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THE VARIABLE EFFECTS OF AMBIENT AND ARTIFICIAL LIGHT:DARK CYCLES ON EMBRYONIC DIAPAUSE IN A LABORATORY POPULATION OF THE ANNUAL FISH *NOTHOBRANCHIUS GUENTHERI*

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SUMMARY

1. The effects of light:dark cycles (L:D) on embryonic development in a laboratory population of the East African annual fish *Nothobranchius guentheri* were studied.

2. Under ambient light conditions (40° N) there was a low frequency of embryos entering diapause between June and October. Beginning in November there was an increasing frequency of diapausing embryos with a peak in December, and a lower frequency by February.

3. Under artificial light conditions there was an increasing frequency of diapausing embryos as the L:D changed from 16:8 to 9:15.

4. When individual fish or groups of fish were followed it was found that, even under the same light conditions, variable frequencies of diapausing and non-diapausing embryos were produced and that the frequencies often changed with time.

5. The L:D cycle under which the embryos were incubated had no effect on diapause. As in some species of insects the 'diapause factor' was of maternal origin.

6. The ability of the fish to produce both diapausing and non-diapausing embryos under the same and variable L:D is most likely an adaptive trait related to the survival of the fish in the harsh environments of alternating rainy and dry seasons.

INTRODUCTION

Nothobranchius guentheri belongs to a group of cyprinodontid fishes known as 'annual fish'. These fishes are found in Africa and South America in temporary bodies of water which dry seasonally (Myers, 1942, 1952). The populations survive the dry

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season as embryos encased in the dry substrate. Embryos of annual fishes have been reported to undergo a developmental arrest, or diapause, at three specific stages of development (Peters, 1963; Wourms, 1964, 1972*b*, *c*; Markofsky & Matias, 1977*a*; Matias & Markofsky, 1978). Diapause I occurs immediately after epiboly (dispersed cell stage); Diapause II just prior to heart contraction (long somite stage); and Diapause III prior to hatching. We recently reported the effects of temperature, season of collection under ambient light conditions, and maternal influence on embryonic diapause in *N. guentheri* (Markofsky & Matias, 1977*a*, *b*; Markofsky & Matias, 1978). Although the seasonal data were limited, they indicated that during the winter months embryos incubated at 25 °C spontaneously entered Diapause II, while during the summer months they bypassed it.

The genus *Nothobranchius* has a distribution primarily along the coastal lowlands of East Africa from the southern portion of Somalia to the Natal region of the Republic of South Africa (Klee, 1965; Jubb, 1969; Scheel, 1975). One species is found in the Chad Republic and one in Zaire. *N. guentheri* is limited in its distribution to Tanzania and Kenya (Jubb, 1969; Bailey, 1972; Scheel, 1975). Although oxygen availability has been shown to be a possible regulatory factor of Diapause I (Peters, 1963), and temperature a regulatory factor of Diapauses I and II (Markofsky & Matias, 1977*a*; Matias & Markofsky, 1978), only for the duration of Diapause II have seasonally dependent variations been reported under constant incubation conditions (Markofsky & Matias, 1977*a*). In order to investigate a possible correlation between the cyclical phenomenon observed in the laboratory with the climatic events occurring in the natural environment, it was necessary to quantify and expand the original observations on the effects of ambient L:D cycles on the induction and duration of embryonic diapause in *N. guentheri*. Furthermore, *N. guentheri* is currently being considered for mosquito control in malarious areas of alternating rainy and dry seasons (WHO, 1973). The successful introduction of this larvivorous fish in nature will be partially dependent upon a relatively complete understanding of its developmental biology provided in part by studies such as these.

MATERIALS AND METHODS

All embryos used for study were obtained from a laboratory stock of the annual fish, *Nothobranchius guentheri* (Class Pisces, Family Cyprinodontidae). Details of the husbandry, spawning, and method of embryo collection have been reported (Markofsky & Perlmutter, 1972, 1973; Markofsky & Matias, 1977*a*; Matias & Markofsky, 1978). The fish were maintained in 9·5 or 19·0 l aquaria. Depending on experimental design, fish were housed as pairs or in varying densities up to 6–8 fish/tank. Preliminary studies indicated that the density of fish had no effect on the duration of Diapause II. It became evident quite early that embryonic development was markedly variable both between and within study populations and even for individual embryos derived from the same female. To ensure that control and experimental groups were maintained under the same conditions throughout the experiment, in all but a few instances embryos from many experimental groups were collected on the same day after uniform spawning episodes.

