

"ASPECTS OF THE BIOLOGY OF FASCICOLA HEPATICA AND ITS  
INTERMEDIATE SNAIL HOST LYMNAEA TRUNCATULA"

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# TABLE OF CONTENTS

	<u>Page No.</u>
SUMMARY	i - ii
ACKNOWLEDGMENTS	iii
PREFACE	1
CHAPTER 1: THE BIOLOGY OF <u>LYMNAEA TRUNCATULA</u>	
A: Environmental Temperature	2 - 37
INTRODUCTION	2 - 7
MATERIALS AND METHODS	8 - 12
RESULTS	12 - 26
DISCUSSION	26 - 37
THE BIOLOGY OF <u>LYMNAEA TRUNCATULA</u>	
B: Soil Moisture	38 - 48
INTRODUCTION	38 - 39
MATERIALS AND METHODS	39 - 42
RESULTS	43 - 45
DISCUSSION	45 - 48
CHAPTER 2: THE BIOLOGY OF <u>FASCIOLA HEPATICA</u>	49 - 83
INTRODUCTION	49 - 58
MATERIALS AND METHODS	58 - 60
RESULTS	60 - 74
DISCUSSION	75 - 83
CHAPTER 3: THE EFFECT OF <u>FASCIOLA HEPATICA</u> ON ITS SNAIL INTERMEDIATE HOST <u>LYMNAEA TRUNCATULA</u>	84 - 99
INTRODUCTION	84 - 87
MATERIALS AND METHODS	87 - 88
RESULTS	88 - 92
DISCUSSION	92 - 99

TABLE OF CONTENTS

	<u>Page No.</u>
CONCLUSION	100 - 102
APPENDIX: A STUDY OF THE EFFECT OF TEMPERATURE ON THE GROWTH OF <u>FASCIOLA HEPATICA</u> IN <u>LYMNAEA TRUNCATULA</u>	103 - 112
REFERENCES	113 - 122

## SUMMARY

The development rates of the eggs of Lymnaea truncatula were determined at specific constant temperatures within the range  $10^{\circ} - 25^{\circ}\text{C}$ ; the relationship between the rate of development and the temperature was described mathematically. The usefulness of this linear relationship as a predictive method was confirmed. The high viability of the snail egg, if maintained within the temperature range  $10^{\circ} - 25^{\circ}\text{C}$ , was demonstrated; prolonged exposure to a temperature of  $5^{\circ}\text{C}$  ( $>15$  days) was found to reduce considerably the egg's ability to develop and hatch.

For snails maintained under conditions of excess food and moisture, the effects of temperature on growth, survival and reproduction were determined at specific constant temperatures within the range  $5^{\circ} - 25^{\circ}\text{C}$ . The effects were found to be complex, the optimum temperature for growth being  $20^{\circ}\text{C}$ , and for reproduction  $16^{\circ}\text{C}$ ; the relationship between temperature and survival was described mathematically. The effect of exposure to short but regular periods (12 hours in every 24 hours) at a temperature of  $5^{\circ}\text{C}$  was found to be complicated; the growth rate was reduced while the survival potential and reproductive rate both increased.

Experiments were completed to investigate the effect on the snail's growth, survival and reproduction of a reduction in the moisture content of the soil on which the snails were maintained. The snail was found to react to a reduction in the moisture level in its micro-environment by altering its behaviour, i.e. it becomes less active and as the moisture level decreases it enters a dormant state (aestivation). The snail remains in this dormant state until either the moisture level is increased or the snail dies.

The development rates of the intramolluscan phase of the parasite Fasciola hepatica were determined at specific constant temperatures within the range  $10^{\circ} - 25^{\circ}\text{C}$ ; the relationship between the rate of development and the temperature was described mathematically. The usefulness of this linear relationship as a predictive method was confirmed. The parasite was found not to be able to survive within snails maintained at a constant temperature of  $5^{\circ}\text{C}$ .

The effects of both the miracidial exposure dose and temperature on the duration of metacercarial production and the numbers of metacercariae produced were investigated. The former was found not to affect the duration of metacercarial production but a miracidial exposure dose of 2 was required to give rise to maximum metacercarial production. It was found to be the action of temperature on the parasite which determined the onset of metacercarial release and the action of the temperature on the snail (determining its survival) which caused the termination of the metacercarial production. The size of the snail appeared to limit the rate of metacercarial production.

The effect of the parasite on its snail host was investigated. No reduction in the host's survival potential was found; however, both castration and gigantism were observed.

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## PREFACE

This thesis has three Chapters and one Appendix. The first Chapter comprises the description of two investigations into the biology of the snail Lymnaea truncatula, determining the effects on the snail's growth, development, reproduction and survival of 1) temperature and 2) the moisture content of the soil on which the snails are maintained. The second Chapter is a description of the effect of temperature and of the number of miracidia to which the snails were exposed, on the development of the intramolluscan stage of the parasite, Fasciola hepatica, the duration of the production of metacercariae and the numbers of metacercariae produced. The third Chapter comprises the description of the effect of the parasite on the growth, reproduction and survival of the snail host and also the determination of whether this effect is modified by temperature. The Appendix is a copy of a paper, prepared with R.A. Wilson, published in Parasitology (1974) Volume 68 (pages 47 to 56) and entitled "A Study of the effect of temperature on the growth of Fasciola hepatica in Lymnaea truncatula".

The aim of the laboratory investigations detailed in the three Chapters of this thesis is to provide information on the biology of the snail Lymnaea truncatula and the intramolluscan larval stage of the parasite Fasciola hepatica. This information is both to aid the formulation of a mathematical model of the snail and parasite and allow future comparison with field observations collected by a fellow investigator (G. Smith).

## CHAPTER 1.

### THE BIOLOGY OF LYMNAEA TRUNCATULA : A Environmental Temperature

#### INTRODUCTION

The snail Lymnaea truncatula often given the descriptive common name of "the mud snail" has for many years been linked with the parasite Fasciola hepatica - the liver fluke. Roberts (1950) reviewed somewhat briefly, the early published evidence of the connections between the snail, the fluke and disease in, and death of sheep. She credits Thomas in England and Leuckart in Germany with, in the early 1880's, the clarification and complete description of the life cycles of both the fluke and the snail, and the former's dependence on the latter.

From the 1880's considerable attention was paid to the biology of Lymnaea truncatula; e.g. Walton (1918), Taylor (1922) and Walton & Jones (1926). However, it was not until Taylor & Mozley (1948) described methods of maintaining the snail in culture in the laboratory, that detailed observations were made possible. Kendall (1953) described how a large breeding colony was maintained and reported on observations made concerning the life cycle.

The life cycle of the snail can be divided into three stages: the egg, the immature (young) and mature (adult). Bailey (1931) suggests that the Lymnaediae are the highest forms of life, in the evolutionary series, in which hermaphroditism occurs. Walton (1918) from field observations and Roberts (1950) and Kendall (1953) from laboratory observations all agreed that Lymnaea truncatula are self fertile and



furthermore report that copulation was never observed. Smith (1978) reports that copulating snails have frequently been observed under laboratory conditions; however, this does not detract from the fact that the snails are self fertile: for a near relative Lymnaea stagnalis has been observed to copulate in both the field and laboratory aquaria and is also self fertile (Kendall, 1950 and van der Steen, 1967).

When the snails are maintained under conditions of high humidity, as in laboratory cultures, the eggs are usually laid at the wet mud/glass interface. Under drier conditions, as found in the field, the eggs are usually laid partially or totally submerged. Kendall (1953) reports that there is no seasonal variation in the oviposition of the snails. The eggs are laid in groups surrounded by a gelatinous matrix, each clutch of eggs aggregated together in this way is termed a mass, the number of eggs per mass varies greatly. Kendall (1953) suggests that the number of eggs per mass is related to the amount of food available, and he reports that under favourable laboratory conditions he observed a mass containing 31 eggs. Van der Steen (1967) from observations of Lymnaea stagnalis suggests the following:

- 1) The mean number of capsules (masses) per snail per day and the mean number of eggs per capsule represent independent functions of reproduction.
- 2) The mean number of eggs per capsule increases with increasing oviposition interval, i.e. the number of days passed since the previous oviposition.
- 3) Atmospheric pressure changes and oviposition activity are unequivocally correlated.

- 4) In some instances, the diet influences total egg production.
- 5) Temperature affects the total egg production and the mean number of eggs per capsule.

Michelson (1961) has demonstrated from laboratory experiments that temperature influences growth and reproduction of the snail Austral-orbis glabratus (the intermediate host of the blood flukes Schistosoma mansoni and Schistosoma haematobium). He suggests that a temperature which accelerates one physiological process may in fact retard or completely inhibit another, citing as evidence his observations that a temperature of 30°C produces maximum growth of the snail while severely inhibiting its reproduction.

Bouillon (1956) from histological studies of the land snail Cepaea nemoralis concludes that temperature has an effect on sexual differentiation; a low temperature (6°C) being more favourable to the development of primary gonocytes in the female direction and a high temperature (23°C) promotes development in the male direction, while a temperature of 0°C totally inhibits the development of the reproductive tissue.

Kendall (1953) states that the fertility of the eggs of Lymnaea truncatula is exceedingly high, noting that this conforms with Olsen's (1944) observations of the breeding of the related Stagnicola bulimoides techella. Vaughn (1953) also records a high % hatching value in a Lymnaea species (Lymnaea stagnalis appressa). The embryo develops within the egg and is afforded some protection from both mechanical injury and desiccation by the gelatinous matrix of the egg mass. The protection from desiccation is, however, strictly limited; Roberts

(1950) reports that the maximum time the eggs, within their mass, can withstand drying is approximately 4 hours.

Both Roberts (1950) and Kendall (1953) agree that the time from oviposition to hatching in Lymnaea truncatula is directly dependent upon the environmental temperature.

Temperature	Time from Oviposition to hatching	Author
17°C	17 to 22 days	Roberts (1950)
25°C	8 to 12 days	Roberts (1950)
11°C	29 days	Kendall (1953)
16°-21°C	12 to 13 days	Kendall (1953)
21°-31°C	11 to 12 days	Kendall (1953)

The results obtained by Vaughn (1953) indicate that this direct dependency can also be demonstrated in Lymnaea stagnalis appressa, at least within the range 9.9° to 28.0°C.

Roberts (1950) suggests that in Lymnaea truncatula embryonic development is completely inhibited at low temperatures (<5°C). She cites confirmatory evidence from Walton & Wright (1926); however, the reference she quotes does not mention the development of the snail egg. If low temperatures have an inhibitory effect on egg development, it could partially explain the field observations reported by Walton (1918); that a large population of very small snails appear in the early spring in apparently adult-free habitats.

The ways in which temperature exerts its effect on the developing snail egg do not appear to have been studied. However, Grainger (1959) from investigations into the effect of temperature on the developing eggs of the frog Rana temporaria discusses the possible ways in which tem-

perature can affect the rate of development of the egg; he concludes that there are two basic effects:

- 1) The Intermediate Effect - acting during the time that the eggs are exposed to a specific temperature.
- 2) The After-Effect - acting on the rate of subsequent development.

The embryo of Lymnaea truncatula when fully developed, breaks free from its egg and mass and commences to feed actively (Kendall; 1953). Under conditions of excess food and moisture with springtime temperatures (whether in the field or in the laboratory) growth is rapid and sexual maturity (i.e. the commencement of oviposition) is attained when the shell is about 4 to 5 mm long (Walton & Jones: 1926 and Kendall: 1953).

If maintained under favourable conditions of food, moisture and temperature the mature snails continue to grow but at a reduced rate and to produce eggs until shortly before they die; a shell length of 12 to 13 mm may often be reached; snails of this size are rarely if ever observed in the field (Kendall; 1953). Roberts (1950) reported that her laboratory reared snails only reached a shell length of 8.5 mm, rather smaller than those collected in the field. Kendall (1953) suggests that the laboratory culture methods used by Roberts do not produce a sufficiently favourable environment to enable the snails to grow to their full potential.

Hodasi (1976) reports that the activity of both young and adult Lymnaea truncatula, provided with excess food and moisture, was markedly reduced during a 3 month period of continual exposure to 5°C in the laboratory, but that hibernation did not occur. The survival

of these snails was high at this low temperature but that there was an almost total cessation of growth and a suppression of reproduction. On return to room temperatures ( $16^{\circ}$ - $22^{\circ}$ C) the young snails became very active, fed voraciously, grew rapidly, tended to live longer and produce more eggs than did controls. In contrast, adult snails given the same treatment showed no appreciable increase in growth rate and produced fewer eggs than controls.

Kendall (1949a) reports that during times of drought Lymnaea truncatula survives in a state of aestivation; in the laboratory newly hatched snails are able to withstand at least 2 months in this inactive state while older snails have been demonstrated to remain alive for more than one year.

The observations thus far related strongly suggest that the "mud snail" is not just a survivor but is able to exploit its extremely unstable environment. As the formal aim of the research work, of which this thesis is a part, is to provide data and advice to help reduce agricultural losses resulting from fascioliasis; then what is required is a fuller understanding and appreciation of the potential of the snail to exploit its environment. Published data whilst providing much of the basic theory, do not quantify the snail's potential; i.e. growth, survival and reproductive capacity. In this first section of this Chapter, a series of experiments and observations are detailed concerning the effects which a basic physiological and environmental parameter - temperature, has on the snail's potential; its growth and development, its reproductive capacity and its survival.

## MATERIALS AND METHODS

### Biological Material

Adult Lymnaea truncatula were obtained from a laboratory-bred strain,\* kept for several generations under conditions which preclude adventitious infection with Fasciola hepatica. Newly laid egg masses were collected over a maximum period of 12 hours, normally less, from groups of stock adult snails, transferred to liberally watered (with aerated tap-water), fresh blue-green algal cultures growing on specially prepared mud. The preparation of the algal cultures will be described in the Methods Section of this Chapter. The egg masses were maintained in aerated tap-water in glass petri dishes (approximately 9 cm in diameter).

Young and adult snails were always maintained on cultures of the blue-green alga, thus under conditions of excess food and moisture.

Egg masses, the young and the adult snails were all exposed to a light regime of 12 hours light : 12 hours dark, thus precluding any possible photoperiodic seasonal effect on the egg or the snail. Kendall (1953) strongly suggests that there is no seasonal effect on the oviposition in Lymnaea truncatula under laboratory conditions and De Witt (1954) reports that in Physa gyrina seasonal periodicity of oviposition disappears in laboratory reared individuals. However, no data has been located concerning the effect of the season other than environmental temperature on growth, development and survival in Lymnaea truncatula.

\*Snails originally obtained from  
Central Veterinary Laboratory,  
Weybridge.

### Controlled Temperature Conditions

Temperature conditions constant to within  $\pm 1^{\circ}\text{C}$  were obtained by using the Departmental constant temperature rooms and cooled incubators. The logical constant temperature series of  $5^{\circ}$ ,  $10^{\circ}$ ,  $15^{\circ}$ ,  $20^{\circ}$  &  $25^{\circ}\text{C}$  was unfortunately not possible; owing to a lack of apparatus it was necessary to substitute  $16^{\circ}\text{C}$  for the  $15^{\circ}\text{C}$  value.

A simulated Day/Night temperature and light regime of 12 hours at  $20^{\circ}\text{C}$  illumination on and 12 hours at  $5^{\circ}\text{C}$  illumination off was obtained by using an illuminated cooled incubator with timed cycling. (Gallenkamp MK 1H 277). This incubator was able to control temperatures to within  $\pm 1^{\circ}\text{C}$  and was able to perform the transition from  $20^{\circ}\text{C}$  to  $5^{\circ}\text{C}$  and vice versa in less than 15 minutes.

### The Preparation of the Algal Cultures

The soil was collected from Millington Pastures, an area of the Yorkshire Wolds, grid reference SE 838531, the most useful soil being that brought to the surface by moles. The soil was stored on the flat roof of the Biology Department on paving stones raised above the surface of the roof to allow rain water to drain away and thus prevent the soil from souring. The soil, which contained large quantities of chalk chips and small stones, was sieved and autoclaved at 15 lb/sq. inch for 25 minutes and then allowed to cool. A small quantity of calcium nitrate and potassium dihydrogen orthophosphate (1 g to 10 kg of soil) was then dissolved in aerated tap-water and added evenly to the soil. Further aerated tap-water was added until the mud assumed the consistency of bread dough. Sufficient of this mud was then placed in 12.5 cm

diameter crystallising dishes, to cover the base to a depth of approximately 2.5 cm. The surface of the mud was then seeded with a sample of blue-green alga of the genus Oscillatoria. The dishes were then covered with lids of glass petri dishes and placed, approximately 50 cm beneath a bank of fluorescent tubes. Small quantities of aerated tap-water were added to ensure that the surface of the mud remained moist. The algal cultures were ready to receive snails, when the surface of the mud was covered by the alga, usually after 6-8 days.

The Determination of the Effect of Temperature on the Hatching Time and the Mortality of the Eggs of Lymnaea truncatula

Batches of fifty egg masses were collected and maintained, as previously described, and exposed to a defined temperature regime. The masses were examined every 24 hours and any snails observed to have hatched were removed; the precise time and the number of snails removed were recorded. When all the eggs had apparently hatched (i.e. when no more mobile embryos could be detected) the masses were examined and the number of undeveloped and dead embryos recorded.

At each temperature regime the number of eggs which failed to hatch was recorded and the time to 50% hatching calculated, using probit transformation (Sokal & Rohlf : 1969); in using this method the assumption is made that hatching is normally distributed about the 50% hatching value.

The basic objectives, the temperature regimes and the number of fifty masses for each investigation were as follows:

- a) to investigate the effect of a range of constant temperature regimes; constant temperatures of 5°, 10°, 16°, 20° & 25°C;



5 batches of fifty masses.

- b) to investigate the effect of a very simple diurnal temperature regime (Day/Night); 10 batches of fifty masses.
- c) to investigate the effect of a random temperature regime, to test predictions from investigation a) above; a 24 hour period at one of the 5 constant temperatures :  $5^{\circ}$ ,  $10^{\circ}$ ,  $16^{\circ}$ ,  $20^{\circ}$  &  $25^{\circ}\text{C}$  chosen at random; 5 batches of fifty masses.
- d) to investigate any specific effect or effects a low temperature ( $5^{\circ}\text{C}$ ) has or have on the snail egg; all combinations (24) of exposure to a specified number of days at  $5^{\circ}\text{C}$  (1, 2, 5, 15, 30 & 60 days) and then maintained at each of the remaining four constant temperatures  $10^{\circ}$ ,  $16^{\circ}$ ,  $20^{\circ}$  &  $25^{\circ}\text{C}$ ; 2 batches of fifty masses.

The Determination of the Effect of Temperature on the Growth, Reproduction Capacity and the Survival of Young and Adult *Lymnaea truncatula*

Growth and Survival

Six batches of forty newly hatched snails were transferred to fresh algal cultures and maintained at one of the following temperature regimes: constant  $5^{\circ}$ ,  $10^{\circ}$ ,  $16^{\circ}$ ,  $20^{\circ}$  &  $25^{\circ}\text{C}$  and Day/night ( $20^{\circ}$  &  $5^{\circ}\text{C}$ ); under conditions of excess food and moisture, with never more than 15 individual snails per dish (surface area approximately  $17.5\text{ cm}^2$ ). The maximum length of the shell of each snail was measured at regular intervals, using a binocular microscope fitted with an eyepiece graticule for the smaller snails ( $<3\text{ mm}$ ) and a micrometer screw gauge for the larger snails ( $>3\text{ mm}$ ). The intervals between measurements were as follows:

<u>Temperature</u>	<u>Interval</u>
5°C	28 days
10°C	14 days
16°C	7 days
20°C	5 days
25°C	4 days
20°/5° (Day/Night)	7 days

The groups of snails were examined more frequently and any dead snails removed and measured.

### Reproduction

Six batches of forty five adult snails of similar shell length and age were divided randomly into three groups of fifteen individuals and maintained as detailed above, one batch at each temperature regime. The dishes were examined every 48 hours, all the egg masses were removed and the number of eggs in each mass determined and recorded, any dead snails were removed, measured and recorded. The maximum shell length of all the snails was determined and recorded periodically.

### RESULTS

#### The Determination of the Effect of Temperature on the Hatching Time and the Mortality of the Eggs of *Lymnaea truncatula*

Using probit transformations as described by Sokal & Rohlf (1969), the time, to the nearest half day, to 50% hatching, the percentage mortality values and the total number of eggs in each experiment at the 10°, 16°, 20° & 25°C constant temperature regimes, together with the value for the Day/Night temperature regime are given in Table 1:1. At the 5°C constant temperature no snail egg hatched; the eggs within their egg masses began to disintegrate after approximately 3 months.

TABLE 1:1

Temperature Regime	Time to 50% hatching; in days	Percentage Mortality	Total Number of Eggs
10°C	31.5	0.77	1,822
16°C	17.5	1.14	1,750
20°C	11.5	1.77	1,411
25°C	9.5	1.38	1,742
Day/Night (20°/5°C)	23.0	1.49	994

The Time to 50% hatching, in days, the Percentage and the Total Number of Eggs maintained at each Temperature Regime.

The development rates of the embryo to hatching, calculated as the reciprocal of the time in days to 50% hatching (i.e. development time) at the four constant temperatures, were plotted against their respective temperature - numerical value in degrees Centigrade - Figure 1:1. Using simple linear regression the equation describing this relationship was calculated (Bailey: 1968). The equation was found to describe a line with a slope of 0.005074 and with an intercept on the y axis (the rate of development) of -0.019793. From this it would appear that over the temperature range 10°C to 25°C there is a positive relationship between rate of development and temperature.

The batches of eggs (total 798) exposed to 24 hour periods at one of the constant temperatures, chosen at random, achieved 50% hatching after 17.5 days, 1.38% failed to hatch. The actual temperatures to which the batches of eggs were exposed are given in Table 1:2.

The time to 50% hatching to the nearest half day, together with the percentage mortality and the total number of snail eggs in the two batches, for all (24) combinations of exposure to a specified number of days to a constant temperature of 5°C (1, 2, 5, 15, 30 & 60) and then maintained at one of the four constant temperatures (10°, 16°, 20° & 25°C) are given in Table 1:3.

