-6	24	3.8	9.5	0.69	0.001	45
1-6	30	6.0	15.3	0.81	0.001	45
6-8	18	0.5	7.5	0.16	n.s.	16
6-8	24	-0.05	36.3	-0.01	n.s.	26
6-8	30	0.9	45.5	0.09	n.s.	26
8-12	18	2.0	-3.8	0.49	0.01	39
8-12	24	2.2	17.5	0.31	0.05	42
8-12	30	2.5	32.8	0.32	0.05	42

Linear regression is used. The eggs are grouped into 3 are classes: 1-6 weeks (early development until katatrepsis), 6-8 weeks (katatrepsis), and 8-12 weeks (development after katatrepsis until final diapause). n.s.: not significant.

rising temperature. Oxygen consumption was also related to the age of the embryo (Fig. 1). Regression analysis showed that there are 3 different periods (Table 1): From oviposition until the 6th week and from the 8th to the 12th week, oxygen consumption increased, between the 6th and 8th week, the time of katatrepsis, it remained constant.

#### Comparison of the oxygen consumption by the different diapause stages

Oxygen consumption and temperature dependence of oxygen consumption differed between eggs in initial, penultimate, and final diapause (Fig. 2). Eggs in initial diapause consumed significantly less oxygen at the same temperatures than eggs in penultimate or in final diapause ( $P < 0.001$ ,  $t$ -test), those in penultimate diapause significantly less than in final diapause ( $P < 0.01$  at 18°C,  $P < 0.001$  at 24-33°C,  $t$ -test). The increase in oxygen consumption with a rise of temperature was least in initial diapause, about twice as much in penultimate and about three times as much in final diapause (Fig. 2).

Oxygen consumption of eggs in initial and in penultimate diapause was compared with that of non-diapause eggs of the same stage, that of eggs in

final diapause with eggs at the beginning of that stage (Table 2). The age of the corresponding non-diapause eggs was 2, 10 and 12 weeks, respectively. After those incubation times, the mean embryonic stage equalled that of the diapause stages. Only eggs in initial diapause consumed significantly less oxygen than subitaneous eggs. Oxygen consumption in initial diapause equalled that of the 1-week-old subitaneous eggs, in which blastoderm formation was not completed; minor differences are not significant ( $t$ -test). In contrast, in penultimate and in final diapause, oxygen consumption did not differ from that of the corresponding active stages significantly (Table 2).

#### DISCUSSION

The results with developing embryos of *Eupholidoptera smyrnensis* confirm the generally observed trend that the oxygen consumption by insect eggs increases with the size of the embryo (e.g. Rakshpal, 1962; Aiouaz *et al.*, 1978; Braune, 1976, Chapman, 1982). In contrast, the results with diapause differ from preceding investigations.

Usually, oxygen consumption by diapausing insects is lower than that by corresponding active stages. There are however considerable differences between species. Mostly, dormant stages of insects consume between 5 and 70% oxygen as the corresponding active stages do (Keister and Buck, 1964; Hayes *et al.*, 1968; Adamek and Fischer, 1985). Thus, the reduction of oxygen consumption is sometimes used as evidence for the incidence of a diapause (Modder, 1978, Gehrken, 1985). During the metamorphosis of a Lepidoptera chrysalis, oxygen consumption usually takes a U-shaped course (Lees, 1955). If it is interrupted by a diapause, oxygen consumption is less than half the minimum of a subitaneous metamorphosis, e.g. in *Platysamia ce-cropia* (Schneidermann and Williams, 1953). Pupae of flesh flies (*Sarcophaga*) in diapause consume even less than 1/10 the rate of comparable morphological stages not in diapause (Denlinger *et al.*, 1972).

With regard to egg diapauses, there is usually a strong increase in oxygen consumption during post-diapause development (see Burkholder, 1934; Boell, 1935; Wright, 1971; Braune, 1980). If compared with preceding stages, the differences are less pronounced. Oxygen consumption by eggs of the cricket *Teleogryllus emma* in diapause is about 1/4 of the maximal rate during prediapause (Izumiyama and Suzuki, 1986), in the Acridoidea *Melanoplus differentialis* and

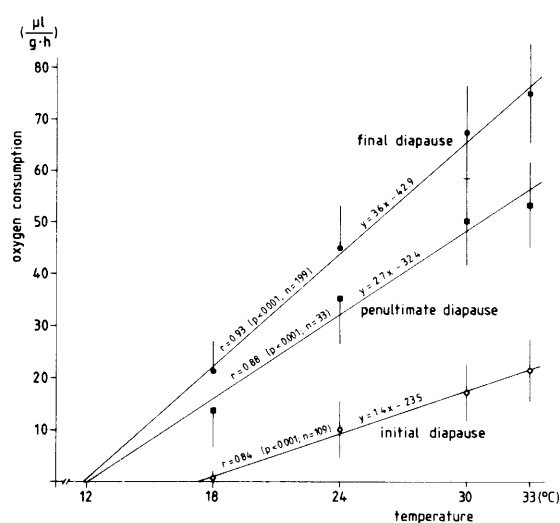


Fig. 2. Oxygen consumption by eggs of *Eupholidoptera smyrnensis* during diapause in different embryonic stages. Means and standard deviations (in both directions) are given. Regression lines show the relations between oxygen consumption and temperature.