Ambient light conditions

Different populations of adults, all derived from our initial stocks, were spawned throughout the year between December 1973 and February 1978 at 23–25 °C, under ambient light conditions. At 40° N on 15 October, the L:D is approximately 11:13; on 15 November, 10:14; on 15 December, 9·5:14·5; 15 January, 9·5:14·5; 15 February, 11:13. On the longest day, 21 June, the L:D is approximately 15:9. The only data used were from eggs collected from one-day to two-week spawning episodes. The only fertilized eggs used were those at Diapause I or at earlier stages. These data, collected over a greater than four-year period, were often assembled from the controls of experiments designed for other purposes. After viewing the data retrospectively, it became apparent that there was a seasonal variation in the frequency of embryos entering Diapause II. Unfortunately data were not available for March, April and May. However, since the frequency of diapausing embryos was declining by February, it would not be unreasonable, in light of the available data, to project a continued decline into the spring months as the day length became increasingly longer.

Artificial light conditions

Artificial light conditions were maintained at either 16:8, 13:11, 11:13 or 9:15, in order to simulate the L:D range observed under ambient light conditions. The light (Gro-Lux, Sylvania Corp., USA) turned on at 0600. Although there were different light intensities in the room it was shown to have no effect on the duration of embryonic diapause. This is also true in insects where the threshold of sensitivity seems to lie just above the intensity of moonlight (Lees, 1955).

Adults were maintained under variable L:D conditions for variable lengths of time. When these studies were initiated neither the effects of prior exposure to different L:D cycles, nor the effects of constant exposure to one L:D cycle since birth, were known. Preliminary data indicated that these were in fact variables which could perturb the interpretation of the data but that a steady state, at an experimental L:D, was usually reached by 3–4 weeks. Since in most cases adults were maintained for at least 6–8 weeks under an experimental light regimen, the data can be considered to be representative. After steady state, the variability among and between individuals overshadowed the variability due to prior L:D history. Therefore, in order to present a general overview, the data in Tables 4 and 5 are presented as the combined results from all of the experiments. In Tables 6–8 an attempt was made to show representative experiments with specific photoperiod histories, since it was impracticable to report data from all of the individual experiments.

Treatment of the embryos post-collection

After collection (Day 0) the embryos were placed into 15 ml round-bottom test tubes, with an initial density of one embryo per 1 ml of aquarium water, with a usual density of ten embryos per tube. The tubes were capped with aluminium foil and stored at between 23 and 26 °C, under ambient light conditions. The post-spawning photoperiod had no effect on the duration of diapause or on the total time for development. Occasionally all of the embryos from a given tank were observed. Usually, however, a representative random sample of 20–30 embryos were observed if adequate

numbers were available. This allowed 2–3 tubes from a given tank to be followed for duplication of the results. The embryos were observed at weekly intervals and the stages of development and abnormalities recorded. When the embryos reached full development (Stage 43) they were placed into a general pool for hatching and other experiments. The embryonic stages of *Austrofundulus myersi*, a South American annual fish, were used for designating the stages of embryonic development, since they are similar to *N. guentheri* and have already been described (Wourms, 1972*a*).

Treatment of the data

The time course of development from each tank and each experiment was placed into a computer. Each experiment was coded for tank identification, temperature, time of year, L:D cycle and the frequency of the developmental stages at 5-day intervals. Since we had observed over 8000 embryos for this and other related studies, the computer allowed rapid sorting for data analysis. The computer was programmed so that if embryos died, they could be either eliminated from, or included in the denominator when the frequency distribution was calculated for the next time interval. Since there were no consistent differences in mortality rates among the groups, the data in the tables are presented excluding the dead embryos. The highest mortality occurred during the first 10 days and would not perturb interpretation of the data. All of the abnormal embryos were included in the data in the tables and their frequency distribution can be calculated as 100 minus the percentage of the population reaching Stage 43 in Tables 2 and 5. The types of abnormalities have previously been described (Markofsky & Matias, 1977*a*; Matias & Markofsky, 1978). In the figures, however, the denominator used to plot the frequency distribution was the total number of normal embryos reaching full development (Stage 43). This standardized the figures so that the abnormality frequencies, which were not constant among the experimental groups, would not perturb the interpretation of the graphically presented data. The durations of the interdiapausal stages were not given but were observed to be relatively constant and did not vary under any of the L:D cycles. They can be calculated from the tables and from previous publications (Markofsky & Matias, 1977*a*; Matias & Markofsky, 1978).