### Snail Growth

The plots of mean maximum shell length (mm) against time (days since hatching) for the six temperature regimes are given in Figure 1:2. For all but the 5°C constant temperature regime the plots are terminated with the recording taken nearest to the 50% mortality value of

FIGURE 1:1 Plot of Egg Development Rate against Temperature  
°C. The Development Rate calculated as the  
reciprocal of the Time in days from oviposition  
to 50% Hatching.

$$s = 3.720$$

$$t = 4.404 \text{ with 2 degrees of freedom}$$

therefore significantly different from

$$b = 0. (95\%).$$

Calculations from Bailey (1968)

Fig.1:1

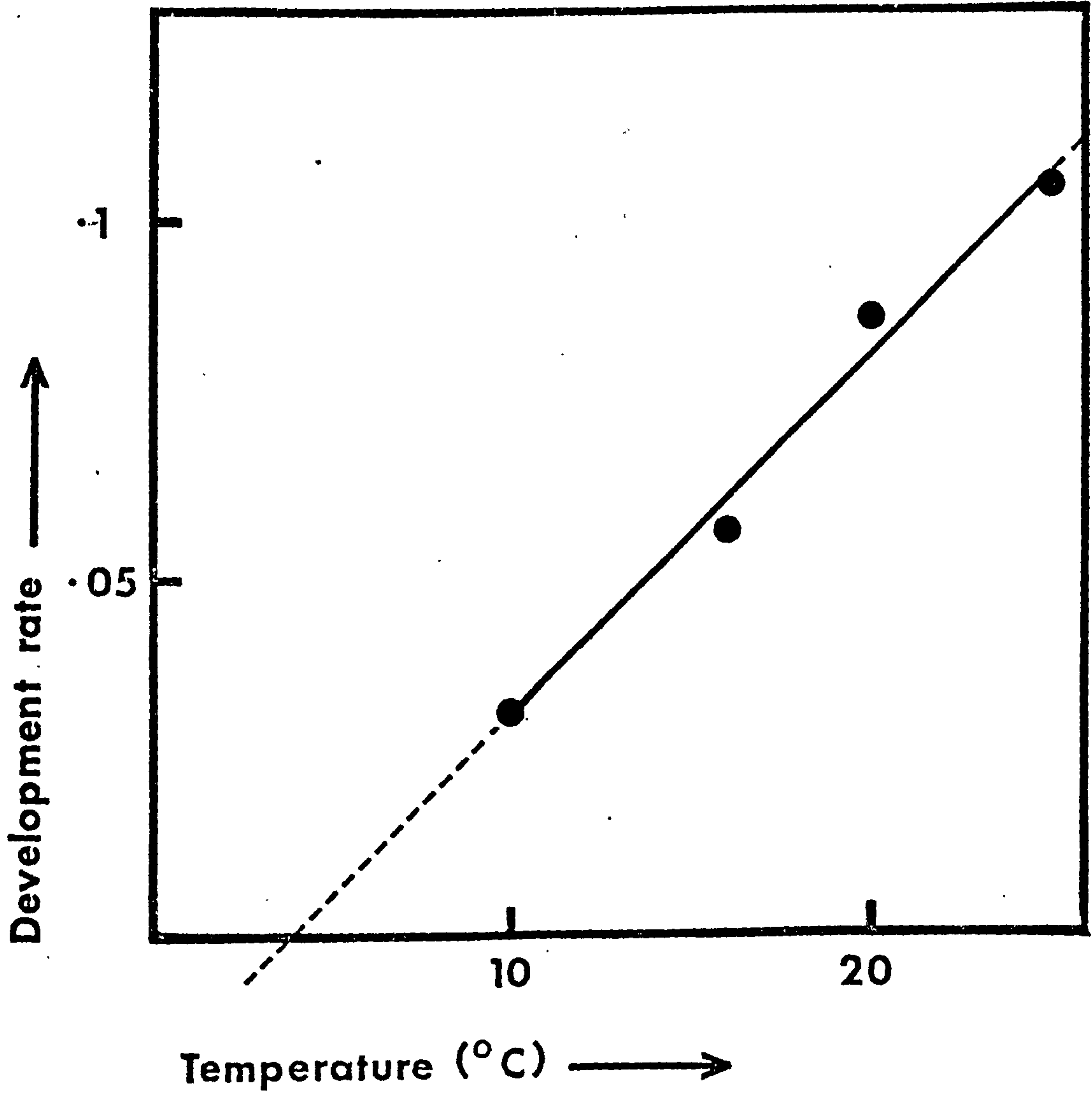


TABLE 1:2

Day Number	Temperature °C	Day Number	Temperature °C
1	20°C	13	20°C
2	20°C	14	20°C
3	16°C	15	5°C
4	10°C	16	25°C
5	20°C	17	20°C
6	20°C	18	10°C
7	10°C	19	10°C
8	5°C	20	25°C
9	25°C	21	10°C
10	16°C	22	25°C
11	10°C	23	20°C
12	5°C	24	16°C

The Constant Temperature to which the Eggs were exposed on each day throughout the experiment.

TABLE 1:3

Constant temperature °C	Number of days exposed to 5°C						
	1	2	5	15	30	60	
10°C	Days to 50% hatching	25.5	28.0	31.0	56.5	70.0	108.0
	% Mortality	4.2	4.2	30.3	29.5	94.0	93.5
	Total Number of eggs	307	240	294	241	294	160
16°C	Days to 50% hatching	16.0	17.0	20.0	29.5	45.5	79.5
	% Mortality	1.3	2.1	4.0	23.7	18.0	74.4
	Total Number of eggs	314	240	273	228	217	180
20°C	Days to 50% hatching	13.0	14.0	17.0	26.5	42.0	71.5
	% Mortality	1.7	3.6	9.7	26.3	26.0	93.2
	Total Number of eggs	290	251	342	213	223	161
25°C	Days to 50% hatching	10.5	10.5	14.0	23.5	39.0	71.0
	% Mortality	1.0	4.4	11.5	20.2	34.6	98.0
	Total Number of eggs	316	274	295	243	208	150

The Time to 50% Hatching, to the nearest half day, the Percentage Mortality and the Total Number of Eggs, for all the 24 combinations of exposure to a specified number of days at a constant temperature of 5°C (1, 2, 5, 15, 30 & 60) and then maintained at one of the four constant temperatures (10°, 16°, 20° & 25°C).



days since hatching. At the lowest constant temperature regime ( $5^{\circ}\text{C}$ ) the plot is shown to terminate on day 308; the snails did continue to grow; the mean maximum shell length on day 308 was 2.145 mm and reached 2.604 mm on day 596 (the nearest recording to the 50% mortality value of days since hatching).

The plots for the remaining five temperature regimes (Figure 1:2) appear to follow a consistent pattern; approximating to the middle and top sections of a logistic curve; i.e. an initial rapid rate of increase in shell length and then a much slower rate terminating in the death of the snails.

At these five temperature regimes the mean maximum shell length reached a value within the range 8.00 mm to 9.25 mm, with the largest snails in these groups attaining shell lengths within the range 9.80 mm to 11.00 mm.

The numerical values of the initial rapid rate of increase in the mean maximum shell length for these five temperature regimes are given in Table 1:4. The values were calculated by estimating from Figure 1:2 the number of days since hatching and the increase in mean maximum shell length from hatching to when the rate of increase in the mean maximum shell length changes from the rapid to the slower rate.

From these results it would appear that:

- a) at a constant temperature of  $5^{\circ}\text{C}$  the increase in shell length is almost totally inhibited.
- b) the optimum constant temperature for increase in shell length is  $20^{\circ}\text{C}$ .

FIGURE 1:2 Plots of Mean Maximum Shell Length in mm  
against Time, Days since Hatching.

□ = 5°C  
△ = 10°C  
▲ = 16°C  
○ = 20°C  
● = 25°C  
X = Day/Night

Fig.1:2

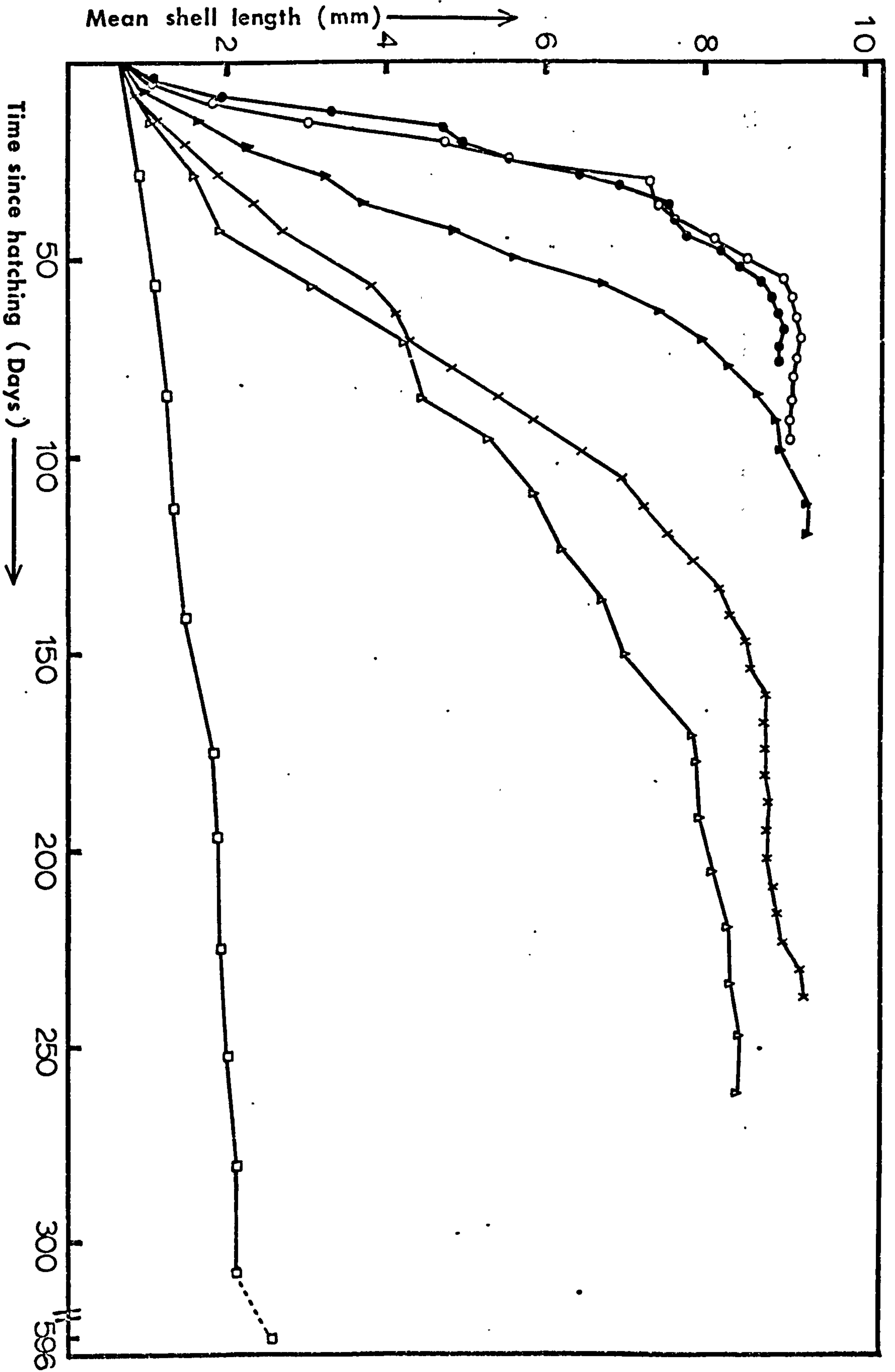


TABLE 1:4

Temperature Regime	Estimated value of the rate of increase in the mean maximum shell length mm/day
5°C	0.003
10°C	0.042
16°C	0.090
20°C	0.151
25°C	0.143
Day/Night (20°C/5°C)	0.056

The Numerical Value of the Estimated Initial Rapid Rate of Increase in the Mean Maximum Shell Length (mm/day) at the six Temperature Regimes.

c) the Day/Night temperature regime (equal time spent each day at 20° and 5°C) gives rise to a rate of increase in shell length considerably less than half that recorded at 20°C.

### Survival

For the snails maintained from hatching at the six temperature regimes, the day on which each snail was found to have died was recorded and the % survival value of the snails at each temperature regime calculated. These % survival values were subjected to a probit transformation (Sokal & Rohlf: 1969) and these values plotted - Figures 1:3a to 1:3f. Lawson (1977) investigating the survival of the cercariae of Schistosoma mansoni utilised a method of probit transformation followed by simple linear regression <sup>to</sup> mathematically describe the survival of this stage of the blood fluke's life cycle. However, from the calculation of the coefficient of determination and from visual observations, it would appear that a simple linear regression does not provide a significant description of all of the total probit transformation plots (Figures 1:3a to 1:3f); for the majority of these plots have a flat initial stage followed by a rapid reduction in the values. Thus, to compare the survival of the snails at each temperature regime, the times, in days, from hatching to 50% mortality - referred to as survival times - were estimated from the actual probit plots; 50% mortality equivalent to probit value 5. The survival times for each temperature regime are given in Table 1:5.

The failure of probit transformation to assist with the clarification of snail mortality, as compared with the usefulness in determining the 50% hatching of the snail egg, may to some extent be explained as the

FIGURE 1:3

Plots of Probit Value of % Survival against  
Time, Days since Hatching.

a = 25°C  
b = 20°C  
c = 16°C  
d = 10°C  
e = 5°C  
f = Day/Night

Fig. 1:3

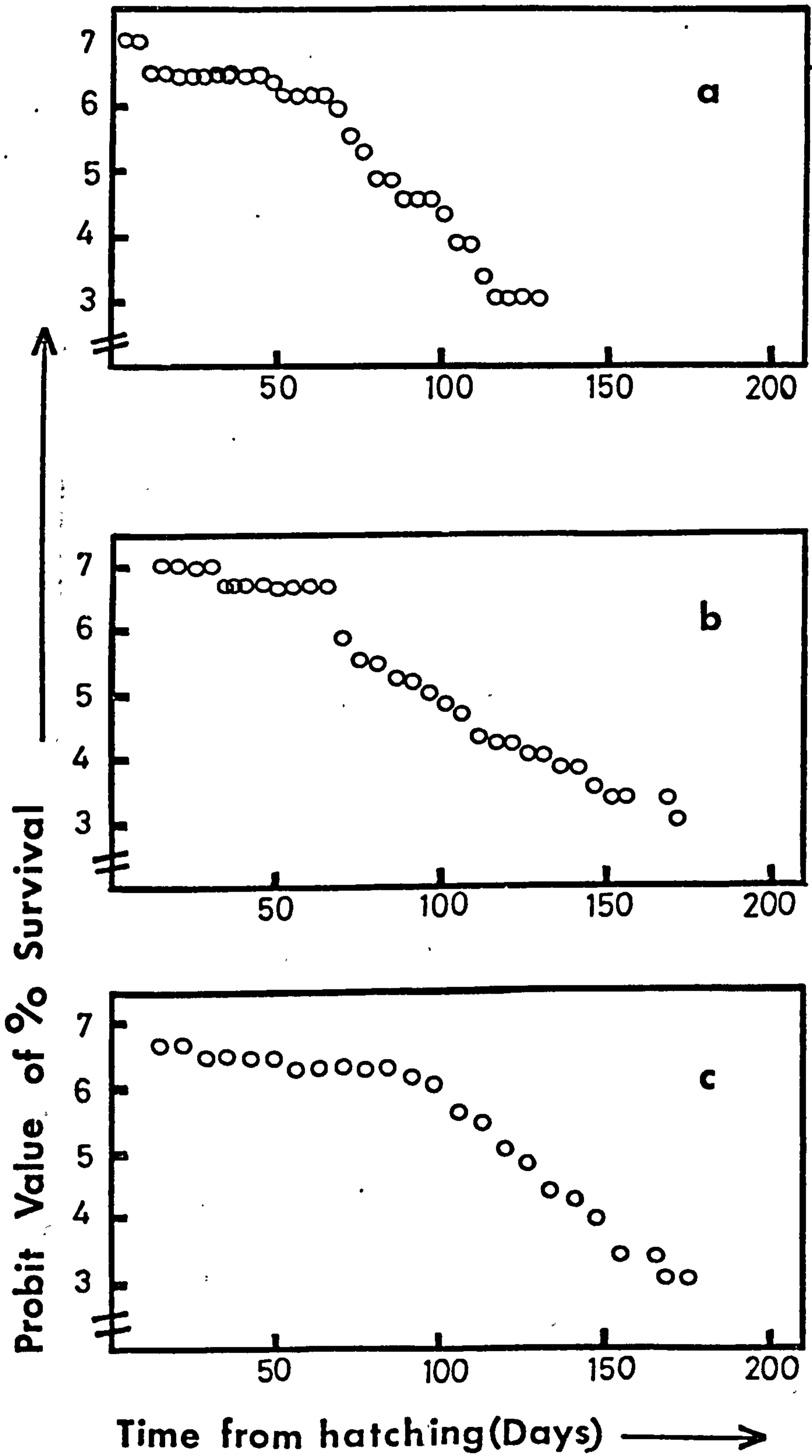


TABLE 1:5

Temperature Regime	Survival Time (Estimated time from hatching to 50% Mortality in days)
25°C	80
20°C	98
16°C	121
10°C	270
5°C	600
Day/Night	230

The Survival Time, in days, for the Snails maintained at the six Temperature Regimes.



effect of experimenter error. In the hatching experiments the eggs and masses were not physically touched, while in the snail survival experiments the snails were handled, i.e. measured and transferred to fresh algal cultures; thus some premature deaths may have been caused by physical damage to the snails. These premature deaths may account for the observed "flat initial stage" of the probits (Figures 1:3a to 1:3f).

If the values from Table 1:5 are plotted, then a negative exponential results - Figure 1:4. To investigate the relationship further, the reciprocal values of the survival times were calculated and plotted against temperature - Figure 1:5. Linear regression (Bailey: 1968) indicates a positive relationship between the reciprocal of the survival time and temperature described by the following equation:

$$y = xb + c$$

where  $y$  is the reciprocal of the survival time,  $x$  the constant temperature and  $b$  and  $c$  constants:  $b$  the slope of the line - numerical value of 0.000489 and  $c$  the intercept on the  $y$  axis - numerical value of 0.00000072.

### Reproduction

The plots of the summation of egg masses produced per snail, for each of the six temperature regimes investigated against time (days since experiments started), are given in Figure 1:6. Estimates of the oviposition rate, i.e. the egg masses produced per snail per day, were obtained by calculating the slope of each plot using simple linear regression (Bailey: 1968). These oviposition rates for the total investigations (i.e. until all the snails died) and up to the 50%

FIGURE 1:4 Plot of Estimated Survival Time, Days from Hatching to 50% Mortality against temperature °C. (See Table 1:5).

Fig.1:4

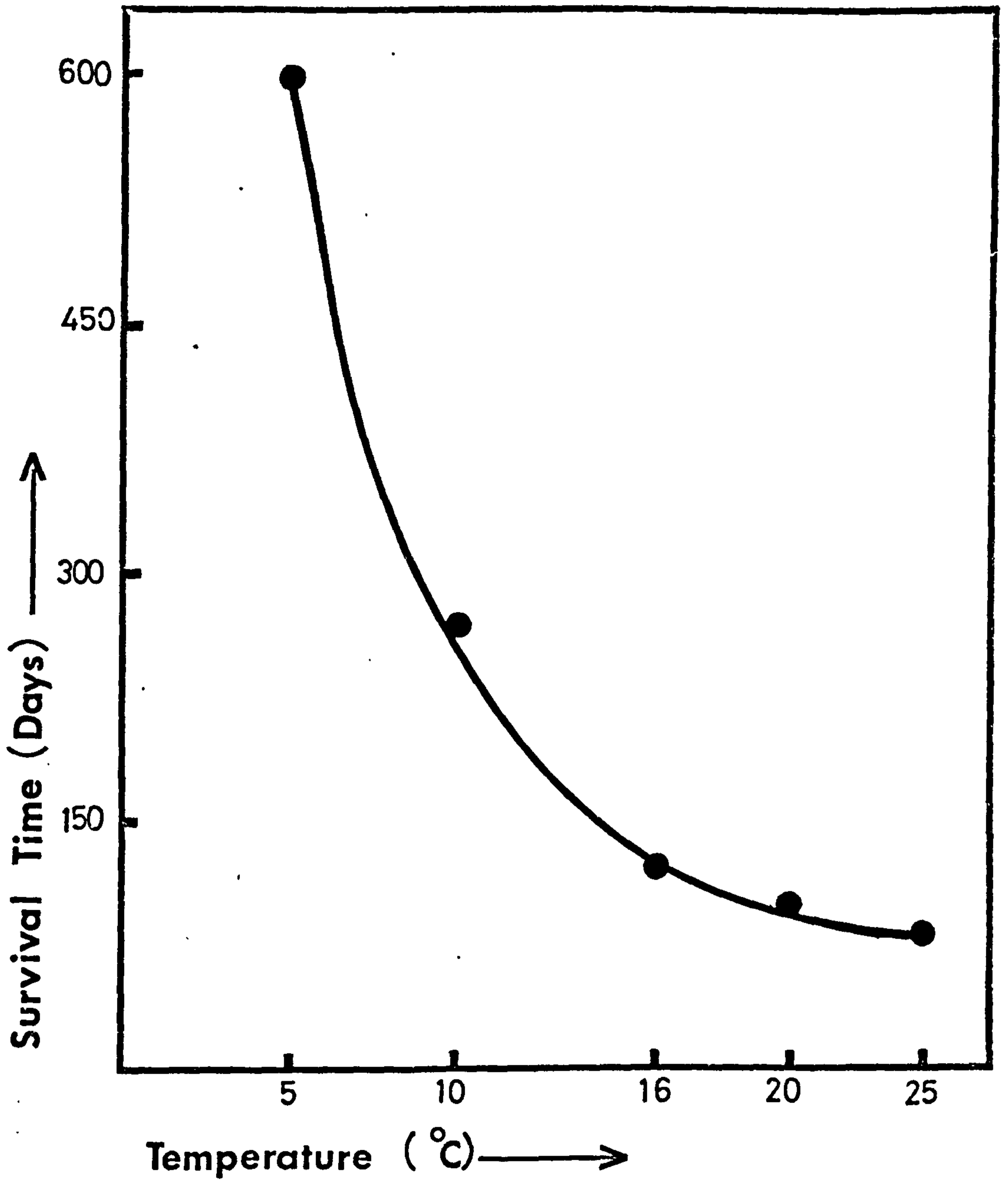


FIGURE 1:5 Plot of Reciprocal of Survival Time against Temperature °C.