Table 2. Comparison of the oxygen consumption by diapause and non-diapause eggs of the same embryonic stage.

Embryonic stage	Temperature (°C)	Oxygen consumption of:		t-test	
		developing eggs $\bar{X} \pm s (n)$	eggs in diapause $\bar{X} \pm s (n)$	t	P
4	18	3.5 ± 5.4 (7)	0.9 ± 1.5 (29)	2.33	0.05
4	24	16.8 ± 4.8 (7)	9.9 ± 5.4 (29)	3.09	0.01
4	30	28.8 ± 5.7 (7)	17.1 ± 5.6 (29)	4.94	0.001
4	33	36.0 ± 11.6 (4)	21.4 ± 6.0 (22)	3.86	0.001
20	18	15.1 ± 2.9 (8)	13.7 ± 7.1 (9)	0.52	n.s.
20	24	37.5 ± 8.7 (8)	35.3 ± 8.9 (9)	0.51	n.s.
20	30	56.1 ± 10.7 (8)	50.2 ± 8.5 (9)	1.27	n.s.
23/24	18	21.0 ± 5.3 (7)	21.2 ± 5.8 (54)	0.09	n.s.
23/24	24	45.0 ± 5.9 (7)	45.0 ± 8.2 (54)	0.01	n.s.
23/24	30	62.7 ± 7.7 (7)	67.4 ± 9.1 (54)	1.30	n.s.

Embryonic stage 4 = initial diapause, 20 = penultimate diapause, 23/34 = final diapause. The age of the corresponding non-diapause eggs was 2, 10 and 12 weeks respectively. The difference of the oxygen consumption by diapause and non-diapause eggs was tested by a t-test for significance; n.s. = not significant.

*Zonocerus variegatus* it is about half of the maximal rate, however higher than immediately after oviposition (Boell, 1935 and Modder, 1978). In eggs of another grasshopper, *Aulocara elliotti*, the rate of oxygen consumption during prediapause and diapause was identical (Roemhild, 1965). For a comparison with *E. smyrnensis*, we have to consider that in contrast to the Tettigoniidae, embryos of the Grylloidea and the Acridoidea diapause during late anatrepsis (e.g. Uvarov, 1966).

The results with *Eupholidoptera smyrnensis* reveal that in this species, oxygen consumption merely depends on the size of the embryo, not on the physiological state or on activity. It increases as the embryo grows, but remains at the same level during katrepsis, when there is no increase in size. Moreover, oxygen consumption is not lowered when the eggs enter penultimate or final diapause. An exception is the initial diapause. In this facultative diapause, oxygen consumption is lower than that of the corresponding active stage. However, one should consider that in non-diapause eggs, embryonic stage 4 (the diapause stage) is only a transient state of short duration. The higher oxygen consumption might be an expression of the continuing development. Oxygen consumption in initial diapause equals that of 1-week-old eggs. At this time, blastoderm formation is not completed, and the embryonic primordium not yet present.

The different diapauses during the embryogenesis of the Tettigoniidae are obviously not equivalent. Only the results with initial diapause confirm the expectation that oxygen consumption is lower in diapause than in developing stages. In contrast, oxygen consumption is not lowered when the embryo enters penultimate or final diapause. Since the final diapause occurs near the end of embryogenesis, oxygen consumption was the highest measured. It should, however, be investigated, if it further rises during post-diapause development and hatching.

The difference in oxygen consumption by eggs of *E. smyrnensis* in initial and in final diapause may be due to differences in demands for energy required for maintenance and to differences in function of the diapauses involved. The initial diapause is a static state. In *E. smyrnensis* it can be prolonged for up to 4 years, in other Tettigoniidae species even up to 7

years (Ingrisch, 1986c). During this period, the egg may experience extremes of temperature and drought. Since the temperature dependence of oxygen consumption is low, the egg needs very little energy, even at high temperatures. Thus, in initial diapause, the egg can maintain vitality for many years. In contrast, the final diapause is a dynamic state. In eggs of Tettigoniidae, the time required for post-diapause development and hatching depends largely on the length of the preceding exposure to cold (Ingrisch, 1985). Obviously, during final diapause, physiological changes take place in the egg. They are accompanied by changes in water uptake (own results, unpublished). Oxygen consumption remains at a high level, and the temperature dependence of oxygen consumption is high. Thus, the embryo would need a large amount of energy just for maintenance during high temperatures in summer. In contrast, at low winter temperatures, oxygen consumption becomes negligibly low. In the eastern Mediterranean species *E. smyrnensis*, the penultimate diapause can help the embryo to save energy in a warm autumn. The present results reveal that it is energetically profitable to the embryo to arrive at the final diapause stage only in late autumn when the temperatures have become low.

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