RESULTS

The frequency distributions with time of embryos at Diapause II (Stage 32) and at full development (Stage 43) under ambient light conditions at 23–25 °C, at different months during the year over the course of 4½ years, are found in Tables 1 and 2 and in Figs. 1 and 2. Very little Diapause II was observed between June and October, and no differences were observed during the entire year for the duration of Diapause I. However, beginning in November there was a spontaneous appearance of Diapause II, with a maximum amplitude and duration in December. By February, a maximum of 34% of the population entered Diapause II. The above data represent the sum of all experiments performed between December 1973 and February 1978. With a few exceptions the data were consistent from year to year. Since it was not practical to present all of the individual data, in Table 3 the data are presented for embryos collected at weekly intervals from one trio of fish (1 male, 3 females) followed

Table 1. *The frequency distribution of embryos at Stage* 32 (*Diapause II*) *of the annual fish* Nothobranchius guentheri *maintained under ambient light conditions*

Age (days)	Jan. (174)*	Feb. (238)	June (173)	July (78)	Aug. (173)	Sept. (44)	Oct. (143)	Nov. (208)	Dec. (208)
0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
6–10	6·0	3·6	7·2	0·0	1·5	0·0	6·0	2·9	8·6
11–15	59·7	28·7	26·4	25·6	32·8	15·2	11·5	54·0	58·4
16–20	61·8	34·0	6·8	5·8	19·4	0·0	4·6	57·1	61·3
21–25	52·8	30·3	1·1	5·8	2·2	0·0	5·3	50·0	71·7
26–30	45·5	29·8	0·0	1·2	1·7	—	0·7	43·0	72·9
31–35	40·3	27·9	—	0·0	0·0	—	0·0	38·3	64·0
36–40	23·9	19·5	—	—	—	—	—	32·9	58·1
41–45	18·8	16·4	—	—	—	—	—	28·9	31·5
46–50	12·6	7·8	—	—	—	—	—	17·8	8·5
51–55	2·3	7·0	—	—	—	—	—	13·3	3·7
56–60	0·0	4·1	—	—	—	—	—	10·2	0·4
61–65	—	2·1	—	—	—	—	—	4·0	0·4
66–70	—	0·0	—	—	—	—	—	0·0	0·0

* The numbers in the parentheses are the total number of embryos alive at the end of the study excluding those found to be abnormal. The percentage values include those found to be abnormal. If embryos died during the study the denominator was reduced by those numbers at the subsequent time point. In the figures, the abnormal embryos were eliminated when the frequency distributions were calculated.

Table 2. *The cumulative frequency distribution of embryos at Stage* 43 (*pre-hatching*) *of the annual fish* Nothobranchius guentheri *maintained under ambient light conditions*

Age (days)	Jan. (174)	Feb. (238)	June (173)	July (78)	Aug. (173)	Sept. (44)	Oct. (143)	Nov. (208)	Dec. (208)
0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
6–10	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
11–15	0·0	6·2	2·8	0·0	0·0	0·0	5·8	0·4	0·4
16–20	9·6	32·4	11·3	20·9	1·7	15·6	17·8	11·3	1·2
21–25	20·8	55·1	50·6	39·5	32·6	72·7	71·7	29·6	12·8
26–30	33·5	57·5	82·4	76·7	69·7	84·1	76·2	49·6	20·7
31–35	46·6	61·9	92·1	83·7	80·3	86·4	83·8	51·2	24·8
36–40	56·8	66·3	98·3*	90·7*	88·2	100·0	91·9	59·1	27·8
41–45	63·6	77·1	—	—	97·2*	—	96·6*	64·4	36·7
46–50	78·9	79·0	—	—	—	—	—	64·9	44·8
51–55	84·6	83·5	—	—	—	—	—	72·0	56·9
56–60	90·8	87·2	—	—	—	—	—	75·6	78·9
61–65	97·1	91·0	—	—	—	—	—	77·3	82·8
66–70	100·0*	95·9	—	—	—	—	—	84·9	84·4
71–75	—	95·9	—	—	—	—	—	92·4*	86·0*
76–80	—	96·7	—	—	—	—	—	—	—
81–85	—	97·9*	—	—	—	—	—	—	—

* In order to determine the above distribution the total number alive at the end of the study, including those that were abnormal, were included in the denominator. As a result the percentage abnormal is the difference between 100 and the final distribution (also see footnote, Table 1).

from 17 October 1977 to 7 February 1978. Little or no diapause was observed until 4 November, and the major onset of diapause occurred by 22 November. Almost always long-day controls were run simultaneously with those under ambient light conditions, and confirmed that the observed diapause was not due to unusual environmental conditions in the laboratory.