$s = 0.0004472$   
 $t = 17.34$  with 3 degrees of freedom  
therefore significantly different from  
 $b = 0$ . (99.9%).  
Calculations from Bailey (1968)

Fig. 1:5

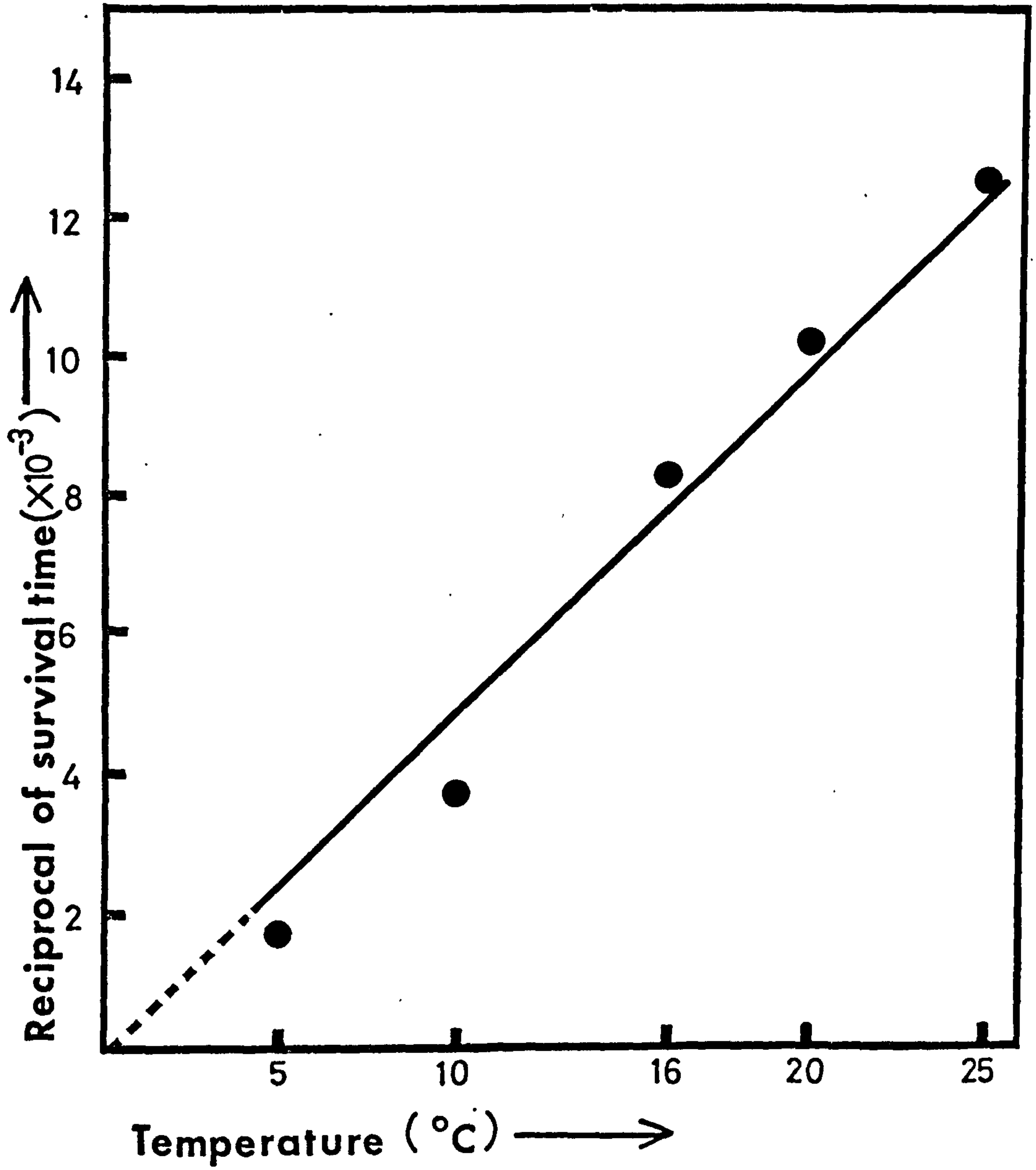
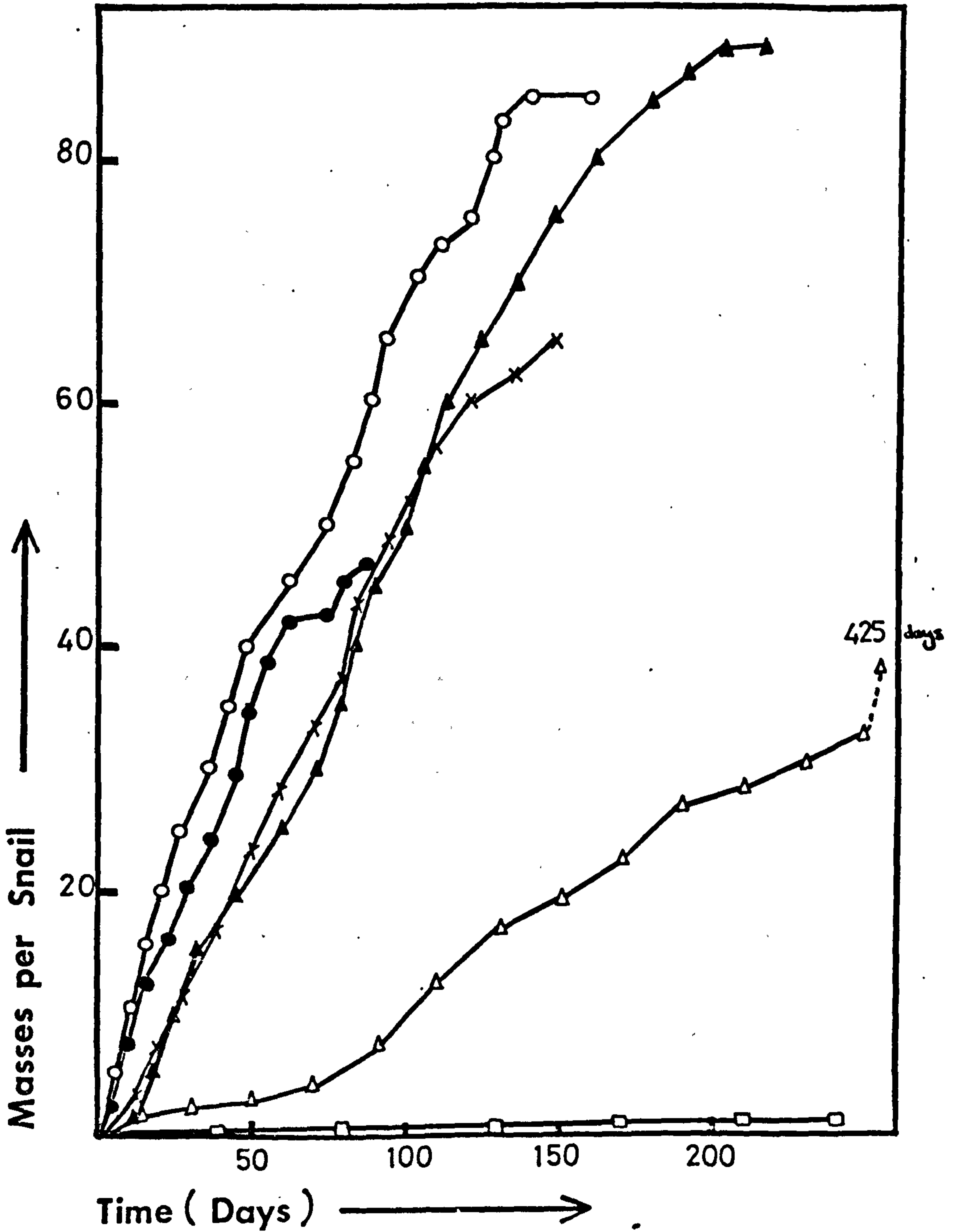


FIGURE 1:6 Plots of Summation of Egg Mass Production per Snail against Time in Days.

□ = 5°C  
△ = 10°C  
▲ = 16°C  
○ = 20°C  
● = 25°C  
X = Day/Night

Fig.1:6



mortality value (i.e. up until 50% of the snails had died) are given in Table 1:6. From these results it would appear that:

- a) at a constant temperature of  $5^{\circ}\text{C}$  oviposition is almost totally inhibited.
- b) at a constant temperature of  $10^{\circ}\text{C}$  the oviposition rate is approximately one order of magnitude greater than that at  $5^{\circ}\text{C}$ .
- c) at the three remaining constant temperatures ( $15^{\circ}$ ,  $20^{\circ}$  &  $25^{\circ}\text{C}$ ) the oviposition rates reach a maximum approximating to the production of a little over one egg mass by each snail every two days.
- d) with the Day/Night temperature regime the oviposition rate is considerably higher than the sum of half the rate at  $20^{\circ}\text{C}$  plus half the rate at  $5^{\circ}\text{C}$  (either 0.317 masses per snail per day at 50% mortality or 0.301 masses per snail per day at 100% mortality), the rate being very close to a production of one egg mass per snail every two days.

The plots of the summation of eggs produced per snail, for each of the six temperature regimes investigated against days (since experiments started), are given in Figure 1:7. Estimates of the reproductive rate, i.e. the eggs produced per snail per day, were obtained by calculating the slope of each plot using simple linear regression (Bailey: 1968). The reproductive rates for the total investigations (i.e. until all the snails died) and up to the 50% mortality value (i.e. up until half of the snails had died) are given in Table 1:7. From these results it would appear that:

- a) at a constant temperature of  $5^{\circ}\text{C}$  the egg production is almost totally inhibited.
- b) at a constant temperature of  $10^{\circ}\text{C}$  the reproductive rate is 150 times greater than at  $5^{\circ}\text{C}$ .



FIGURE 1:7 Plots of Summation of Eggs Produced Per Snail against Time in Days.

□ = 5°C  
△ = 10°C  
▲ = 16°C  
○ = 20°C  
● = 25°C  
X = Day/Night

Fig. 1:7

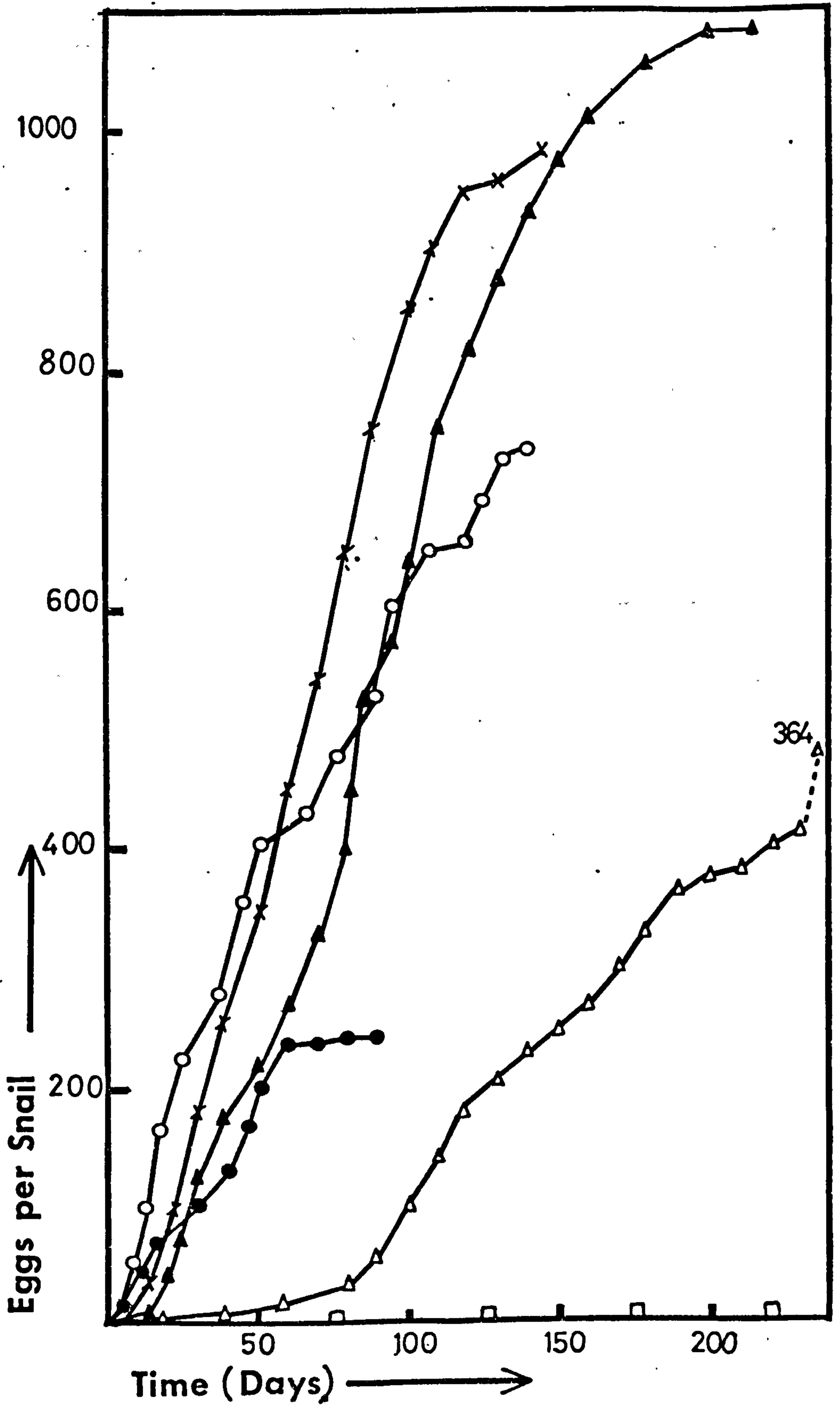


TABLE 1:6

Temperature Regime (°C)	Oviposition rate (masses produced per snail per day)	
	To the 50% Mortality value	The total Investigation
5	0.012	0.015
10	0.135	0.122
16	0.546	0.491
20	0.621	0.587
25	0.671	0.542
Day/Night (20/5)	0.499	0.492

The Oviposition Rate, masses produced per snail per day, to the 50% Mortality Value and for the Total Investigation at each of the six Temperature Regimes.

TABLE 1:7

Temperature Regime (°C)	Reproductive Rate (eggs produced per snail per day)	
	To the 50% Mortality value	The total Investigation
5	0.013	0.010
10	1.897	1.669
16	7.303	6.352
20	5.560	5.090
25	3.823	3.033
Day/Night (20/5)	7.957	7.801

The Reproductive Rate, eggs produced per snail per day, to the 50% Mortality Value and for the Total Investigation at each of the six Temperature Regimes.

- c) of those investigated the constant temperature of  $16^{\circ}\text{C}$  is the optimum constant temperature for egg production.
- d) with the Day/Night temperature regime the reproductive rate is considerably higher than the sum of half of the rate at  $20^{\circ}\text{C}$  plus half of the rate at  $5^{\circ}\text{C}$  (either 2.787 eggs per snail per day at 50% mortality or 2.550 eggs per snail per day at 100% mortality), the rate is even higher than the rate observed at the constant temperature of  $16^{\circ}\text{C}$ .

The range of the number of eggs per mass is very large, from zero to 36; the largest mass was produced by a snail exposed to the Day/Night temperature regime.

The plots of the mean number of eggs per mass produced over variable periods (constant for each temperature regime: 2 days at  $25^{\circ}\text{C}$ , 4 days at  $20^{\circ}\text{C}$ , 6 days at  $16^{\circ}\text{C}$ , 10 days at  $10^{\circ}\text{C}$  and 3 & 4 days at the Day/Night regime) against time: in days since the start of the experiment to the time of 50% mortality are given in Figures 1:8a to 1:8e.

Included with these are the plots of the mean maximum shell lengths of the snails producing these eggs, also against time: in days since the start of the experiment. From these plots the general trend would appear to be as follows:

The mean number of eggs per mass increases with time, reaching a peak value, and then decreases as the snails die.

This increase in the mean number of eggs per mass corresponds to the period of rapid increase in the mean maximum shell length, and the peak corresponds to the period when the mean maximum shell length first reaches a plateau.

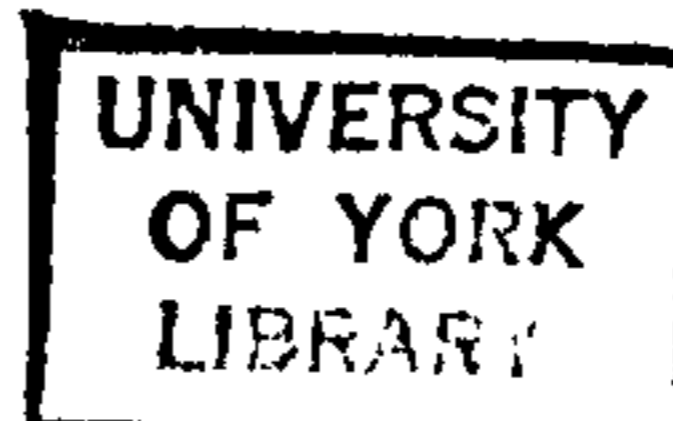
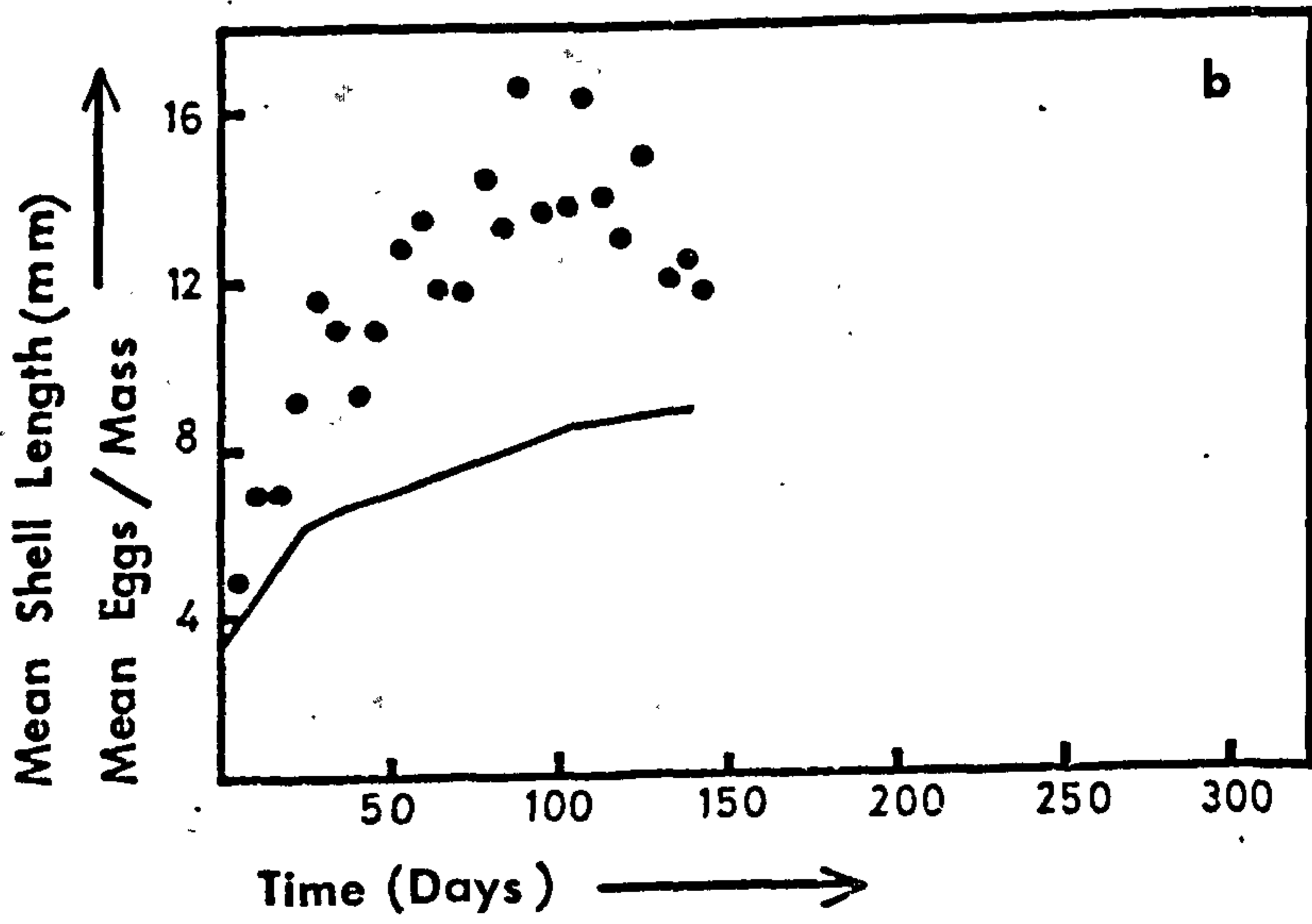
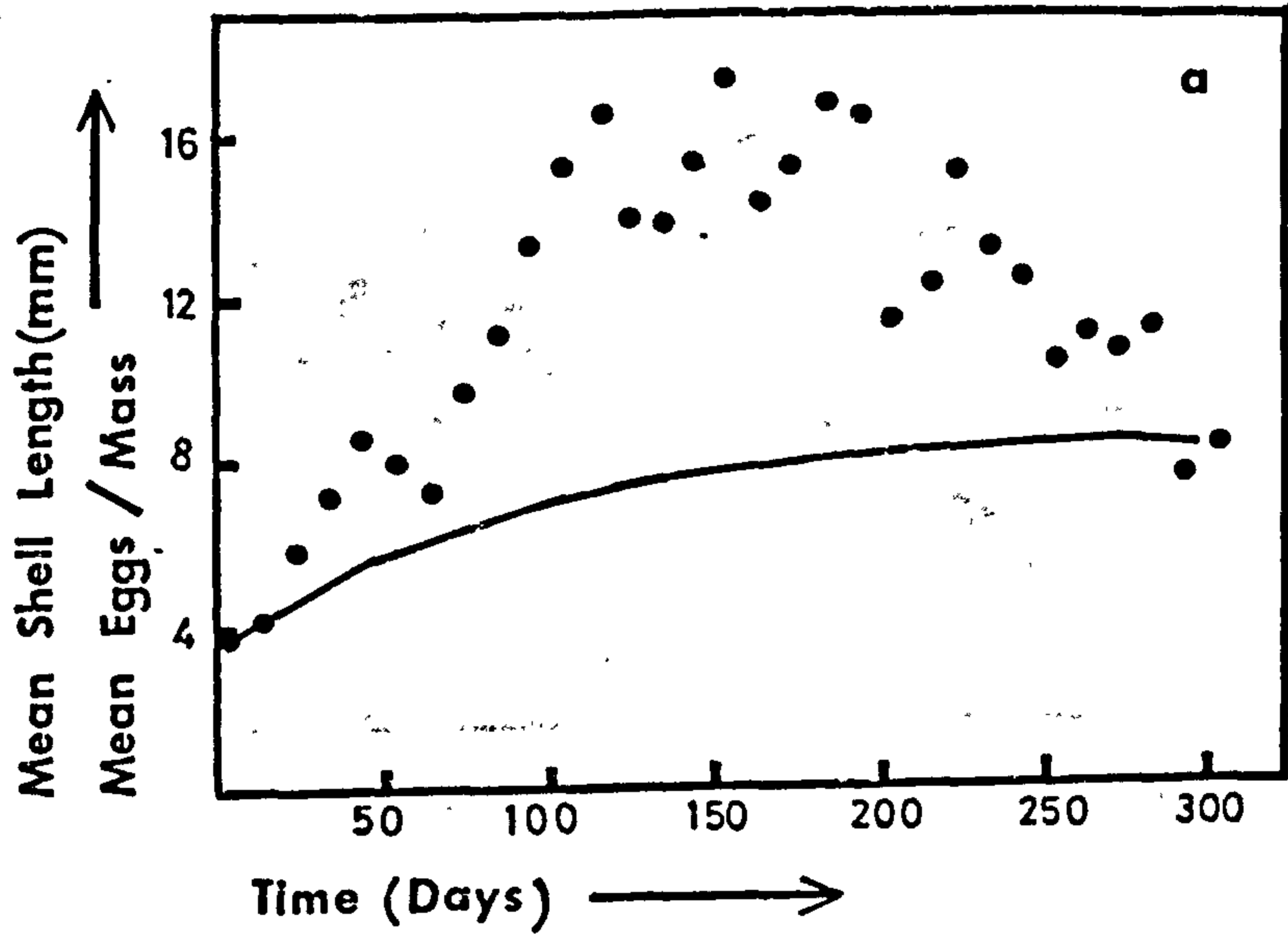