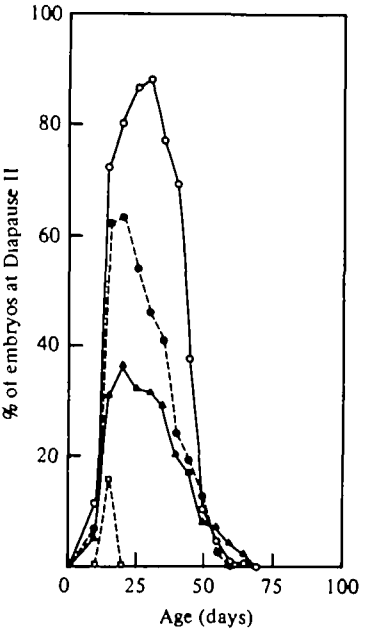

Fig. 1. The frequency distribution of embryos at Diapause II (Stage 32) under ambient light conditions during the year. ○, December; ●, January; △, February; □, September.

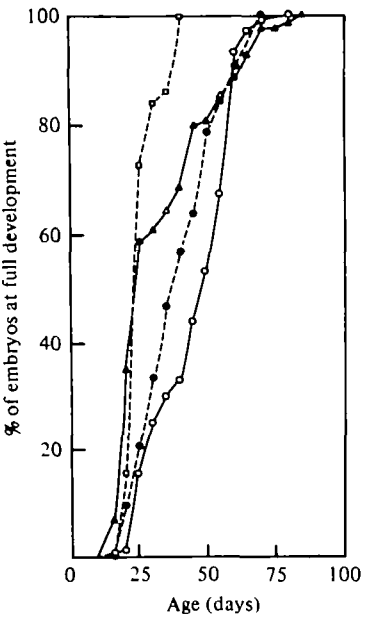

Fig. 2. The cumulative frequency distribution of embryos at full development (Stage 43) under ambient light conditions during the year. ○, December; ●, January; △, February; □, September.

Table 3. *The effects of ambient L:D conditions on the frequency distribution of Diapause II. Observations of one tank followed longitudinally from October* 1977 *to February* 1978

Age (days)	17 Oct. (24)*	26 Oct. (18)	4 Nov. (7)	9 Nov. (5)	22 Nov. (26)	27 Dec. (28)	11 Jan. (24)	20 Jan. (26)	29 Jan. (23)	7 Feb. (16)
0–5	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
6–10	0·0	0·0	60·0	0·0	0·0	0·0	0·0	13·8	0·0	0·0
11–15	0·0	42·1	20·0	0·0	92·9	100·0	100·0	88·5	53·9	6·3
16–20	7·4	0·0	30·0	100·0	88·9	100·0	88·5	50·0	0·0	0·0
21–25	7·4	—	20·0	0·0	84·9	100·0	84·6	26·9	—	—
26–30	0·0	—	0·0	—	65·4	100·0	80·0	3·9	—	—
31–35	—	—	—	—	57·7	92·9	56·0	0·0	—	—
36–40	—	—	—	—	46·2	82·1	24·0	—	—	—
41–45	—	—	—	—	46·2	50·0	0·0	—	—	—
46–50	—	—	—	—	7·7	14·3	—	—	—	—
51–55	—	—	—	—	7·7	0·0	—	—	—	—
56–60	—	—	—	—	3·8	—	—	—	—	—
61–65	—	—	—	—	0·0	—	—	—	—	—

* See footnote to Table 1.

Table 4. *The frequency distribution of embryos at Stage* 32 *(Diapause II) of the annual fish* Nothobranchius guentheri *maintained under artificial light:dark (L:D) conditions*

Age (days)	9:15 (896)*	11:13 (346)	13:11 (992)	16:8 (478)
0	0·0	0·0	0·0	0·0
6–10	9·7	1·7	19·9	5·7
11–15	36·3	38·8	38·9	16·8
16–20	63·2	58·3	31·1	17·1
21–25	58·8	64·9	20·3	11·4
26–30	47·6	50·5	13·6	6·6
31–35	40·3	35·4	7·3	4·3
36–40	35·3	20·4	4·4	1·6
41–45	29·7	15·6	2·9	0·4
46–50	14·5	7·7	0·2	0·0
51–55	10·4	6·0	0·0	—
56–60	5·4	1·4	—	—
61–65	2·7	0·0	—	—
66–70	1·3	—	—	—
71–75	·0·7	—	—	—
76–80	0·1	—	—	—
81–85	0·0	—	—	—

* See footnote to Table 1.

The frequency distributions with time of embryos at Diapause II and at full development under artificial light conditions at 23–25 °C are found in Tables 4 and 5 and Figs. 3 and 4. In these tables and figures the data from all of the experiments are pooled and no distinctions are made for experimental design other than the duration of the L:D cycle. Nevertheless, it can be seen that as the length of day increased the duration and frequency of diapause decreased. As with the data for ambient light conditions, it was not practical to present the data from each of the individual experiments. As a result, key experiments were culled from the large amount of data and the results presented in Tables 6–8. Attempts were always made to follow the same adults for a period of time and to have several groups in each experimental design.