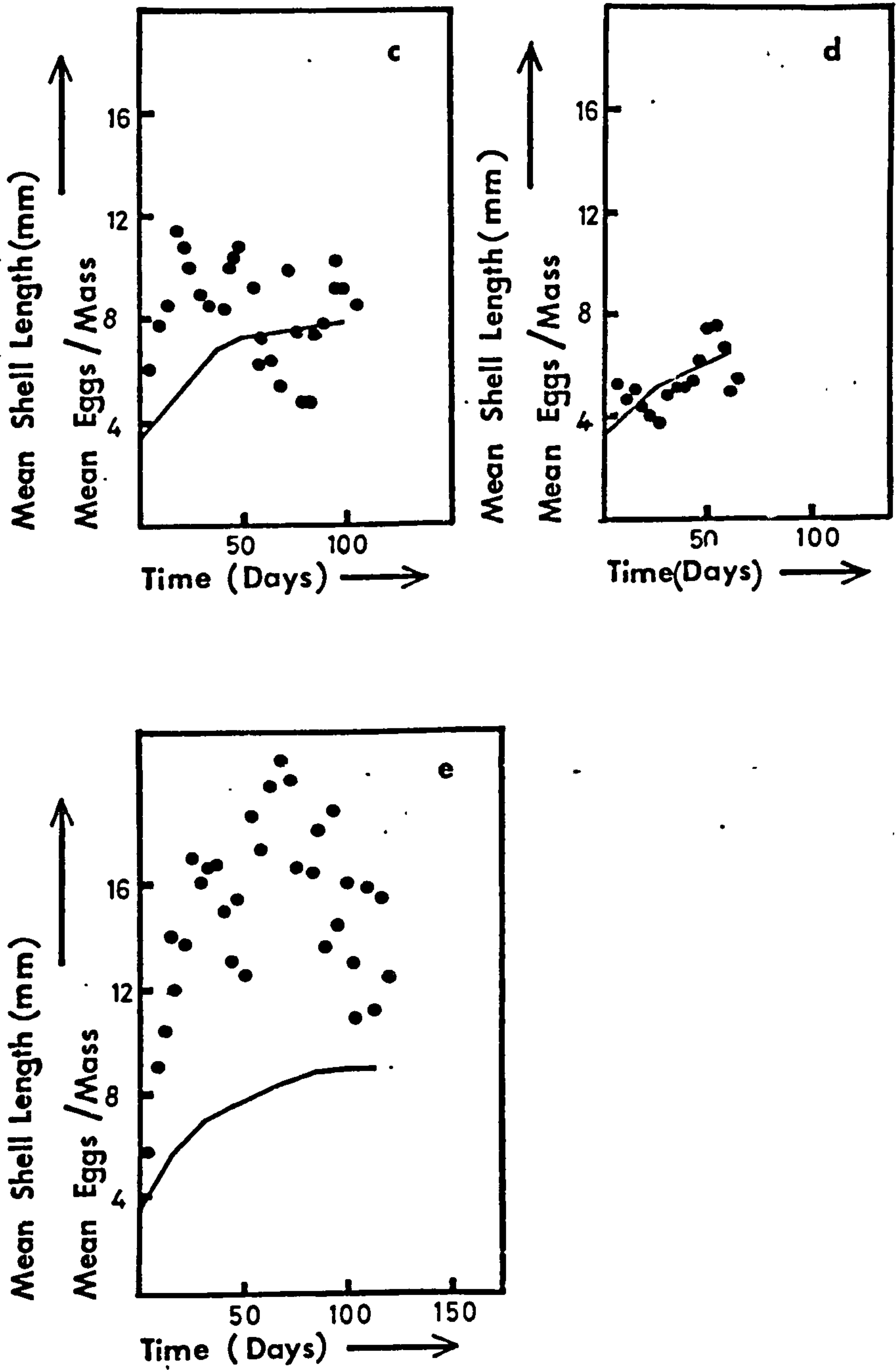
FIGURE 1:8 Plots of the Mean Eggs per Mass against Time in Days - points. Plots of the Mean Shell Length (mm) against Time in Days - single line.

- a - 10°C
- b - 16°C
- c - 20°C
- d - 25°C
- e - Day/Night

Fig. 1:8



Fig,1:8





The optimum constant temperatures for the production of large egg masses, from these plots, are 10° and 16°C; while at the Day/Night temperature regime larger still mean values are observed, this is in agreement with the earlier findings that at the Day/Night temperature regime the highest reproductive rate (eggs produced per snail) was observed.

### DISCUSSION

The results obtained from maintaining eggs of Lymnaea truncatula at constant temperatures, Table 1:1 and Figure 1:1, indicate that the rate of development of the egg is directly dependent upon the environmental temperature, at least over the range 10° to 25°C. This relationship can be described mathematically as the equation:

$$DR = Tb + c$$

where DR is the development rate at the temperature T°C and b & c are constants: b is the slope of the line and c is the intercept on the y axis (the development rate axis). The numerical values of the constants are: b = 0.005074 and c = -0.019793.

The findings of Rowcliffe and Ollerenshaw (1960), concerning the effect of temperature on the development of the egg phase of the parasite Fasciola hepatica, provide a precedent for the formulation of a linear relationship between temperature and the rate of development over a specific temperature range.

It must be stated, before discussing the use of this relationship between temperature and the rate of development of the snail egg, that its formulation has involved both experimental and mathematical error. The temperature measurements are only accurate to a value of  $\pm 1^\circ\text{C}$  and

the time to 50% hatching to the nearest half day, also the linear regression was executed with only 4 datum points. However, the results obtained experimentally, plus the values given by Roberts (1950) and Kendall (1953) when compared with estimates calculated using the equation (Table 1:8), indicate the validity of the equation in predicting the time in days to 50% hatching, for only one value (17°C Roberts, 1950) is outside the range calculated using a range of  $\pm 1^\circ\text{C}$  on the specified temperature.

If the assumption is made that the rate of development of the snail egg at a specified temperature is constant throughout the egg phase, then the above equation can be used to estimate the time to 50% hatching under a fluctuating temperature regime using the following method:

the proportion of each day spent at each specified temperature is calculated; then using these temperature values and the equation, describing the rate of development with respect to temperature, the amount of development that occurs in each day is determined. The daily values of development are then added in chronological order until their sum is equal to the numerical value of full development (i.e. one). The time to 50% hatching is thus the number of daily values needed to produce full development.

The Day/Night temperature regime of 12 hours at 20°C and 12 hours at 5°C is a simple fluctuating temperature regime. The predicted time to 50% hatching is calculated by summing a daily value of half the development rate at 20°C and half the development rate at 5°C, until the value reaches one, i.e. full development.

TABLE 1:8

Temperature (°C)	Experimentally determined values of the time to 50% hatching (in days)	Values of the time to 50% hatching estimated from the equation (in days)	Range if allow $\pm 1^{\circ}\text{C}$ (in days)
10°C	31.5	32.3	27.8 to 38.7
16°C	17.5	16.3	15.1 to 17.8
20°C	11.5	12.2	11.5 to 13.1
25°C	9.5	9.3	8.9 to 9.8
17°C *	17 to 22	15.1	14.0 to 16.3
25°C *	8 to 12	9.3	8.9 to 9.8
11°C **	29	27.8	24.3 to 32.3
16°C-21°C **	12 to 13	11.5 to 16.3	10.9 to 17.8
21°C-30°C **	11 to 12	7.5 to 11.5	7.2 to 12.2

\* Roberts (1950)

\*\* Kendall (1953)

The Time, in days, to 50% Hatching, Experimentally Determined, Estimated from the Derived Equation and the Range if a Variation of  $\pm 1^{\circ}\text{C}$  is allowed, for the experiments described in this Chapter and from the data given by Roberts (1950) and Kendall (1953).

Unfortunately, at a constant temperature of 5°C the snail eggs failed to hatch, thus this poses a question concerning the effect a temperature of 5°C has on the egg. If the inhibitory effect of this low temperature on the development of the egg is total then the development rate should be zero and thus the numerical value for the development rate at the Day/Night temperature regime will be half of that at 20°C; using the experimentally determined value for 20°C (0.08696) then the estimated time to 50% hatch is 23.0 days.

However, if the effect of 5°C is only to reduce the development rate then a numerical value for the development rate at 5°C can be obtained by extrapolating the relationship (Figure 1:1) of rate of development against temperature. The numerical value is thus 0.005577, equivalent to a time to 50% hatching of 179.3 days (approximately 6 months). Using the calculated values for 20°C and 5°C the daily value will be  $\frac{1}{2} \times 0.081687 + \frac{1}{2} \times 0.005577 = 0.043632$ ; giving an estimate of 22.9 days to 50% hatching. The two estimates thus calculated (23.0 & 22.9 days) compare very favourably with the experimentally determined value of 23.0 days (Table 1:1).

To test further this predictive method a group of eggs was subjected to 24 hour periods of exposure to a constant temperature chosen at random, see Table 1:2. The time to 50% hatching was estimated to be 17.0 days  $\pm \frac{1}{2}$  day; the time experimentally determined was 17.5 days  $\pm \frac{1}{2}$  day. Thus, as with the results obtained from the Day/Night temperature regime, this method of predicting the time to 50% hatching of snail eggs exposed to fluctuating temperatures within the range 5° to 25°C from the equation describing the relationship of the rate of development to temperature would appear to be valid.

The basic effect of temperature within the range  $10^{\circ}\text{C}$  to  $25^{\circ}\text{C}$  on the development of the snail egg would appear to be of the immediate type (Grainger; 1959).

The mortality values of the eggs, maintained at constant temperatures within the range  $10^{\circ}$  to  $25^{\circ}\text{C}$ , maintained at the Day/Night temperature regime, and maintained at the single days at random temperatures, all appear to be very low, i.e. 2%; in full agreement with the statement made by Kendall (1953) "the fertility of the eggs of Lymnaea truncatula is exceedingly high"; and Olsen's (1944) observations of the related species Stagnicola bulimoides techella.

The effect of a temperature of  $5^{\circ}\text{C}$  on the development of the snail eggs was further investigated, to determine whether time spent at this low temperature had any "after-effect" (Grainger; 1959) on the eggs' development. Groups of eggs were exposed to  $5^{\circ}\text{C}$  for a period and then allowed to develop at one of the 4 constant temperatures previously investigated ( $10^{\circ}$ ,  $16^{\circ}$ ,  $20^{\circ}$  &  $25^{\circ}\text{C}$ ). The resulting times to 50% hatching and % mortality values are given in Table 1:3. If the assumption is made that time spent at  $5^{\circ}\text{C}$  suspends development, then the values of time to 50% hatching minus the time spent at  $5^{\circ}\text{C}$  should be consistent at each of the constant temperatures at which the eggs were allowed to hatch. Table 1:9 contains the values of the time to 50% hatching minus the time spent at  $5^{\circ}\text{C}$  at each constant temperature, together with the estimated range of the time to 50% hatching calculated from the equation, assuming a  $\pm 1^{\circ}\text{C}$  range for each constant temperature.

Before the information contained in Table 1:9 can be used to either indicate acceptance or rejection of the hypothesis; that time spent at a

TABLE 1:9

Constant Temperature	Estimated range of time to 50% hatching from the equation	Number of days spent at 5°C The value of the time to 50% hatching minus the time spent at 5°C					
°C	in days	in days					
		1	2	5	15	30	60
10°	27.8 to 38.7	24.5	26.0	26.0	41.5	40.0	48.0
16°	15.1 to 17.8	15.0	15.0	15.0	14.5	15.5	19.5
20°	11.5 to 13.1	12.0	12.0	12.0	11.5	12.0	11.5
25°	8.9 to 9.8	9.5	8.5	9.0	8.5	9.0	11.0

The Time to 50% Hatching for a  $\pm 1^\circ\text{C}$  Range estimated from the Derived Equation at each of the four Temperature Regimes. Also the Time to 50% Hatching minus the specified time spent at  $5^\circ\text{C}$  at each of the four Temperature Regimes.

temperature of 5°C acts only to delay the hatching of snail eggs; consideration must be given to the effect exposure to 5°C has on the mortality of the eggs (Table 1:3). If the mortality values are plotted against time spent at 5°C, Figure 1:9, then there appears to be a general trend; i.e. the longer the exposure to 5°C the greater the resulting egg mortality value. Using linear regression (Bailey, 1968) this relationship can be described as a simple linear equation; the numerical value of the slope being 1.444 and of the intercept on the % mortality axis being 2.3615.

This equation can be utilised to predict the % mortality expected to result when snail eggs are exposed to a temperature of 5°C for a single period and then allowed to develop at a temperature within the range 10°C to 25°C. The equation also indicates that the maximum exposure to a constant temperature of 5°C a snail egg can stand is 67.6 days.

Unfortunately, it is not possible to state whether the effect on snail egg mortality of exposure to 5°C is additive when more than one period of low temperature exposure is involved. However, the % mortality value obtained from the Day/Night temperature regime (Table 1:1; 1.49%) where the periods of 12 hours exposure to 5°C occurred in every 24 hours and the value obtained from the random temperature day exposure (1.38%), do not appear to indicate an additive effect, but further investigations are needed to clarify this point.

Returning to the effect a temperature of 5°C has on the time to 50% hatching and taking into account the fact that five of the values in Table 1:9 are derived from groups of eggs of which a large proportion (74.4 to 98.0%) failed to hatch, i.e. those 4 groups of eggs exposed to

FIGURE 1:9 Plot of Percentage Mortality of Snail Eggs against Time spent at 5°C in Days. Line fitted by linear regression. Points from maintenance temperature.

△ = 10°C  
▲ = 16°C  
○ = 20°C  
● = 25°C

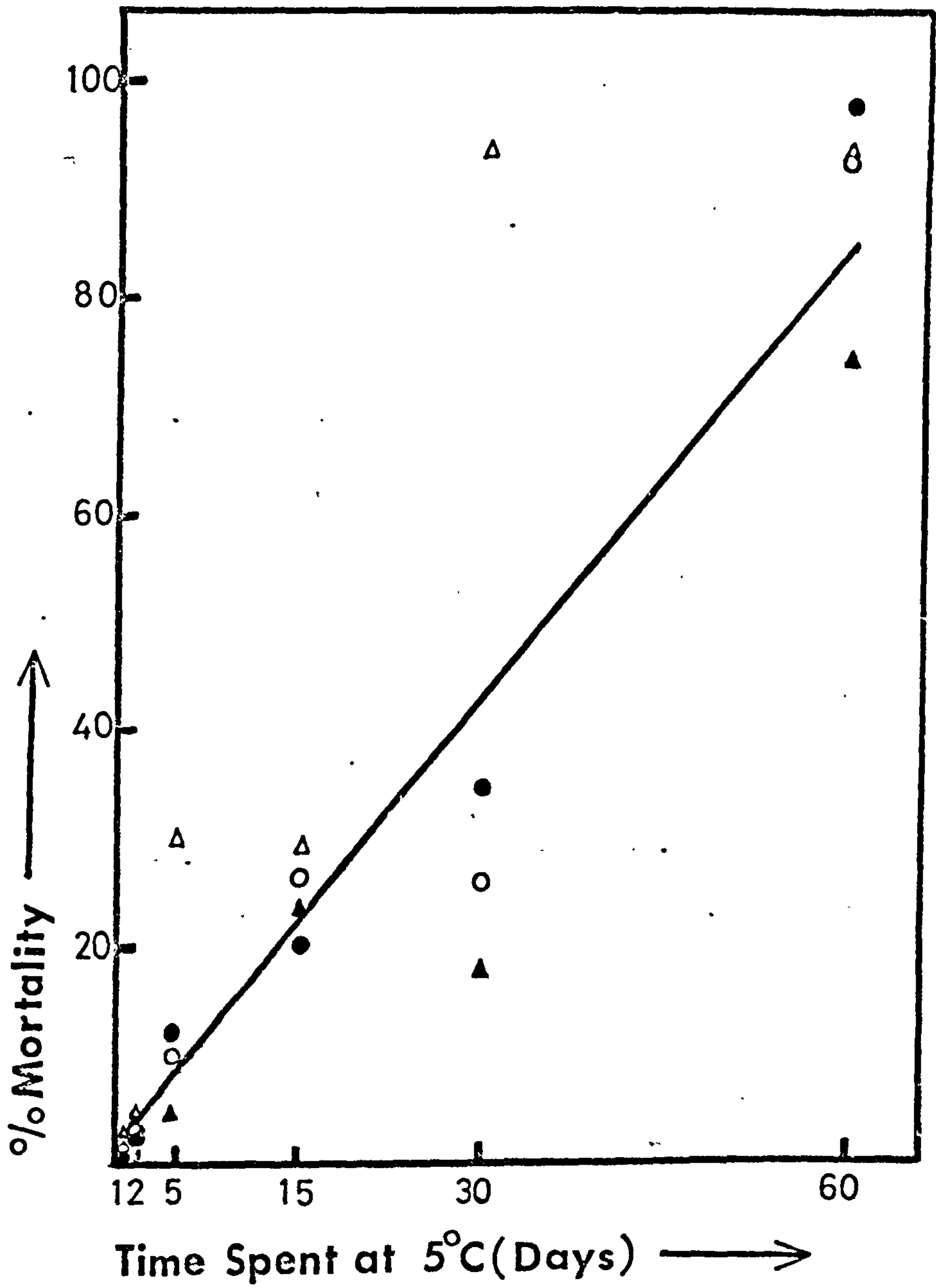
$$s = 34.556$$

$t = 4.283$  with 22 degrees of freedom  
therefore significantly different from  
 $b = 0$ . (99.9%).

Calculations from Bailey (1968)



Fig.1:9



60 days at 5°C together with the group exposed to 30 days at 5°C and then maintained at 10°C - Table 1:3; it would appear that for the 3 high temperatures (16°, 20° & 25°C) the length of time spent at 5°C had little or no effect on the subsequent time to 50% hatching, thus no "after-effect" (Grainger; 1959). However, the information obtained from the maintenance temperature of 10°C is not clear; the 3 shorter times at 5°C (1, 2 & 5 days) appear to produce a reduced time to 50% hatching than expected, while exposure to 15 days at 5°C appears to lengthen this expected time.

Taking an overview of the information obtained concerning the snail egg: the development of the egg and thus the duration of the period from oviposition to hatching is dependent upon the environmental temperature. This controlling action of temperature within the range 10° - 25°C can be expressed as a simple linear equation concerning the rate of development and the temperature. This equation can be used, with some reservations, to predict the hatching of eggs exposed to a specified temperature regime. The reservations concern the observed effect which a temperature of 5°C has on the eggs' survival; prolonged exposure to a constant temperature of 5°C (15 days) considerably affects the snail's ability to hatch. That is to say, the longer the period of exposure to this low temperature the higher becomes the % mortality value.

This final point concerning the effect of a temperature of 5°C indicates that the "large populations of small snails which appear in early spring in apparently adult-free habitats" Walton (1918), are very unlikely to be the result of over-wintering eggs.

Length of shell has been used in many studies of life histories of Mollusca, since it furnishes a definite, easily measurable index of growth, for example Vaughn (1953) and McCraw (1970). The results obtained in this study, by measuring the maximum shell length of snails maintained at defined temperatures from hatching to death, Figure 1:2 and Table 1:4, indicate that within the range 5° to 20°C, the growth of Lymnaea truncatula is directly affected by the environmental temperature. This is in agreement with the findings of Vaughn (1953) investigating Lymnaea stagnalis temperature range 11° to 28.2°C and Michelson (1961) investigating Australorbis glabratus temperature range 5° to 30°C.

The plots of mean shell length against time at the following temperature: 25°, 20°, 16°, 10°C and 20°/5° (Day/night), Figure 1:2, indicate that the snail growth expressed as increase in shell length, approximates to the middle and upper sections of a logistic plot, thus giving two rates of growth: an initial rapid value and a much reduced value as the mean shell length reaches 7.5 to 9.0 mm. Thomas (in preparation) using the results obtained in this study states that the following logistic equation gives an excellent description of snail growth at each temperature:

$$l_t = A / (1 + Ke^{-Art})$$

where:

$l_t$  is the shell length at time  $t$

$A$  is the maximum length

$r$  is a rate constant

$K$  is a constant fixed by the initial shell length

Estimates of the value of the rapid growth rates given in Table 1:4 indicate that the optimum temperature for growth is 20°C, this concurs

with the view expressed by McDonald (1973) from results obtained by Berry and van der Schalie that 20°C is the optimum temperature for the growth of Lymnaea stagnalis, a near relative of Lymnaea truncatula.

It is of interest to note that the estimate of the initial rapid growth rate for the snails maintained at the Day/Night regime is less than half the value estimated for the constant temperature of 20°C indicating that the exposure to 5°C induces an "after-effect" as defined by Grainger (1959) which reduces the overall growth rate.

The effect of the environmental temperature, within the range 5° to 25°C, on the survival of the snail has, from the results obtained in this investigation, been shown to be negative, i.e. as the temperature increases the survival time (time in days from hatching to 50% mortality) decreases. This relationship can, within this temperature range, be described as a linear equation in which the reciprocal of the survival time depends directly on the temperature.

From this equation the estimated survival time for snails maintained at the Day/Night temperature regime is 167 days, considerably less than the actual value obtained from the experiment (Table 1:5) of 230 days. This indicates that exposure to 5°C induces an "after-effect" as defined by Grainger (1959), in this case a prolongation of life. It may also be a manifestation of the reduced growth rate observed to result under these conditions.

The reproductive capacity of Lymnaea truncatula maintained under optimum laboratory conditions can from Figure 1:7 be seen to be exceedingly high, at 16°C the summation of the mean values of egg production per

snail giving a value of over 1000 eggs produced over a lifetime.

The observation by van der Steen (1967) that in Lymnaea stagnalis the mean number of masses per snail per day and the mean number of eggs per mass represent independent functions of reproduction appears to hold true for Lymnaea truncatula. However, his observation that the mean number of eggs per mass increases as the oviposition interval increases does not hold true for Lymnaea truncatula, for from Figure 1:6 the oviposition rate is constant and thus the oviposition interval remains constant, while the mean number of eggs per mass from Figures 1:8a to 1:8e increases as the mean shell length increases (i.e. snail growth) falling only after the snail has reached its maximum length.

As expected from further suggestions by van der Steen (1967), temperature does affect the reproduction of Lymnaea truncatula, the most pronounced effect being on the egg production (Table 1:7), the oviposition rates (Table 1:6) appear to remain at a maximum value over a wide temperature range (16° to 25°C).

In this series of experiments, the highest value of eggs produced per snail observed was at the Day/Night temperature regime. This agrees in part with the findings of Hodasi (1976) that young snails exposed to 5°C for 3 months when returned to favourable temperatures produce more eggs than do controls; it would thus seem that even a short exposure to 5°C (12 hours) induces the snail to produce more eggs.

Temperature is an environmentally relevant aspect of an organism's life. It is a major parameter of virtually all biological activities, affecting chemical reaction rates which in turn affect an organism's physio-

logy and ultimately its behaviour. From the results obtained and described in this chapter it has been demonstrated that the snail Lymnaea truncatula is strongly affected by the temperature of its environment. The maximum values of development, growth, reproduction and survival determined at a range of constant temperatures covering the middle and upper range to which the snail will be exposed in its natural environment and also when the snail is subjected to a regular Day/Night temperature regime, indicate that the actual effects of temperature on the snail are complex with different temperatures giving maximum values for the aspects of its life cycle investigated. The only stage which proves to be simple to describe as a mathematical model is the egg stage; I propose that this is the only stage at which the snail's behaviour is not an important factor. The "after-effect" on growth induced by exposure to 5°C, i.e. reduction from that expected, can be explained by the low temperature not only affecting physiological/biochemical processes but also affecting the snail's behaviour, e.g. by reducing its feeding activity, which would then reduce the amount of food available to support growth. No observations were made concerning the snail's activity under different temperatures, however, Heppleston (1972) reports that the activity of Lymnaea truncatula, in the Orkneys, drops considerably at temperatures below 6°C.

INTRODUCTION

In the previous section of this Chapter the experimental investigations described all involved the maintenance of Lymnaea truncatula under conditions of excess food and moisture. In the field, however, the snails will frequently have to contend with times of drought.

The reaction of this species of pulmonate snail to reduced moisture levels (i.e. its ability to become dormant) has been described many times: for example, Thomas (1883) found that some snails could survive six weeks of drought under laboratory conditions; Mehl (1932) states that the snail can resist desiccation for up to four and a half months and Peters (1938) from field observations, estimates that the snails may be able to survive drought for three to four months.

Kendall (1949a), from his laboratory experiments, details the considerable survival potential of both young and adult Lymnaea truncatula under drought conditions. He concludes that: 1) the snails survive by entering a state of aestivation, i.e. they become inactive and both growth and reproduction cease; and 2) this non-operculate snail appears to reduce its water loss drastically by covering over its aperture by attaching itself to the mud surface or some solid object.

The after-effect of aestivation on the snail has been discussed by Hodasi (1971). In his review of the available literature he concludes that there is some considerable disagreement, mainly occasioned by the varied observations on the ability of different age groups to resist desiccation. Hodasi's own laboratory observations suggest that after

aestivation, growth in young snails becomes irregular and their reproductive capacity tends to be reduced when compared with non-aestivated control snails.

Thus, published data demonstrate that Lymnaea truncatula is able to resist drought conditions, often for considerable periods. However, no indication is given as to what constitutes drought conditions, i.e. at what soil moisture content do the snails cease to lead an active life? What, in general, is the effect on the snail of conditions between excess moisture and drought?

The aim of the investigations described in this section is to determine the growth, reproductive capacity and survival of the snails maintained on algal cultures with a range of soil moisture contents at a single constant temperature (20°C). Initially this was to be effected by maintaining snails on soils with a range of fixed moisture contents: however, after many unsuccessful attempts to obtain such controlled conditions, the snails were maintained on algal cultures whose soil moisture content was periodically determined and adjustments made to keep them within the five different limited ranges.

#### MATERIALS AND METHODS

##### Biological Material, Controlled Temperature Conditions, Light Regime and the Preparation of the Algal Cultures

The newly hatched Lymnaea truncatula, the constant temperature of 20°C ( $\pm 1^\circ\text{C}$ ) and the 12 hours light/12 hours dark diurnal regime were obtained as described earlier in this Chapter. The algal cultures were prepared as described in detail, earlier in this Chapter, differ-



ing only in that the crystallising dishes were replaced by square flat-bottomed plastic dishes (Stewart Plastics) of area 410 cm<sup>2</sup>.

The Preparation of a Variety of Algal Cultures whose Soil Moisture Content is maintained within one of Five Different Limited Ranges

The soil moisture content of an algal culture was determined by taking a core (approximately 1.25 cm in diameter) from the algal culture (i.e. a core of soil with the algae growing on the surface), weighing it, allowing it to dry to constant weight in an oven set at 105°C. The soil moisture content is then expressed mathematically as a percentage calculated thus:

$$\left[ \text{Weight Loss (Water)} / \text{Total Wet Weight of Soil} \right] \times 100.$$

The algal cultures having excess moisture on the surface, as used for the experiments in the first section of this Chapter, were found to have a soil moisture content of > 45%. Algal cultures which were allowed to dry out, until cracks appeared and on which both young and adult snails became less active, were found to have a soil moisture content of < 30%. Thus, the 5 limited ranges of soil moisture content used for this investigation were chosen to be:

- 1) > 45%
- 2) 40-45%
- 3) 35-40%
- 4) 30-35%
- 5) < 30%

An algal culture with a specific soil moisture content was prepared by a "trial and error" method: its actual soil moisture content was determined and either aerated tap-water added or the lid of the dish removed to allow evaporation to occur at a faster rate. The new value was then determined and the necessary action taken until the required value was obtained.

These soil moisture content values are only relevant to the specific soil used in these investigations, as soil moisture content is dependent on the soil structure (Russell, 1973). To enable these observations to be compared with any obtained by maintaining snails on other soils, samples of the soil were dispatched to Dr. D. Whitehead (then working at Rothamsted Experimental Station) who determined the Soil Moisture Characteristic Curve which relates the soil moisture content to the suction pressure of the water in the soil expressed as pF; pF is  $\log_{10}$  of the suction, in cm of water (Schofield: 1935). This relationship for this soil is given as Figure 1:10.

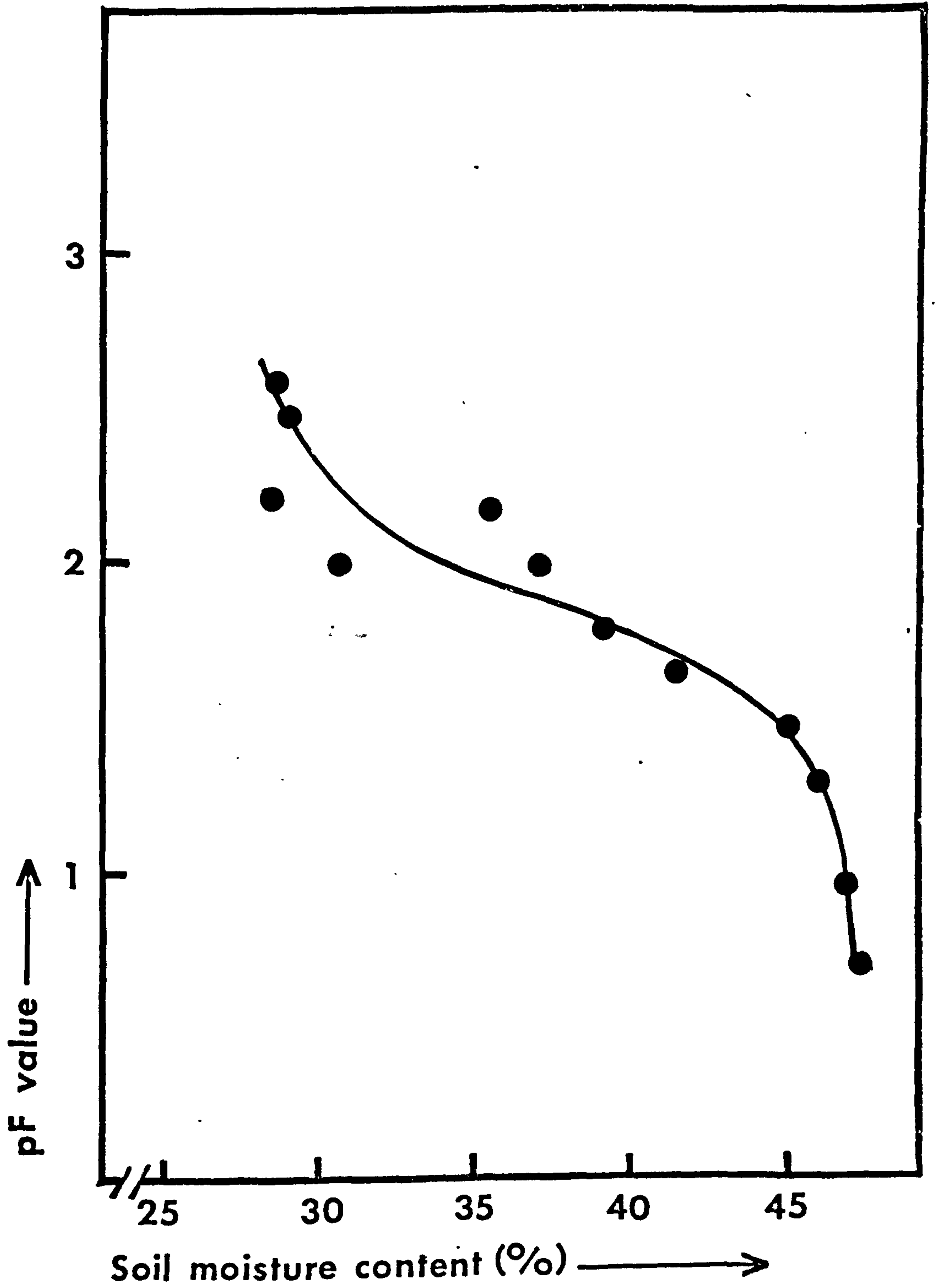
The method used by Whitehead was as follows: the soil was put into a small tray with a filter paper bottom and placed firmly on a ceramic plate and left to become saturated with water. By raising the plate and sample to measured heights above a reservoir of water, suction was applied and the resultant weight of tray and soil measured. Four days were allowed for equilibration at each height. After the final measurement was taken the sample was dried in a forced draught oven for 24 hours at  $110^{\circ}\text{C}$  and the % soil moisture content calculated (mathematical method described earlier).

It must be noted that this curve (Figure 1:10) was obtained from the drying cycle; the re-wetting cycle will show some hysteresis (Yong & Warkentin: 1975).

All the soil moisture content values detailed in this section can thus be converted to pF values from Figure 1:10.

FIGURE 1:10 Plot of pF Values against Soil Moisture Content (%).

Fig.1:10



The Determination of the Growth, Reproductive Capacity and Survival of Snails maintained on the Various Algal Cultures of Different Soil Moisture Contents

Twenty-five newly hatched snails were placed, with great care using a sable paint brush, onto the surface of the five algal cultures. Each week the following data were collected:

- a) the soil moisture content value of each dish; this was calculated by taking the mean of the soil moisture content at the beginning and end of the week.
- b) the number of snails alive (any snails which died were removed from the dish).
- c) the rate of increase in the mean maximum shell length (mm/day), the method of determining this parameter was as described previously in this Chapter.
- d) the oviposition and reproductive rates for the snails in each group. These rates were calculated as the mean number of egg masses produced per snail per day (oviposition rate) and the mean number of eggs produced per snail per day (reproductive rate).

If the soil moisture content of a dish fell below or rose above its specific range, or the algae appeared to be depleted, then the snails were transferred to another dish with a relevant soil moisture content and with a virgin surface film of algae.

This series of experiments was repeated.

## RESULTS

### The Determination of the Effect of the Soil Moisture Content of the Algal Cultures on the Growth, Reproductive Capacity and Survival of the Snail

#### Growth

The rates of increase in the mean maximum shell length (mm/day) calculated for each batch of snails (10) each week, until 50% of their number died, were grouped into those corresponding to each 1% of the soil moisture content value, (range 22% to 50%). The mean of each of these groups of rates plotted against the soil moisture content value (%) is given in Figure 1:11.

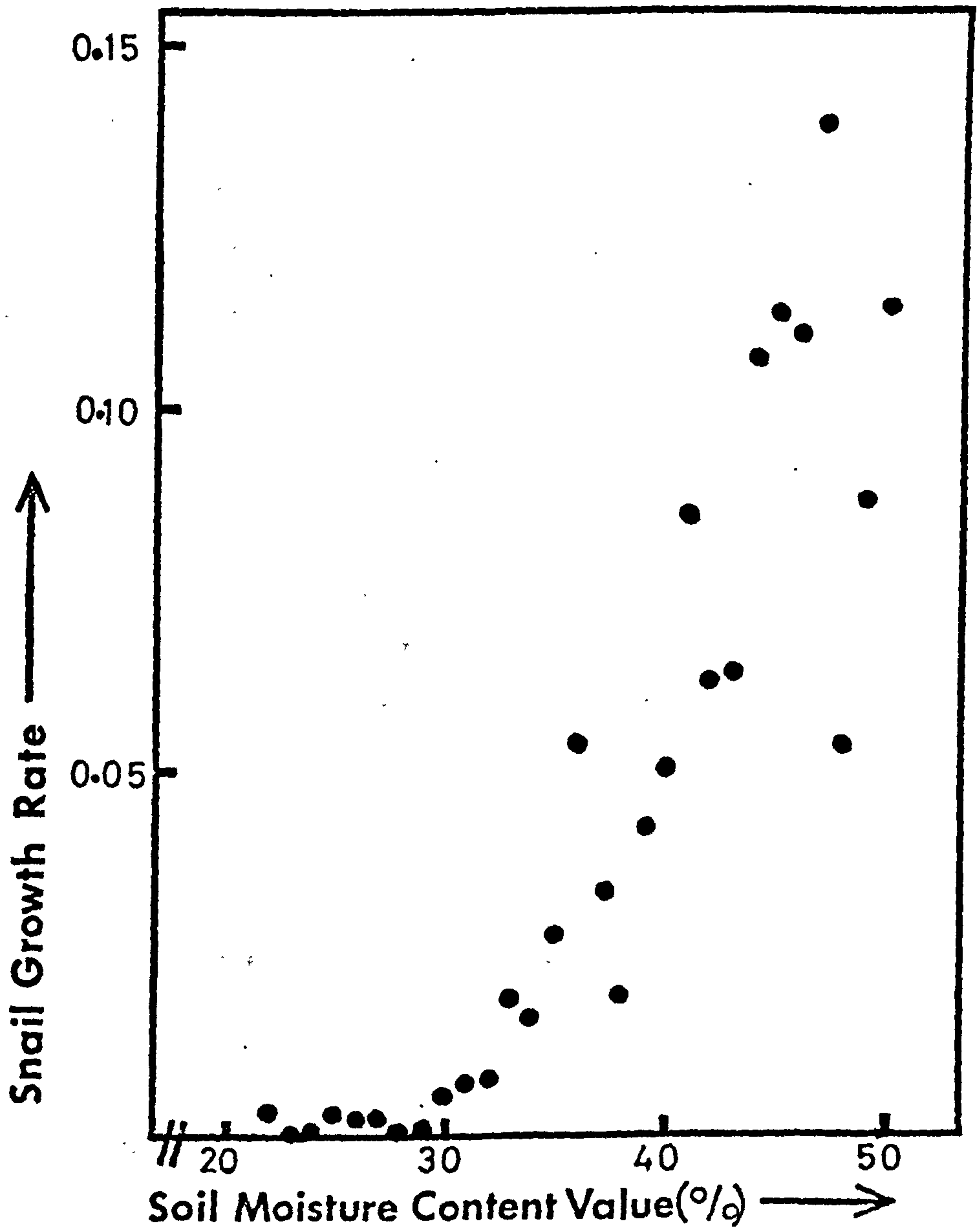
As noted in the previous section of this Chapter, increase in shell length provides a useful measure of snail growth. Thus from Figure 1:11 it would appear that the moisture content of the algal cultures has an effect on the growth of the snail. At soil moisture content values of < 30% snail growth does not occur to any appreciable extent, while at values of > 30% the snail growth increases as the soil moisture content increases, almost reaching the rate of increase in shell length estimated from experimentally maintaining snails at 20°C, under conditions of excess moisture (0.151 mm/day, from Table 1:4).

#### Reproductive Capacity

The oviposition and reproductive rates calculated for each batch of snails (respectively egg masses/snail/day and eggs/snail/day) each week, until 50% of their number died, were grouped into those corresponding to each 1% of the soil moisture content value, (range 30% to 48%).

FIGURE 1:11 Plot of Snail Growth Rate (rate of increase in the mean maximum shell length mm per day) against Soil Moisture Content Value (%).

Fig.1:11





The mean of each of these groups of rates plotted against their respective soil moisture content value (%) are given in Figures 1:12a & 1:12b. The oviposition and reproductive rates for snails maintained at 20°C, under conditions of excess moisture, as obtained from the experiments detailed in the previous section of this Chapter, are respectively in the region of 0.587 to 0.621 masses per snail per day (Table 1:6) and 5.090 to 5.560 eggs per snail per day (Table 1:7).

From Figure 1:12a it would appear that for oviposition to occur the soil moisture content value must be at least approximately 34%. Above this switchover point the rate of oviposition reaches very nearly that rate obtained from the optimum conditions of excess moisture at 20°C, it then remains at approximately this level.

From Figure 1:12b the reproductive rate naturally does not begin to rise at soil moisture content values < 34%, as there can be no eggs without egg masses. However, the subsequent rise is much slower, with no apparent switchover point, only reaching the expected maximum at soil moisture content values > 40%.