Table 5. *The cumulative frequency distribution of embryos at Stage 43 (pre-hatching of the annual fish* Nothobranchius guentheri *maintained under artificial light:dark (L:D) conditions*

Age (days)	9:15 (896)	11:13 (346)	13:11 (992)	16:8 (478)
0	0·0	0·0	0·0	0·0
6–10	0·0	0·0	0·0	0·0
11–15	0·2	1·6	10·7	0·0
16–20	13·9	7·3	24·9	41·2
21–25	28·1	22·6	52·5	64·6
26–30	37·6	30·7	65·3	74·7
31–35	46·6	41·9	80·9	85·2
36–40	53·1	46·1	87·4	90·7
41–45	58·4	66·4	92·8	96·0
46–50	67·4	72·9	94·0	96·2
51–55	76·8	77·4	96·0	96·6*
56–60	87·3	80·3	97·6	—
61–65	91·0	85·0	98·1*	—
66–70	95·0	85·9*	—	—
71–75	95·6	—	—	—
76–80	96·8	—	—	—
81–85	98·2	—	—	—
86–90	98·4*	—	—	—

* See footnote to Table 2.

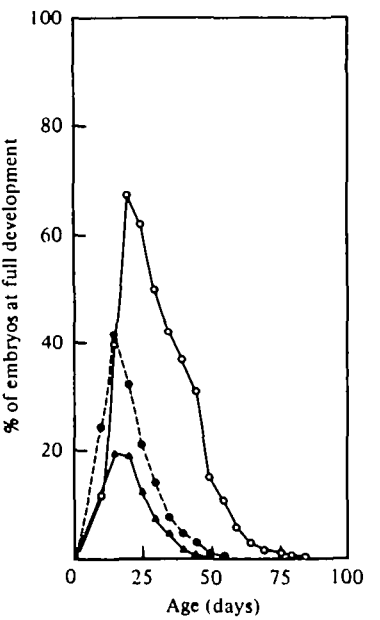

Fig. 3. The frequency distribution of embryos at Diapause II under artificial light conditions. ○, 9:15; ●, 13:11; △, 16:8.

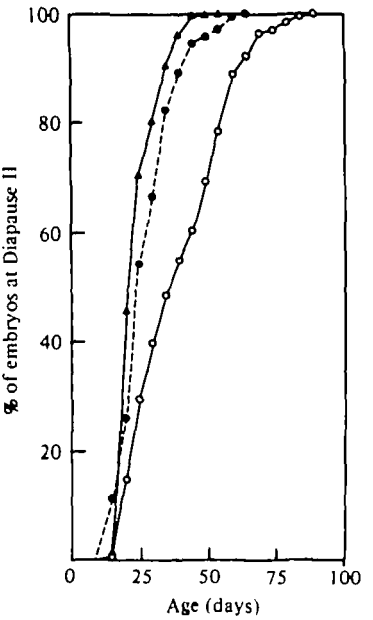

Fig. 4. The cumulative frequency distribution of embryos at full development (Stage 43) under artificial light conditions. ○, 9:15; ●, 13:11; △, 16:8.

Table 6. *The effects of* 9:15 *L:D conditions on the frequency distribution of Diapause II. Observations of two tanks followed longitudinally for* 8 *weeks after transfer from ambient light conditions**

Age (days)	Wk 4 A (11)	Wk 14 B (29)	Wk 5 A (29)	Wk 15 B (27)	Wk 6 A (18)	Wk 16 B (27)	Wk 9 A (55)	Wk 19 B (14)	Wk 12 A (16)	Wk 22 B (28)
0–5	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
6–10	0·0	0·0	0·0	63·3	0·0	80·0	5·0	0·0	0·0	0·0
11–15	6·3	16·1	3·3	65·5	5·3	13·8	67·9	100·0	6·3	27·6
16–20	61·5	27·6	96·7	55·2	68·4	3·5	87·5	100·0	93·8	100·0
21–25	66·7	27·6	90·0	0·0	77·8	3·5	43·6	57·1	93·8	100·0
26–30	72·7	10·3	80·0	—	77·8	3·5	21·8	42·9	75·0	69·0
31–35	72·7	0·0	79·3	—	55·6	0·0	14·6	35·7	75·0	58·6
36–40	72·7	—	79·3	—	50·0	—	9·1	28·6	75·0	55·2
41–45	72·7	—	72·4	—	44·4	—	9·1	28·6	37·5	48·3
46–50	9·1	—	72·4	—	33·3	—	7·3	0·0	25·0	10·3
51–55	0·0	—	65·5	—	0·0	—	5·5	—	0·0	0·0
56–60	—	—	13·8	—	—	—	5·5	—	—	—
61–65	—	—	0·0	—	—	—	5·5	—	—	—
66–70	—	—	—	—	—	—	5·5	—	—	—
71–75	—	—	—	—	—	—	3·6	—	—	—
76–80	—	—	—	—	—	—	0·0	—	—	—

* Tank A was placed in the cycle on 14 May 1977 and Tank B on 2 March 1977. Eggs were collected from each tank on the same day, and incubated under the same conditions.

See footnote to Table 1.

Table 7. *The effects of* 11:13 *L:D conditions on the frequency distribution of Diapause II. Observations of two tanks followed longitudinally for* 4 *weeks after being maintained at* 9:15 *since birth*

	Week 3		Week 4		Week 5		Week 6	
Age (days)	C (28)*	D (13)	C (16)	D (12)	C (22)	D (29)	C (25)	D (29)
0–5	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
6–10	0·0	0·0	5·6	64·3	0·0	0·0	0·0	0·0
11–15	0·0	0·0	93·8	0·0	70·8	86·7	100·0	58·6
16–20	86·2	66·7	93·8	25·0	60·9	89·7	90·0	86·2
21–25	82·8	76·9	87·5	0·0	56·5	89·7	83·3	86·2
26–30	82·8	46·2	87·5	—	17·4	69·0	46·7	10·3
31–35	41·4	46·2	87·5	—	0·0	10·3	6·7	10·3
36–40	7·1	38·5	0·0	—	—	10·3	0·0	0·0
41–45	7·1	0·0	—	—	—	10·3	—	—
46–50	0·0	—	—	—	—	0·0	—	—

* See footnote to Table 1.

Table 8. *The effects of* 13:11 *L:D conditions on the frequency distribution of Diapause II. Observations of two tanks followed longitudinally after transfer from* 11:13

	Week 1		Week 2		Week 3		Week 4		Week 8	
Age (days)	C (17)*	D (27)	C (5)	D (15)	C (6)	D (20)	C (26)	D (29)	C (3)	D (13)
0–5	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
6–10	0·0	0·0	60·0	—	0·0	3·3	3·3	6·7	0·0	26·7
11–15	5·9	40·0	0·0	—	62·5	4·0	63·0	0·0	100·0	53·9
16–20	0·0	0·0	—	—	83·3	0·0	51·9	—	66·7	53·9
21–25	—	—	—	—	66·7	—	51·9	—	66·7	15·4
26–30	—	—	—	—	66·7	—	51·9	—	0·0	15·4
31–35	—	—	—	—	66·7	—	34·6	—	—	0·0
36–40	—	—	—	—	66·7	—	15·4	—	—	—
41–45	—	—	—	—	66·7	—	0·0	—	—	—
46–50	—	—	—	—	0·0	—	—	—	—	—

* See footnote to Table 1.

In Table 6 the data are presented for embryos derived from two sets of adults followed for different lengths of time at an L:D of 9:15. These adults were born on the same day and maintained under ambient light conditions until they were transferred to 9:15. Embryos were collected at weekly intervals on the same day over the course of 8 weeks. Although it was possible to induce Diapause II under these conditions, there was variability in the frequency distributions with time. The variability could not be accounted for by environmental conditions in the laboratory, since many experiments were carried out simultaneously under the same conditions which showed consistent results. Although it took about 4 weeks to induce a high frequency of diapausing embryos, after long durations at this short-day L:D there was large variability in the numbers of diapausing embryos and the duration of diapause. This was also confirmed with embryos maintained from birth at 9:15. In Table 7 the data are presented for two groups of adults born on the same day and followed for 4 weeks at 11:13 after

being maintained at 9:15 since birth. Except for week 4 there was general agreement between the two sets of adults. At week 4 embryos from adult group D exhibited a prolonged duration of Diapause I (the only time this was noted) in 25% of the embryos, and none entered Diapause II. Similar to the observations at 9:15, at prolonged durations at 11:13 there was an increase in the variability among the tanks for the frequency of diapausing embryos. In Table 8 the data are presented for the same group of fish described in Table 7 immediately after their transfer to 13:11 after a 6-week duration at 11:13. No diapause was observed for the first two weeks but began to appear by the third week. Again, there was variability between the tanks which could not be attributed to environmental conditions in the laboratory since the embryos were collected on the same day and stored under the same conditions.