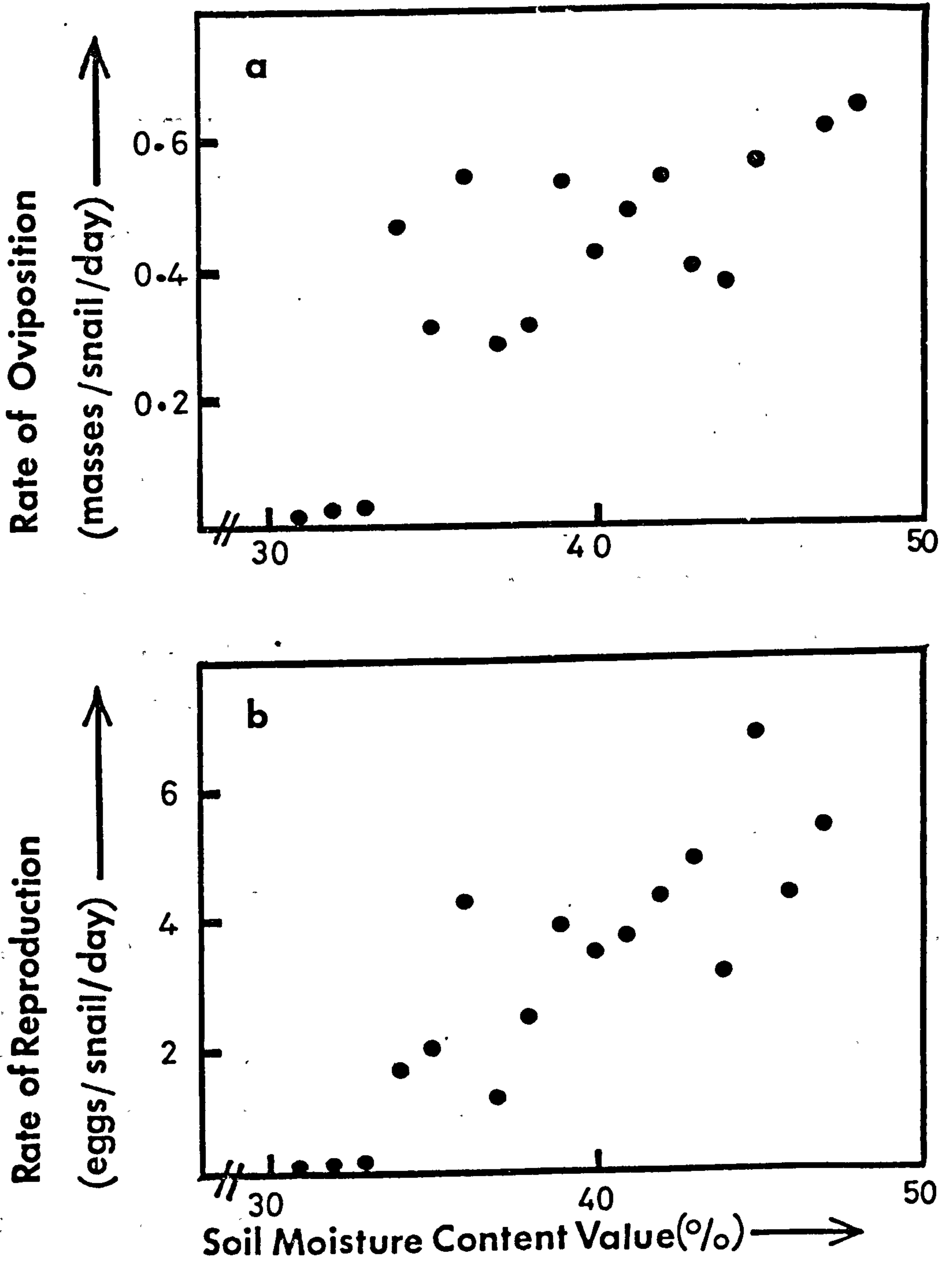
### Survival

For the ten groups of snails the time to 50% mortality was calculated from the data collected; i.e. the number of snails alive at the end of each week in each group. These values, plotted against the mean of the soil moisture content values for each group over the relevant time (i.e. to 50% mortality) are given as Figure 1:13; also included on this plot is the time to 50% mortality for snails maintained at 20°C, under conditions of excess moisture, (as determined in the previous

FIGURE 1:12a Plot of Rate of Oviposition (masses per snail per day) against Soil Moisture Content Value (%)

FIGURE 1:12b Plot of Rate of Reproduction (eggs per snail per day) against Soil Moisture Content Value (%).

Fig. 1:12



section of this Chapter (Table 1:5)) i.e. 98 days. Using this survival time as a guideline, then from Figure 1:13, soil moisture contents of <34% result in a reduced survival time, those >34% to 40% result in an increased survival time, while those >40% result in a survival time approximating to the guideline value.

## DISCUSSION

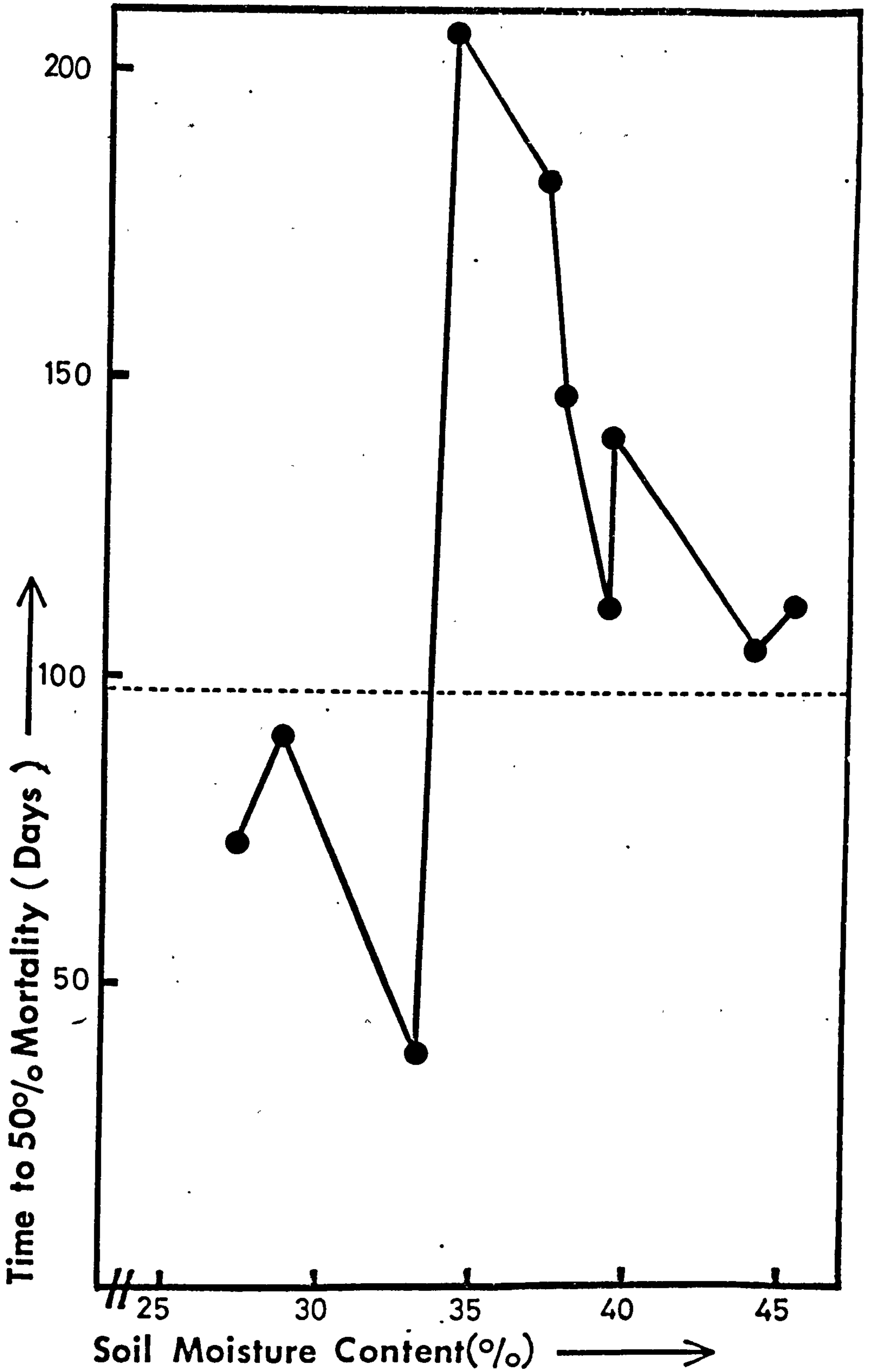
From the laboratory investigations here described it can be seen that the soil moisture content of the algal cultures, on which the Lymnaea truncatula is maintained, exerts an effect on the snail's growth, reproductive capacity and survival.

The data obtained on the growth of the snail at different soil moisture content values (Figure 1:11) indicates that at soil moisture content values of <30% (pF >2.3) snail growth is inhibited. As the soil moisture content value increases over the range 30% to 50% (pF 2.30 to <1), the snail's growth also increases, until the expected optimum level is reached, i.e. that estimated from maintaining the snails under conditions of excess moisture at 20°C.

A possible explanation of this effect is as follows: a lack of moisture induces the snail to become less active which thus results in a reduction in the food intake which, in turn, inhibits growth. At low moisture levels (soil moisture content values <30%) aestivation (i.e. total dormancy) is induced and no growth occurs. As the moisture level is raised (30% to 50%) either the periods of dormancy are shorter or the cessation of snail activity takes longer to occur, and thus some feeding takes place, resulting in some growth. At the highest moisture levels (>45% excess moisture) growth is maximised, as the snails do not enter the dormant state.

FIGURE 1:13 Plot of Time to 50% Mortality (days) against  
Soil Moisture Content Value (%).

Fig. 1:13



The data obtained on the reproductive capacity of the snail at different soil moisture content values (Figures 1:12a & 1:12b) indicate that oviposition is inhibited at soil moisture content values  $< 34\%$  ( $pF > 2.0$ ). However, at values  $> 34\%$  ( $pF < 2.0$ ) the estimated maximum rate of oviposition (Table 1:6) is reached and maintained; egg production (reproduction) is naturally inhibited at soil moisture content values of  $< 34\%$ , but unlike the oviposition rate it increases as the soil moisture content value increases reaching the estimated maximum (Table 1:7) at values  $> 44\%$  ( $pF < 1.5$ ), i.e. approximating to excess moisture.

Excluding the inhibition of all aspects of reproduction at soil moisture content values of  $< 34\%$  ( $pF > 2.0$ ), the above observations indicate that the main effect of a reduction in the soil moisture content is to reduce the reproductive capacity of the snail, by means of a reduction in the number of eggs per mass. This statement would seem consistent with the postulate made concerning snail growth, i.e. that the reduction in soil moisture content induces a reduction in the snail's feeding activity; this would then result in less material and energy being available to allow growth and/or egg production.

A comparison between the survival times of the snail at different soil moisture content conditions (Figure 1:13) and the expected survival time estimated from the snails which were maintained at  $20^{\circ}\text{C}$ , under conditions of excess moisture (Table 1:5) indicates possible effects:

- a) Soil moisture contents of  $< 34\%$  ( $pF > 2.0$ ) result in a reduced survival potential.

- b) Over the range  $>34\%$  to  $44\%$  (pF 2.0 to 1.5) there is an increased survival potential.
- c) At  $> 44\%$  (pF  $< 1.5$ ), survival is as expected from the estimated value of 98 days.

It must be noted that the data on soil moisture content given in Figure 1:13 are means of the soil moisture content values to which each group of snails was exposed: this must therefore increase the experimental error factor for this set of experiments. However, the observations do appear to be in agreement with the observations on growth and reproduction.

A possible explanation of the increased survival potential over this middle range of soil moisture contents could be as follows: the snail has a fixed length of active life, but when activity ceases, i.e. when dormant, its "life clock is switched off" and only re-starts when activity re-commences; this is modified by the effect of low soil moisture contents ( $< 34\%$ ), i.e. there is a separate fixed time to death caused by dehydration or depletion of food reserves. In the middle range of soil moisture contents there were periods when the snails were subjected to soil moisture contents near the upper limit (i.e.  $> 44\%$ ). They (the snails) were then able to replenish their body water and food reserves, thus resulting in a large increase in their survival potential.

The aim of this investigation was to study the effect on Lymnaea truncatula of various soil moisture content conditions. As with much experimental biology, it is not possible to make rigid conclusions: however, some observations and information have been obtained, at least for snails maintained under laboratory conditions. Detailed explanations



of these observations are not possible without further experiments. However, it can be postulated that the snail's reaction to conditions of low environmental moisture is behavioural; i.e. it reduces its activity and becomes dormant (aestivation) and remains in this inactive state until either the environmental moisture increases or it dies.

## CHAPTER 2.

### THE BIOLOGY OF FASCIOLA HEPATICA

#### INTRODUCTION

The life cycle of the liver fluke Fasciola hepatica can, for convenience, be divided into six phases: 1) egg, 2) miracidial, 3) intramolluscan, 4) cercarial, 5) metacercarial and 6) intramammalian. The first five phases, under normal circumstances, would be expected to be subjected to variations in temperature; the final (adult) phase would not, because its host is a homoeotherm.

The effect of temperature on the egg phase of the life cycle has been investigated by Rowcliffe & Ollerenshaw (1960) and Al Habbib, as quoted by Hope Cawdrey, Gettinby & Grainger (1978). They concluded that the rate of egg development in moist faeces-free conditions is clearly related to temperature: a direct linear relationship occurs between temperature and the reciprocal of the duration of development. This relationship is confined to the temperature range  $9.5^{\circ}$  to  $30^{\circ}\text{C}$ : above  $30^{\circ}\text{C}$  development is inhibited, and below  $9.5^{\circ}\text{C}$  development ceases.

The effect of temperature on the mortality of the eggs is found to be more complex but in general high temperatures increase the mean mortality and low temperatures aid survival. Work with Fasciola gigantica (Dinnik & Dinnik 1959), Fascioloides magna (Swales 1935) and various nematode species (Belle: 1959, and Crofton: 1965), provides agreement concerning these basic features of temperature effects.

Stirewalt (1954), investigating the effect of temperature on the infectivity of the miracidia of Schistosoma mansoni, found that by raising

the environmental temperature an increased percentage infection resulted. A possible explanation of this finding is that the higher temperatures increased the miracidial swimming speed, thus increasing the search area per unit time and in turn enhancing the chance of locating a snail host. Once the temperature exceeds an optimum value it is possible that the life-span of the miracidium will be reduced because of too rapid utilization of its food reserves, and thus its chance of locating a snail host will be reduced. Wilson & Taylor (1978), investigating the effect of temperature on the ability of miracidia of Fasciola hepatica to infect Lymnaea truncatula also found that, within the temperature range 10° to 25°C, an increase in temperature resulted in an increased level of parasitisation. Since this relationship was not exponential (as was expected), they postulate that, within this temperature range, the expected increase in parasitisation due to an increased swimming speed induced by a raised temperature is almost cancelled out by a resultant reduction in the survival time also induced by the raised temperature.

However, as the duration of the miracidial phase is very short, it would probably be of limited significance with respect to the whole life cycle (Harris & Charleston: 1971). One important limiting effect of temperature is that miracidia do not hatch at temperatures below 10°C (Ollerenshaw: 1976).

The intramolluscan phase consists of three morphologically distinct stages: 1) the sporocyst, which metamorphoses from the miracidium; 2) the redia, which develops within the sporocyst before being released, then migrates through and feeds on the host's tissue; and

3) the immature cercaria which develops within the redia and is then released into the host's haemocoel. Roberts (1950), Ollerenshaw (1971b), Nice & Wilson (1974) and Al. Habbib as quoted by Hope Cawdrey et al (1978) have shown that the rate of development of the intra-molluscan phase is directly dependent on temperature.

Fasciola hepatica cercariae reach maturity within the snail's haemocoel. The observations of Kendall & McCullough (1951) suggest that the emergence of the cercariae from Lymnaea truncatula is largely passive and that they are expelled as a result of unusual activity of the mantle causing increased pressure on the terminal part of the perivisceral space. This emergence was found to be limited to temperatures above 9°C. Above this threshold, indiscriminate emergence occurs over a wide range of temperatures. It is also suggested that other physical conditions, for example pH (within the range 5.5 - 8.5) and illumination do not appear to affect this emergence. De Jesus (1935) reported that emergence rarely occurred before noon. This is in agreement with the observations on Schistosoma mansoni parasitising Australorbis glabratus, that cercariae emerge during the period 11.00 - 17.00 (Barbosa, Coelho & Dobbin 1954). De Jesus (loc cit) was making observations on the snail Lymnaea philippinensis, the main intermediate host of Fasciola hepatica in the Philippines. This therefore does not invalidate the observations of Kendall and McCullough, since the snail Lymnaea philippinensis might react to some environmental stimulus by expelling cercariae where Lymnaea truncatula does not.

Cercariae, once free from their snail host, encyst on vegetation, if available, often forming vertical zonation with respect to the surface

of the water (Hodasi 1972a). Alternatively, encystment may occur on the surface of the water, the snail shell or on the soil surface. Cercariae remain free-swimming for only a few hours; they then require 24 hours to mature and become infective metacercariae (Boray 1963).

The metacercarial phase of the fluke life cycle forms the second resistant phase, the first being the egg. Metacercariae do not undergo any further development, remaining dormant until ingested by a suitable mammalian host, or until they die. The effect of temperature (within the range  $-20^{\circ}$  to  $+35^{\circ}\text{C}$ ) on the survival of Fasciola hepatica metacercariae has been investigated by Boray & Enigk (1964). They found that: 1) if exposed to  $-20^{\circ}\text{C}$  for 12 hours metacercariae undergo irreversible changes, becoming uninfected; 2) above  $30^{\circ}\text{C}$  the survival of metacercariae is significantly impaired; 3) over the range  $10^{\circ}$  -  $30^{\circ}\text{C}$  the survival of metacercariae is inversely dependent on temperature; and 4) when exposed to repeated freezing ( $-5^{\circ}\text{C}$ ) and thawing ( $+10^{\circ}\text{C}$ ), metacercariae remain infective for at least 70 days. These findings thus indicate that, assuming moist conditions, metacercariae are able to survive an average Western European Winter. This is confirmed by the field observations of Ollerenshaw (1967), that metacercariae encysting on herbage in September remain alive and infective throughout the winter; 50% of them able to survive until March.

Published data leaves unanswered questions about the effect of temperature on the duration of metacercarial production and on the numbers produced when Lymnaea truncatula is parasitised by Fasciola hepatica.

Table 2:1 includes the data available on the numbers of Fasciola hepatica metacercariae produced per snail. It is not possible, if

TABLE 2:1

Host Snail Species	Metacercariae per Snail		Author
	Maximum	Mean	
<u>Pseudosuccinea collumella</u>	629	278	Krull (1941)
<u>Lymnaea tormentosa</u>	3390	212	Boray (1963)
<u>Lymnaea truncatula</u>	1789	594	Hodasi (1972a)

A Summary of the published data on the Number of Metacercariae produced when three species of snail are infected with Fasciola hepatica.

valid, to compare the different sets of data available, since the snails are not of the same species and they were maintained at 'laboratory' or unspecified temperatures.

The effect of temperature on the fluke life cycle can be seen from the preceding paragraphs to be important with respect both to the rate of development and the survival of the parasite. A distinction must, however, be made as to whether the effect of temperature on these aspects of the fluke life cycle is 'immediate' (acting only during the specified conditions) or if there is an 'after-effect' (previous conditions acting in some way to modify the effect of subsequent conditions), (Grainger 1959).

From the work of Rowcliffe and Ollerenshaw (1960) and Al Habbib (as quoted by Hope Cawdrey et al: 1978) the effect of temperature on the development of the fluke egg would appear to be of an 'immediate' nature within the range  $10^{\circ} - 30^{\circ}\text{C}$ . They were, however, able to demonstrate a possible 'after-effect'; that of the inhibitory action of temperatures above  $30^{\circ}\text{C}$ . Eggs maintained at  $37^{\circ}\text{C}$  and then incubated at  $25^{\circ}\text{C}$  were found to have extended development times, this extension being directly dependent on the time spent at  $37^{\circ}\text{C}$ . This inhibitory effect of a temperature similar to the mammalian body temperature may prevent the eggs developing before leaving the host. Krull (1941) reports that fluke eggs refrigerated ( $2^{\circ} - 10^{\circ}\text{C}$ ) for  $2\frac{1}{2}$  years and then incubated at room temperature for 18 days hatch and infect their snail host successfully. If one assumes the 'room temperature' quoted to be approximately  $20^{\circ}\text{C}$ , then this finding gives confirmation of the 'immediate' effect. It also expands the range given above ( $10^{\circ} - 30^{\circ}\text{C}$ ) to

between 2° and 30°C, since 18 days is approximately the time required for the complete development of 'fresh' eggs at 20°C (Rowcliffe & Ollerenshaw, 1960).

Ollerenshaw (1971b) and Nice & Wilson (1974) make the assumption that the effect of temperature on the development of the intramolluscan phase of the fluke life cycle is of an 'immediate' type, at least over the range of temperatures encountered by the parasite in the field. However, Wilson & Draskau (1976) describe a possibly important 'after-effect' of exposure to low temperatures (10°C); that of the production of daughter rediae. The findings of Boray & Enigk (1964) would appear to indicate that within the range -10° to +30°C, the effect of temperature on the survival of Fasciola hepatica metacercariae is of an 'immediate' type. Since temperatures outside this range cause loss of infectivity, this could be termed a 'fatal after-effect'.

- Other aspects of the environment that are important when investigating the biology of Fasciola hepatica are the presence or absence of free water, and the relative humidity of the parasite's microclimate. The presence of a film of water is necessary not only for the survival of the egg but also for the hatching and survival of the miracidium, (Rowcliffe & Ollerenshaw, 1960) and successful location of the host (Wilson & Taylor: 1978). The intramolluscan phase of the life cycle is to some extent protected by its snail host from the effect of drought conditions. Kendal (1949a) reports that not only does the parasite survive within the aestivating snail but that it also undergoes development. Cercariae develop, but remain dormant within the snail until there is sufficient water present to break the snail's



state of aestivation and allow the snail to release the cercariae. The metacercariae have only limited resistance to desiccation. Their survival can be seen (Table 2:2) to be dependent on the interaction of both the relative humidity and the temperature of their microenvironment (Boray & Enigk, 1967).

The effect of the size of the miracidial exposure dose on the development of the intramolluscan phase of Fasciola hepatica has to some extent been investigated by Kendall (1949b). Individual Lymnaea truncatula were exposed either to a single miracidium or to a large (unspecified) number. Kendall concludes that retarded development (expressed as a reduction in the numbers of mature cercariae per redia) results from multiple infections.

Chu, Sabbaghian & Massoud (1966) investigated the effects of variation in miracidial exposure dose of Schistosoma haematobium infecting Bulinus truncatus. They report that: 1) the development time of the intramolluscan stages was inversely proportional to the exposure number of miracidia; 2) snails exposed to a single miracidium produced fewer cercariae than others exposed to two or more; and 3) the peak of cercarial shedding occurred 40 to 90 days after the shedding had commenced.

Massoud (1972) exposed Lymnaea gedrosiana to infection by Ornithobilharzia turkestanicum and he concluded that: 1) the number of cercariae produced when snails are exposed to 1 or 2 miracidia was lower than with 5, 10 or 20; and 2) cercarial numbers increased to a peak value at which the infected snails died.

TABLE 2:2

Temperature, (°C)	Relative Humidity, (%)	Time to Loss of Infectivity, (Days)
10	75-80	31
10	90	122
20	75-80	3
20	90	27

The Time to the Loss of Infectivity in Fasciola hepatica Metacercariae with respect to Temperature and Relative Humidity (Boray & Enigk, 1967).

The experimental work described in this chapter consists of three investigations:

- 1) The effect of temperature on the development of the intramolluscan phase of the fluke life cycle.
- 2) The effect of temperature on the duration of metacercarial production and on the numbers of metacercariae produced.
- 3) The effect of variation in the miracidial exposure dose on the parasite development during the intramolluscan phase, and also on the duration of the metacercarial production and on the numbers produced.