DISCUSSION

In his survey of the diapause characteristics of the subfamily, *Rivulinae* (Family Cyprinodontidae), Wourms (1972*b*) found inter- and intra-generic distinctions with regard to the extent and duration of embryonic diapause under constant laboratory conditions. What seemed evident was an almost continuous evolutionary sequence of developmental features from the typical teleostean non-diapausing development (e.g. *Epiplatys* sp.) to that of the diapausing development typical of the annual fishes (e.g. *Nothobranchius* sp.). Both Peters (1963) and Wourms (1972*c*) incorporated this embryonic diapause into working hypotheses for annual fish survival in arid areas of alternating rainy and dry seasons. It has already been noted that temperature and oxygen are environmental stimuli which act directly on the embryo in determining, at least in part, the durations of Diapause I and II (Peters, 1963; Markofsky & Matias, 1977*a*). These factors, however, do not necessarily predict the onset or duration of the rainy and dry season. Since the embryo has no apparent way of predicting the duration of the variable environmental conditions, it may be postulated that the L:D cycle is the link, through maternal influence (Markofsky & Matias, 1977*b*, 1978), between diapause induction and the duration of the rainy season.

It is generally the case that rainy season activity coincides with the period of highest solar incidence (Trewartha, 1951). In the southern hemisphere along the Tropic of Capricorn, the period of high sun occurs during the winter solstice (21 December), and occurs progressively later northward towards the equator. Therefore, in Tanzania (2–10° S), the period of high sun can be estimated to occur between January and March depending on the latitude. This is consistent with Bailey's (1972) observation that in one habitat (approximately 6° S) water began to accumulate in February and was completely absent by August. We may therefore speculate that embryos derived from spawnings early in the rainy season are not programmed to enter Diapause II via the influence of L:D cycle. If the pond dries prematurely and all of the adults die, a population of embryos is available for hatching if the rainy season resumes shortly after this dry period. In contrast, at the end of the rainy season a large number of embryos are produced which spontaneously enter Diapause II. In the event of a rain at the end of the rainy season which would not sustain a population to sexual maturity, the vast majority of embryos would not develop but would be available for the next rainy season. As a result, L:D can inhibit or accelerate develop-

ment and thus contribute an element of variability which is significant to survival of the population.

The effect of L:D cycle on embryonic diapause in the annual fish *N. guentheri* is a unique observation in the Vertebrata. As in some insects, the diapause factor was found to be dependent on the maternal L:D cycle and independent of that of the male (Markofsky & Matias, 1977*b*, 1978; Ryan, 1965; Saunders, 1966). Embryos maintained under L:D cycles ranging from all-light to all-dark conditions demonstrated diapause which was only dependent on the maternal L:D cycle. Since Wourms (1972*a*) maintained his adult *N. guentheri* at an L:D of 16:8, based on the current findings, it was not unreasonable that he did not observe Diapause II in his studies. However, since the duration of Diapause II in *N. guentheri* is also temperature dependent (Wourms, 1972*c*; Markofsky & Matias, 1977*a*), perhaps the other species he observed which did enter diapause were showing either a temperature- or an L:D cycle-dependent diapause. A more detailed comparative analysis of the habitats and meteorological conditions of the various species of annual fish and their relationships to embryonic diapause would be most informative. Peters (1963) reported Diapause II in *N. guentheri* but did not report the L:D cycle.

As indicated in the Results, the effects of ambient and artificial L:D cycles are not simple predictable phenomena. In our initial studies it took about 2 weeks for the embryos to demonstrate a response to an artificial L:D (Markofsky & Matias, 1978). The results, however, were inconsistent when attempts were made to reproduce the experiment several months later. Initially we could not explain the inconsistencies. It later became apparent that the frequency of diapausing embryos was dependent on L:D cycles but that (1) there were individual differences among the fish, (2) these differences changed with time under the same L:D, (3) under ambient conditions there was also variability with time especially during the short-day winter months, (4) there were some differences from year to year (to be reported), and (5) the time of year the artificial L:D experiments were initiated may also have been a factor. These conclusions were based on the results of many experiments run simultaneously in order to eliminate environmental factors in the laboratory which might have influenced the onset and duration of diapause. We often would find differing frequencies of Diapause II for embryos derived from fish born on the same day and housed under the same conditions even though the embryos were collected on the same day and incubated under the same conditions. In addition, at an L:D of 13:11 individual females, over the course of only one week's time, produce both diapausing and non-diapausing embryos (personal observation).