## MATERIALS AND METHODS

### Biological Material

Mature adult specimens of Fasciola hepatica were obtained from infected cattle livers from the York Abattoir. Eggs were teased from the uteri of the flukes and washed in distilled water. The eggs were then allowed to develop in distilled water, in the dark at a temperature of 25°C, for a minimum of 12 days.

Adult Lymnaea truncatula were obtained and maintained as described in Chapter 1 of this thesis.

The controlled temperature conditions were as described in Chapter 1.

### Infection Procedure

Fully-developed parasite eggs were centrifuged in distilled water at 3,000 r.p.m. for 30 seconds, the supernatant removed and replaced with aerated tap-water adjusted to pH 7 by the addition of distilled water.

Mass hatching of these eggs was produced by exposure to light. Individual snails were cleaned with aerated tap-water and, unless otherwise stated, exposed to 5 miracidia in a solid watch glass containing aerated tap-water, for a minimum of 2 hours at laboratory temperature ( $18^{\circ} - 22^{\circ}\text{C}$ ). The snails were then transferred to their appropriate algal dishes.

The Determination of the Maximum Development Rate of the Intramolluscan Phase with respect to Temperature

45 adult snails of similar age and shell length were individually infected, divided randomly into 3 groups of 15 snails and maintained at controlled temperatures. The individual shell lengths were recorded periodically, the groups of snails were examined daily and the onset of cercarial emergence recorded. The maximum development rate of the intramolluscan phase, expressed as the reciprocal of the time (in days) from infection to the onset of cercarial release, was calculated for the relevant temperature regimes.

The Determination of the Duration of the Metacercarial Production and the Numbers Produced with respect to Temperature

After the onset of cercarial release the groups of snails described in the previous paragraph were maintained under the same conditions. The individual shell lengths were recorded periodically and the groups were examined at least once each week. At these weekly examinations of the dishes and the snails the metacercariae produced were counted with the aid of a binocular microscope, removed and destroyed. In addition, any dead snails were removed, the shell length recorded and the snail dissected. The duration of the metacercarial production and the numbers

produced per snail at each temperature regime were calculated from these results.

The Determination of the effect of Variation in the Miracidial Exposure Dose on the Development of the Intramolluscan Phase, the Duration of the Metacercarial Production and on the Numbers Produced

100 adult snails of similar age and shell length were randomly divided into 5 groups of 20 snails. The snails in each group were then infected in the normal way, except that the miracidial exposure dose for each group was varied. The exposure doses for the 5 groups were respectively 0, 1, 2, 4 and 8 miracidia. The groups were then sub-divided into two sub-groups and maintained at 20°C. The maximum development rate of the intramolluscan phase, the duration of the metacercarial production and the numbers produced were calculated for each group as described previously.

RESULTS

The Maximum Development Rate of the Intramolluscan Phase with respect to Temperature

The values of the minimum duration of the intramolluscan phase of the fluke life cycle, i.e. the times, in days, from infection to the onset of cercarial emergence, for the constant temperatures 10°, 16°, 20° and 25°C are given in Table 2:3. Included in this table, for comparison, are the results obtained by Ollerenshaw (1971b) and Al Habbib (Hope Cawdrey et al: 1978) for constant temperatures within the range 10°C to 30°C. The three sets of results appear to be in general agreement, with the exception that development and cercarial production was observed at 10°C in this investigation, but not by Ollerenshaw (1971b) and Al Habbib.

TABLE 2:3

Constant Temperatures  °C	Days to Cercarial Emergence		
	Ollerenshaw (1971b)	Al Habbib	Own Experiments 3 groups
10	No development	No development	207, 209, 209
15	82	70	- - -
16	-	-	72, 71, 71
17.5	53	-	- - -
20	40	37	37, 39, 39
22.5	34	-	- - -
25	25	-	31, 30, 30
26	-	27	- - -
27	22	-	- - -
30	-	23	- - -

The Time from Infection to Cercarial Emergence at Constant Temperatures.

Two attempts were made to maintain an infection at the constant temperature of  $5^{\circ}\text{C}$ . The first, following the usual procedure, failed to produce an infection. The second, which was modified by maintaining the newly-infected snails at  $20^{\circ}\text{C}$  for the first 48 hours to ensure successful miracidial penetration and sporocyst establishment (Roberts, 1950), also proved unsuccessful. All the snails were dissected when they died, but none was found to contain an infection at any developmental stage.

The values of the minimum duration of the intramolluscan phase of the fluke life cycle for the simulated Day/Night investigation, were found to be 88, 92 and 88 days respectively for the 3 groups, thus giving a mean value of 89 days (89.33).

The maximum values of the development rate of the intramolluscan phase (calculated as the reciprocal of the minimum duration of the development) at the four constant temperatures, were plotted against their appropriate temperature value (Figure 2:1). Using simple linear regression the equation describing this relationship was calculated.

The equation was found to describe a line with a slope of 0.001942 and an intercept on the y axis (the rate of development axis) of -0.014944. Using an analysis of variance (ANOVA) statistical test, it is possible to test the significance of this positive relationship.

For this test the Null Hypothesis was that the slope is zero, i.e. that there is no relationship between the two variables; for this hypothesis to be accepted the F value from the ANOVA at the 1% probability level with the appropriate degrees of freedom (1 and 10) must be less than

FIGURE 2:1 Plot of the Maximum Values of the Rate of Development of the Intramolluscan Phase against Temperature °C. Line fitted by Linear Regression.

$$s = 0.001732$$

$$t = 12.3223 \text{ with 2 degrees of freedom}$$

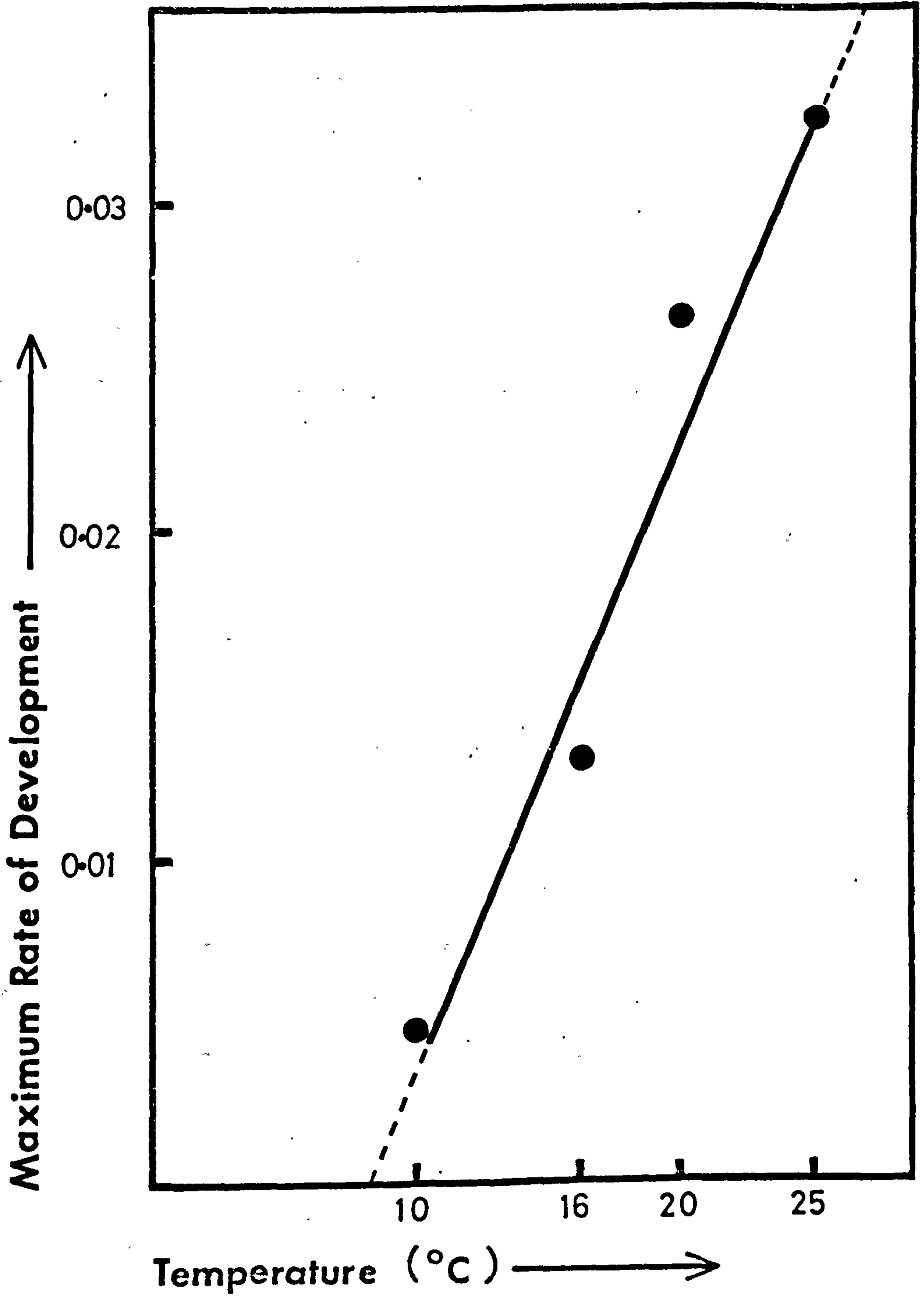
therefore significantly different from

$$b = 0. \text{ (99\%).}$$

Calculations from Bailey (1968)



Fig. 2:1



10.04 (Bailey, 1968). The F value calculated was 350.64, and thus the Null Hypothesis rejected. It may be concluded that, over a temperature range of 10 - 25°C, there is a positive relationship between the rate of development and the temperature. This relationship has been estimated by linear regression to have a numerical value of 0.001942 per degree Centigrade.

The Duration of the Metacercarial Production and the Numbers Produced with respect to Temperature

The dissection of the snails which died after the onset of cercarial emergence revealed the presence of both rediae and cercariae. This indicates that the duration of the metacercarial production from an individual snail would appear to be dependent on the survival of that snail.

The values of the duration of the metacercarial production (in days), with respect to temperature for both the 50% and the 100% mortality values of the snails in which an infection reached maturity, are given in Table 2:4.

The number of metacercariae produced per snail, summed and plotted against time for each of the 5 temperature regimes, is expressed in Figure 2:2. From these plots it would appear that the rate of metacercarial production is constant at each temperature regime.

As before, using an analysis of variance statistical test (ANOVA), it is possible to determine the significance of the relationship between the two variables (the summed value of the metacercariae produced per snail, and the time in days since infection). The calculated F

FIGURE 2:2 Plots of the Summation of the Numbers of Metacercariae Produced Per Snail against Time Since Infection, in Days, for each Temperature Regime.

$\Delta$  = 10°C  
 $\blacktriangle$  = 16°C  
 $\circ$  = 20°C  
 $\bullet$  = 25°C  
 $\times$  = Day/Night

The 50% and 100% Mortality Times for the Snails are indicated.

Fig. 2:2

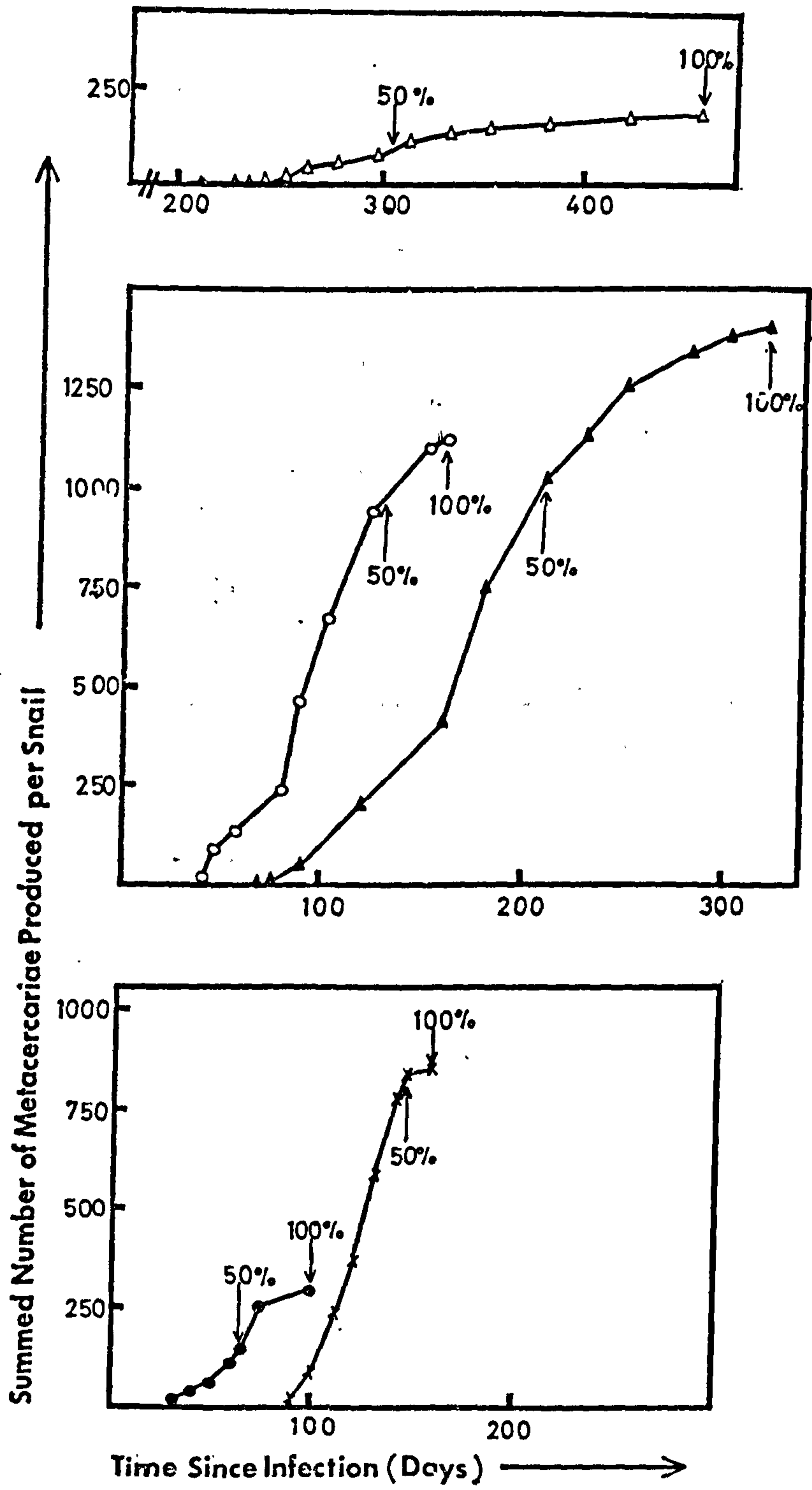


TABLE 2:4

Temperature Regime	Duration of Metacercarial Production (Days)	
	To 50% Mortality	To 100% Mortality
Constant		
10°C	94	269
16°C	138	250
20°C	88	121
25°C	35	69
Day/Night		
20°/5°C	58	68

The Duration of Metacercarial Production with respect to Temperature.

values, the appropriate degrees of freedom and the F values at the 1% probability level below which the Null Hypothesis will be accepted, are given in Table 2:5.

From the .F values in Table 2:5 it can be seen that at each temperature regime there is a relationship between the two variables which is described by the numerical value of the slope of the line as calculated by simple linear regression. The numerical value of this slope is the rate of metacercarial production per snail (metacercariae per snail per day). The values obtained by linear regression are given in Table 2:6. These values for the metacercarial production rate per snail do not indicate the existence of a simple mathematical relationship between this rate and the temperature regime.

Table 2:7 gives the mean shell length of the snails for the temperature regimes, at 3 stages: 1) the time of infection; 2) the onset of cercarial release and 3) the 50% snail mortality level.

#### The Effect of the Variation of the Miracidial Exposure Dose

The survival time (i.e. the number of days that elapsed between the infection and the death of the snail) was recorded for each snail at each of the 5 miracidial exposure doses. The mean values of these survival times are given in Table 2:8. From the calculation of the students' t values (Bailey, 1968) no significant difference was found ( $P > 0.01$ ) between the mean survival times. At the 5% probability level, those snails given an exposure dose of 4 were found to survive longer than those snails used as the controls (i.e. those snails not exposed to miracidial infection).

TABLE 2:5

Temperature Regime	Calculated F Value	Degrees of Freedom	Null Hypothesis F Value (1% P) (Bailey 1968)
Constant			
10°C	1153.75	1, 11,	9.65
16°C	288.10	1, 13,	8.29
20°C	433.31	1, 11,	9.65
25°C	286.67	1, 4,	21.20
Day/Night			
20°/5°C	808.79	1, 15,	8.68

Results from Analysis of Variance Statistical Tests on the significance of the Relationships given in Figure 2:2.

TABLE 2:6

Temperature Regime	Rate (Metacercariae produced per snail per day)	Numbers (Metacercariae produced per snail)
Constant		
10°C	0.953	89.58
16°C	7.665	1057.77
20°C	10.389	914.23
25°C	3.407	119.25
Day/Night		
20°/5°C	16.258	912.96

The Rates and Numbers of Metacercariae Produced per Snail to the 50% Snail Mortality Level with respect to Temperature.



TABLE 2:7

Temperature Regime	Mean Shell Length (mm)		
	Infection	Onset of Cercarial Release	50% Mortality Level
Constant			
10°C	3.63	7.65	7.49
16°C	3.13	8.40	9.91
20°C	3.40	7.44	9.61
25°C	3.08	6.90	8.42
Day/Night			
20°/5°C	3.60	9.20	9.78
Variation in Miracidial Numbers			
20°C	3.12	7.85	10.06

The Mean Shell Length of Snails at Infection, the Onset of Cercarial Release and 50% Mortality level, at the Various Temperature Regimes.

TABLE 2:8

Miracidial Exposure Dose (Miracidia per Snail)	Mean Snail Survival Time (Days)	Percentage Infection (%)	Minimum Duration of the Intramolluscan Phase (Days) 2 Sub-Groups	
0	125.18	0	-	-
1	151.00	90	48,	52.
2	151.30	100	48,	49.
4	161.65	100	45,	47.
8	134.55	100	46,	46.

The Mean Survival Time of Snails, the Percentage Infection and the Minimum Duration of the Intramolluscan Phase, with respect to the Miracidial Exposure Dose.

The percentage of snails infected in each of the 5 miracidial exposure dose groups is expressed in Table 2:8. The values indicate the high efficiency of the miracidia under the conditions involved in the infection procedure. In the group exposed to one miracidium per snail, the infection rate was only 90%, and so in order to enable the calculation of the metacercarial production per snail, the number of uninfected snails was subtracted from the total.

The values of the minimum duration of the intramolluscan phase of the fluke life cycle, i.e. the time (in days) from infection to the onset of cercarial emergence, for the relevant miracidial exposure dose are also given in Table 2:8. These values are not significantly different from each other when tested using an analysis of variance (Bailey, 1968): the F value was 0.920 with 3 and 4 degrees of freedom. The mean value for the duration (47.63 days) was found to be not significantly different from the value for an exposure dose of 5 miracidia per snail, predicted from the relationship given in Figure 2:1 (43.5 days): the t value was 1.742 with 7 degrees of freedom. Thus, at a temperature of 20°C, the maximum development rate of the intramolluscan phase of the fluke life cycle is not significantly affected by the exposure dose, within the range 1 to 8 miracidia per snail.

The dissection of the snails that died after the onset of cercarial emergence revealed, as before, the presence of both rediae and cercariae. Again, this indicates that the duration of the metacercarial production from an individual snail is limited by the survival time of that snail and not by the exhaustion of the redial supply.

The values of the duration of the metacercarial production (in days) to the 50% snail mortality level for the relevant miracidial exposure doses are given in Table 2:9. These values are not significantly different from each other when tested using an analysis of variance (Bailey, 1968): the F value was 1.363 with 3 and 4 degrees of freedom. The mean value for the duration (104.63 days) was found to be not significantly different from the value for an exposure dose of 5 miracidia per snail, (88 days from Table 2:4): the t value was 1.625 with 9 degrees of freedom. Thus at a temperature of 20°C, the duration of the metacercarial production is not significantly affected by the exposure dose, within the range 1 to 8 miracidia per snail.

The numbers of metacercariae produced per snail summed and plotted against time for each of the 4 relevant miracidial exposure doses are expressed in Figure 2:3. The number of metacercariae produced per snail with respect to time, for each of the 4 miracidial exposure doses, appears to be constant, as was the situation previously described at 20°C with an exposure dose of 5 miracidia per snail. As before, using analysis of variance, the F values were calculated for each miracidial exposure dose. These values, plus the degrees of freedom and the F values at the 1% probability level (Bailey, 1968) below which the Null Hypothesis (that there is no relationship between the variables) will be rejected, are given in Table 2:10. From these values, it can be seen that for each miracidial exposure dose there is a direct relationship between the two variables, which is described by the numerical value of the slope of the line calculated by simple linear regression. The numerical values of these slopes are given in Table 2:10, as rates of metacercarial production per snail.

FIGURE 2:3 Plots of the Summation of the Numbers of Metacercariae Produced Per Snail against Time Since Infection, in Days, for each Miracidial Exposure Dose.