It may be concluded, therefore, that variability in the frequency of diapausing embryos produced within the population, under both constant and varying light conditions, is a critical factor contributing to the survival potential of the species in the erratic and harsh environments in which they are found. As a result, these unique fish have developed a survival mechanism so that anything short of a major catastrophe allows for some members of the population to survive.

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REFERENCES

BAILEY, R. G. (1972). Observations on the biology of *Nothobranchius guentheri* (Pfeffer) (Cyprinodontidae), an annual fish from the coastal region of East Africa. *Afr. J. trop. Hydrobiol. Fish.* **2**, 33–43.

JUBB, R. A. (1969). The *Nothobranchius* (Pisces, Cyprinodontidae) of Southern Africa and a new species from Lake Chilwa, Malawi. *An. Cape Prov. Mus.* (*Nat. Hist.*) **8**, 1–11.

KLEE, A. (1965). A quick review of *Nothobranchius*. *J. Am. Killifish Ass.* **2**, 2–16.

LEES, A. D. (1955). *The Physiology of Diapause in Arthropods*, p. 20. Cambridge University Press.

MARKOFSKY, J. & MATIAS, J. R. (1977*a*). The effect of temperature and season of collection on the onset and duration of diapause in embryos of the annual fish *Nothobranchius guentheri*. *J. exp. Zool.* **202**, 49–56.

MARKOFSKY, J. & MATIAS, J. R. (1977*b*). The effects of light:dark cycles on embryonic diapause in the East African annual fish *Nothobranchius guentheri*. *Chronobiologia* **4**, 130.

MARKOFSKY, J. & MATIAS, J. R. (1978). Effects of light:dark cycles and temperature on embryonic diapause in the East African annual fish, *Nothobranchius guentheri*. *Proc. XIII int. Conf. int. Soc. Chronobiology*. (In the Press.)

MARKOFSKY, J. & PERLMUTTER, A. (1972). Age at sexual maturity and its relationship to longevity in the male annual cyprinodont fish, *Nothobranchius guentheri*. *Exp. Gerontol.* **7**, 131–135.

MARKOFSKY, J. & PERLMUTTER, A. (1973). Growth differences in subgroups of varying longevities in a laboratory population of the male annual cyprinodont fish, *Nothobranchius guentheri*. *Exp. Gerontol.* **8**, 65–73.

MATIAS, J. R. & MARKOFSKY, J. (1978). The survival of embryos of the annual fish *Nothobranchius guentheri* exposed to temperature extremes and the subsequent effects on embryonic diapause. *J. exp. Zool.* **204**, 219–227.

MYERS, G. S. (1942). Studies on South American fresh-water fishes. I. *Stanford ichthyol. Bull.* **2**, 89–114.

MYERS, G. S. (1952). Annual fishes. *Aquarium J.* **23**, 125–141.

PETERS, N. (1963). Embryonale Anpassungen oviparer Zahnkarpfen aus periodisch austroknenden Gewässern. *Int. Revue ges. Hydrobiol.* **18**, 257–313.

RYAN, R. B. (1965). Maternal influence on diapause in a parasitic insect *Coeloides brunneri* Vier. (Hymenoptera: Braconidae). *J. Insect. Physiol.* **11**, 1331–1336.

SAUNDERS, D. S. (1966). Larval diapause of maternal origin. II. The effect of photoperiod and temperature on *Nasonia vitripennis*. *J. Insect. Physiol.* **12**, 569–581.

SCHEEL, J. (1975). *Rivulins of the Old World*. TFH Publ., Jersey City, N.J.

TREWARTHA, G. T. (1951). *An Introduction to Climate*. McGraw-Hill, N.Y.

WHO (1973). World Health Organization's *Manual on Larval Control Operations in Malaria Programmes*, Geneva, Switzerland.

WOURMS, J. P. (1964). Comparative observations of the early embryology of *Nothobranchius taeniopygus* (Hilgendorf) and *Aplocheilichthys pumulis* (Boulenger) with special reference to the problem of naturally occurring embryonic diapause in teleost fishes. *A. Rep. E. Afr. Freshwat. Fish. Res. Org.* (Appendix H), 68–73.

WOURMS, J. P. (1972*a*). Developmental biology of annual fishes. I. Stages in the normal development of *Austrofundulus myersi* Dahl. *J. exp. Zool.* **182**, 143–168.

WOURMS, J. P. (1972*b*). The developmental biology of annual fishes. II. Naturally occurring dispersion and reaggregation of blastomeres during the development of annual fish eggs. *J. exp. Zool.* **182**, 169–200.

WOURMS, J. P. (1972*c*). The developmental biology of annual fishes. III. Pre-embryonic and embryonic diapause of variable duration in the egg of annual fishes. *J. exp. Zool.* **182**, 389–414.