- = 1 miracidium per snail
- △ = 2 miracidia " "
- ▲ = 4 " " "
- ◻ = 8 " " "

Fig. 2:3

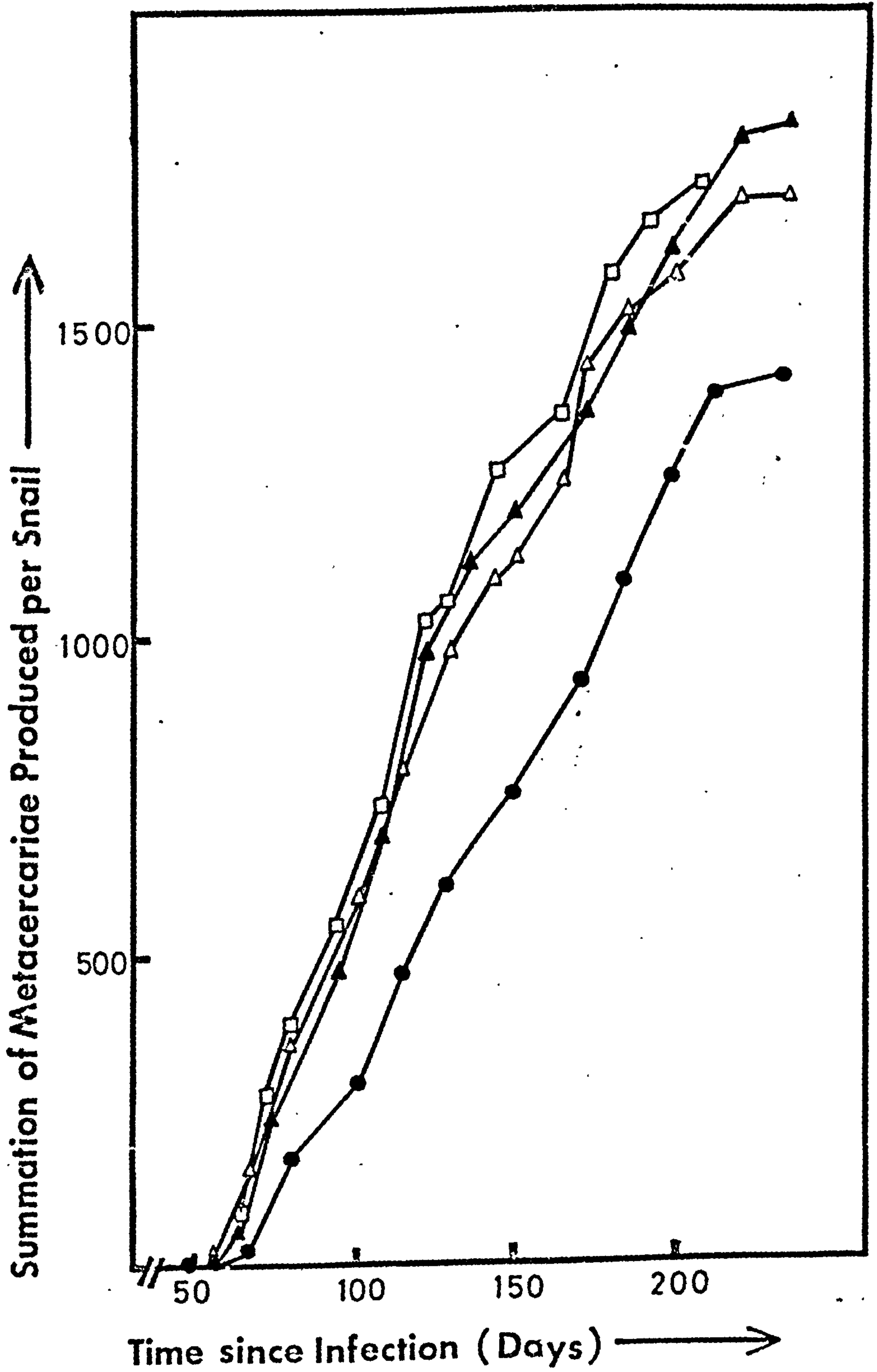


TABLE 2:9

Miracidial Exposure Dose (Miracidia per Snail)	Duration of the Metacercarial Production to the 50% Mortality Level Two Sub-Groups (Days)	
1	81,	79,
2	104,	114,
4	126,	114,
8	115,	104,

The Duration of Metacercarial Production to the 50% Snail Mortality Level with respect to Miracidial Exposure Dose.

TABLE 2:10

Miracidial Exposure Dose (Miracidia per Snail)	Calculated 'F' Value	Degrees of Freedom	Null Hypothesis 'F' Value (P 1%) (Bailey, 1968)	Slope of the Regression Line (Metacercariae produced per snail per day)
1	743.50	1, 15,	8.68	8.109
2	1028.20	1, 14,	8.86	11.971
4	570.84	1, 14,	8.86	12.619
8	878.92	1, 14,	8.86	13.468

The 'F' values calculated by analysis of variance, their degrees of freedom, the 'F' value above which the Null Hypothesis is rejected, and the Rate of Metacercarial Production per Snail; for the 4 Miracidial Exposure Doses.



The rates of metacercarial production per snail were compared using a modification of the students' *t* test, which enables the comparison of pairs of regression coefficients (Bailey, 1968). The single miracidium exposure dose was found to result in a significantly lower ( $P < 0.001$ ) metacercarial production rate per snail than the other doses; e.g. the *t* value when comparing the single miracidium metacercarial production rate with that obtained from the 2 miracidia per snail dose was 8.159 with 29 degrees of freedom. The 2, 4 and 8 miracidia per snail doses were found not to give rise to production rates that were significantly different ( $P < 0.01$ ); e.g. the *t* value when comparing the '2' and the '8' was 2.347 with 28 degrees of freedom. Thus, within the range of miracidial exposure doses of 2 to 8 per snail, the resulting metacercarial production rate per snail would not appear to be altered, but below 2 miracidia per snail the resulting metacercarial production per snail is reduced. It must be noted that a miracidial exposure dose of 2, 4 or 8 does not necessarily guarantee a successful infection by that number of miracidia.

The individual mean shell lengths for the groups of snails exposed to 1, 2, 4 and 8 miracidia per snail were found, from the calculation of students' *t* values, (Bailey: 1968) not to be significantly different. Thus, only the total mean shell length for these infected snails at the time of their infection, and estimated values of the mean lengths at the onset of cercarial release and at 50% snail mortality level are included for comparison in Table 2:7.

## DISCUSSION

The rate of development of the intramolluscan phase of the life cycle of Fasciola hepatica has been found to be directly dependent on temperature, at least over the range 10 to 25°C (Figure 2:1). The relationship can be described mathematically as the equation:

$$DR = Tb + c$$

where DR is the development rate at the temperature T°C and b & c are constants: b is the slope of the line and c is the intercept on the y axis (the development rate axis). The numerical values of the constants are: b = 0.001942 and c = -0.014977.

The findings of Rowcliffe and Ollerenshaw (1960), concerning the effect of temperature on the development of the egg phase of Fasciola hepatica, provide a precedent for the formulation of a linear relationship between temperature and the rate of development over a specific temperature range. It is of interest to note that the temperature range over which they have shown egg development to occur is very similar to the range predicted by the formulation equation for the intramolluscan phase. The ranges are respectively 9.5° to 30°C and 7.6° to 25+°C.

It must be stated, before discussing the use of the relationship between temperature and the rate of development of the intramolluscan phase, that its formulation has involved both experimental and mathematical error. The temperature measurements are only accurate to a value of  $\pm 1^{\circ}\text{C}$  and the time to the onset of cercarial release to the nearest day; also the linear regression was executed with only 12 datum-points.

If the assumption is made that the rate of development of the parasite at a specific temperature is constant throughout the intramolluscan phase, then the above equation can be used to predict the minimum time to the onset of cercarial release under a fluctuating temperature regime. It is not possible to state that this assumption is totally valid, but my findings from an investigation into the growth of the intramolluscan phase of the parasite strongly indicate the validity of this assumption (Nice & Wilson, 1974). (This investigation published with R.A. Wilson is included in this thesis as Appendix 1).

The minimum time to the onset of cercarial release under a fluctuating temperature regime can be calculated by the following method: the proportion of each day spent at each temperature is calculated from temperature data; then, using these temperature values and the equation describing the rate of development with respect to temperature, the amount of development that occurs in each day is determined. The daily values of development are then added in chronological order until their sum is equal to the numerical value of full development (i.e. one). The time is thus the number of daily values required to produce full development. For example, with a simple regime in which 12 hours of each day was spent at 20°C and 12 hours at 5°C, the daily value will be:  $\frac{1}{2} \times 0.02386 + \frac{1}{2} \times 0 = 0.01193$ . The minimum time will thus be predicted to be 84 days.

An experiment to determine the value of the minimum time to the onset of cercarial release for a fluctuating temperature regime was carried out. The temperature regime used was a simulated Day/Night regime with the first 12 hours of each day at 20°C and the remainder at 5°C. The result of this was a mean minimum time of 89 days.

Taking into consideration the experimental errors previously stated and the error in the temperature regime (i.e. that approximately 30 minutes of each day is spent in temperature transition), the resultant value gives a good indication of the usefulness of this predictive method. It is not possible to use a simple statistical test to confirm the validity of this predictive method: the small number of datum-points from which the equation was formulated, plus the existence of only 3 values to calculate the mean of the experimental result, would be beyond the scope of simple statistical methods.

The result obtained from the simulated Day/Night temperature regime gives an indication of the physiological reaction of the parasite to time spent at a temperature at which development is not expected to occur. It would thus seem that time spent at 5°C does not affect the rate of development that would be expected when the temperature changes to 20°C. Under the circumstances of this experiment there thus appears to be no after effect due to temperature (Grainger, 1959) acting on the timing of the parasite life cycle.

If the findings of Chu et al (1966) from their investigations into the parasitisation of Bulinus truncatus by Schistosoma haematobium are applied to Lymnaea truncatula parasitised by Fasciola hepatica, then it might be expected that the minimum duration of the intramolluscan phase will be reduced by an increased miracidial exposure dose. The resulting numerical values of the duration of the intramolluscan phase with respect to miracidial exposure dose (Table 2:8) might indicate a reduction in the duration with increasing exposure doses. However, statistically there is no significant reduction. If the data given by Chu

et al (1966) are examined statistically, then within a similar range of exposure doses (i.e. 1 to 10 miracidia per snail), there is also no significant reduction with increasing exposure doses; (e.g. comparing the duration values given from the single miracidia per snail with that from the 10 miracidia per snail, the  $d$  value is 0.623 with 10.02 degrees of freedom).

The postmortem dissection of each snail in which an infection reached maturity revealed the presence of both rediae and cercariae. These findings strongly indicate that the limiting factor acting on the duration of cercarial release is the survival of the snail host and not the exhaustion of the redial supply. The values of the duration of metacercarial production with respect to temperature as given in Table 2:4 must thus be reconsidered, since temperature acts not only on the parasite but on its snail host. For example, the survival of snails has been shown to be affected by temperature (Chapter 1). The values of the duration of metacercarial production as given are thus related to the resultant effect of each specified temperature. It is not really possible, if even valid, to formulate any simple relationship between the duration of metacercarial production and temperature from the data available (Table 2:4). However, it would seem that the onset of cercarial release is governed by the action of temperature in determining the parasite's rate of development, and the termination of this cercarial release by the action of temperature determining the snail's survival potential. The effect of the parasite on the survival potential of the snail host will be discussed in the following Chapter.

The duration of metacercarial production in snails maintained at 20°C

was found not to be significantly affected by variation in the miracidial exposure dose, at least within the range of 1 to 8 miracidia per snail. However, the rate at which the metacercariae were produced per snail was affected. The rate that resulted from a miracidial exposure dose of one per snail was significantly less than those rates that resulted from exposure doses of 2, 4 and 8 miracidia per snail. The possible positive relationship between miracidial exposure dose and the rate of metacercarial production indicated in Table 2:10 was found not to continue above an exposure dose of 2. The exposure doses of 2, 4 and 8 miracidia gave rise to metacercarial production rates that were not significantly different.

A possible explanation for the observation that when the miracidial exposure dose is above 2, the metacercarial production rate appears to be limited to a maximum value, is that the size of the snail host in some way limits the population size of the parasites within it. It would seem logical that the snail size limits the spatial parameters of the parasite's habitat and limits its food supply (snail tissue).

The mean shell length (a measure of snail size) was monitored throughout the life of the infected snail. The miracidial exposure dose within the range 1 to 8 per snail was found not to affect the snail shell lengths significantly. Evidence that the snail size limits the metacercarial production rate is furnished from the snail shell measurements and the metacercarial production rates observed at 20°C. The mean shell length of the snails exposed to 5 miracidia per snail over the period from the onset of cercarial emergence to the 50% snail mortality, was smaller than the mean lengths of the snails exposed to

other miracidial exposure doses, (7.44 to 9.61 mm and 7.85 to 10.06 mm respectively, Table 2:7). The metacercarial production rate of the former group of snails when compared with the range observed from snails exposed to 2 or more miracidia per snail, is again smaller (10.389 metacercariae per snail per day and 11.971 to 13.468 metacercariae per snail per day).

The mechanism by which the parasite population and thus the metacercarial production is limited cannot be determined from the investigations presented in this thesis. However, Heyneman & Umathe-ry (1968) conclude that the trematode redial stage can act as a predator which seeks out and devours the developmental stages of other flukes. Thus, multiple infections with the same parasite might self-limit the size of the parasite population.

The numbers of metacercariae produced per snail up to the 50% snail mortality level for the snails exposed to infection by 5 miracidia per snail at specific constant temperature regimes (Table 2:6) must, as with the duration of the cercarial release, be considered as the resultant effect of temperature. This temperature effect must also be considered in conjunction with the effect of snail size.

A visual representation of the action of temperature on the snail and the parasite is given as a three-dimensional graph with axes: 1) the mean snail shell length (mm); 2) time (days since infection); and 3) the total number of metacercariae produced per snail. The line 'A' is constructed by joining the 4 points corresponding to the mean shell length and the time since infection at which the cercariae first emerge, for each of the 4 constant temperatures (10°, 16°, 20° & 25°C). The

line 'B' is similarly constructed and relates time and shell length at the 50% snail mortality level. The line 'C' is constructed by joining the values of the total metacercarial production per snail up to the 50% snail mortality level for each of the 4 constant temperatures. All three lines are in the form of irregular arcs.

The line 'A' describes the resultant action of temperature on the increase in shell length (a measure of shell growth) and on the development of the parasite. The line 'B' describes only the action of temperature on the snail host, as the survival of the snail host determines the duration of cercarial release. The duration of cercarial production is given by the difference between the lines 'A' and 'B' on the Time axis. The line 'C' describes the resultant action of temperature on the total metacercariae produced per snail.

The description of the action of temperature on the snail and the parasite (Figure 2:4) is limited by the small number of temperature values investigated. However, the optimum temperature range for the production of metacercariae per snail would appear to be approximately 16° to 20°C. This estimated optimum range assumes that the snails are infected at an approximate shell length of 3.3 mm, by exposure to 5 miracidia per snail.

The rate of metacercarial production from the snails infected and maintained at the simulated Day/Night temperature regime was calculated to be 16.258 metacercariae per snail per day. This rate is rather high when compared with the other rates given in Tables 2:6 and 2:10. It would appear to be even higher if the observations of Kendall & McCullough (1951) are considered; these report that cercariae only



FIGURE 2:4 A Three-Dimensional Representation relating Mean Snail Shell Length (mm), Total Number of Metacercariae produced per Snail and Time since Infection in days at the four constant Temperatures.

$\Delta$  = 10°C  
▲ = 16°C  
○ = 20°C  
● = 25°C

Total Number of Metacercariae per Snail

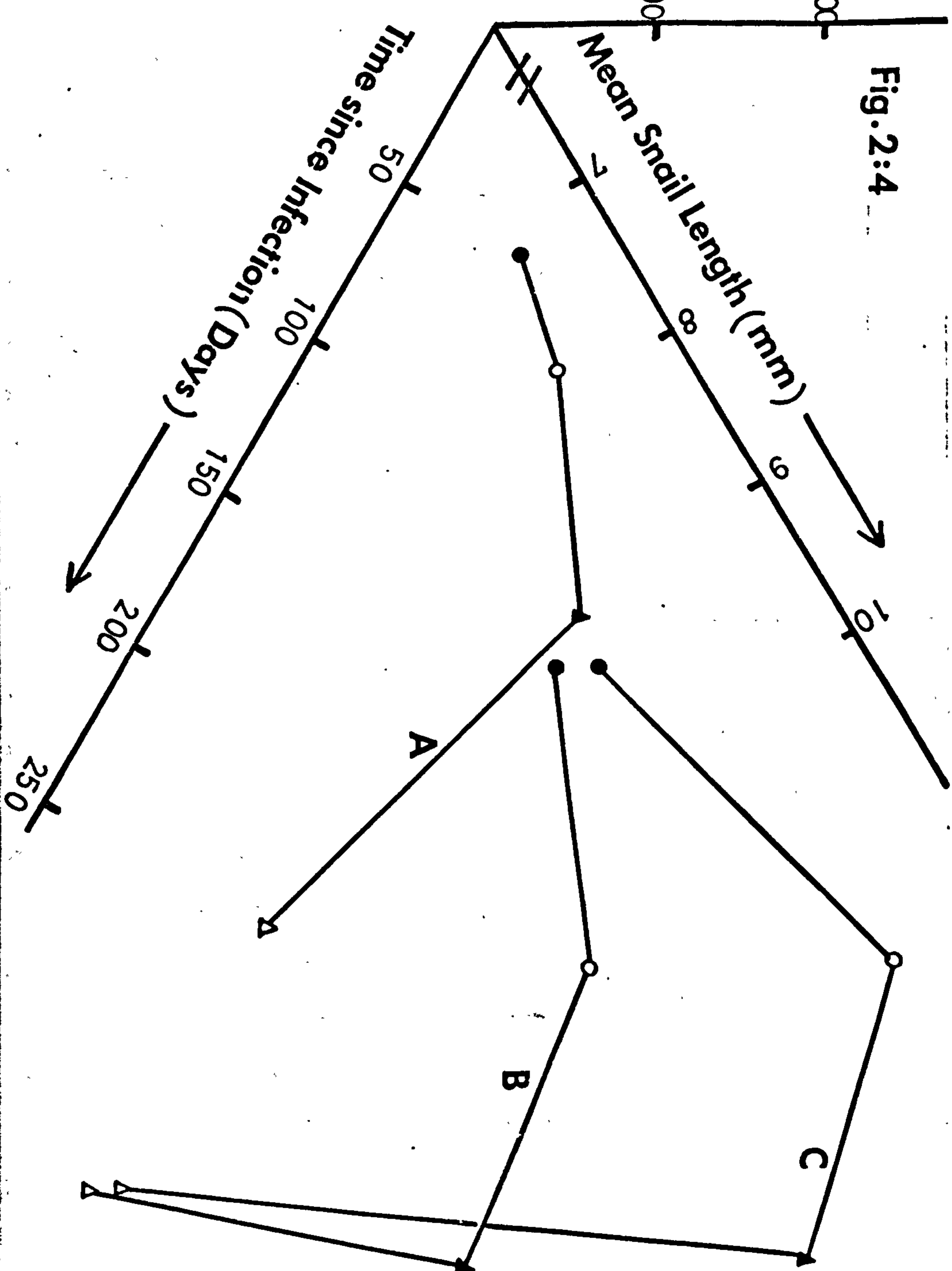


Fig. 2:4

emerge at temperatures above  $9^{\circ}\text{C}$ , which indicates that the rate during the 12 hour Day period (at  $20^{\circ}\text{C}$ ) would have been 32.516 metacercariae per snail per day.

Two possible explanations of the high production rate observed are:

1) the low temperature ( $5^{\circ}\text{C}$ ) during the Night period causes a stimulation of daughter redial production, as described by Wilson & Draskau (1976), thus resulting in an increased cercarial production; 2) the mean shell length of the snails at the onset of cercarial release is considerably greater than the lengths observed at this stage at the other temperature regimes (Table 2:7), increasing the parasite's habitat size and food supply, and thus resulting in an increased cercarial production.

If the first explanation is correct, the end result of the daughter redial production would be the same as that obtained by increasing the number of sporocysts. As described previously, an increased rate of metacercarial production does not result when the exposure dose is increased beyond 2 miracidia per snail. The snails in the Day/Night simulation were exposed to 5 miracidia per snail; thus the maximum possible metacercarial production rate from the sporocysts available is five times the production rate observed from the single miracidium exposure dose (numerical value:  $5 \times 8.1 = 40.5$ ). However, this maximum rate was not reached, and thus the increased rate that was observed can be explained solely in terms of an increase in the parasite's habitat size and food supply. Daughter redial production might be important in increasing the rate of metacercarial production if the snail is only infected by one miracidium, but the data available do not

allow the relative importance of the daughter redial production to be quantified.

The data presented in this Chapter give a good indication of the relationship between metacercarial production and temperature. However, until more information is available for intermediate temperatures, especially  $13^{\circ}$ ,  $18^{\circ}$  and  $22^{\circ}\text{C}$ , and until there is a more detailed understanding of the effect of snail size on the metacercarial production, it is not possible to formulate a mathematical expression to interrelate all the variables.

## CHAPTER 3.

### THE EFFECT OF FASCIOLA HEPATICA ON ITS SNAIL INTERMEDIATE HOST LYMNAEA TRUNCATULA

#### INTRODUCTION

In studying aspects of the population dynamics of the vector snail of a parasitic trematode in the field, various questions will arise which can only be answered by laboratory investigations under controlled conditions (cf. Sturrock & Sturrock, 1970). This rationale applies to the question of 'how do the larval stages of a digenetic trematode affect the physiological processes, for example, survival, growth and reproduction of its snail host?'

Much of the experimental data on the effects of trematode infections on the physiological processes of their snail hosts, concerns the relationship between the blood flukes Schistosoma mansoni and Schistosoma haematobium, and the snails Biomphalaria glabrata (Australorbis glabrata) and Biomphalaria pfeifferi.

Generally a higher mortality rate (Pan, 1963, 1965; B.M. Sturrock 1966; Perlowagora - Szumlewicz 1968, and Meuleman, 1972) and an increased susceptibility to adverse conditions, for example, high temperature (Etges & Gresso, 1965), desiccation (Brumpt, 1941; Olivier, Barbosa & Coelho, 1954) and molluscicides (Paulini, 1958), has been reported for infected snails.

Sturrock & Sturrock (1970) report a reduced growth rate in snails infected at an age of two weeks (maintained for two weeks after hatching

at a temperature range of 22° to 28°C). In other publications infection was found to induce a temporary acceleration in growth rate (Pan, 1963, 1965; B.M. Sturrock 1966 and Meuleman 1972). The accelerated growth rate was not found to produce gigantic snails: their ultimate size was found to be within the range attained by the uninfected controls. In some cases infected snails eventually became stunted (Pan, 1963, 1965).

Very young snails were found to become completely sterile after infection (Sturrock & Sturrock, 1970). Infection of nearly mature snails was found to cause reduced oviposition prior to the onset of cercarial release, and complete inhibition from then on (Pan, 1963, 1965; Perlowagora - Szumlewicz 1968; Sturrock & Sturrock, 1970 and Meulcman, 1972). In mature snails infection was found to cause either a great reduction of oviposition (Brumpt, 1941; Coelho, 1954 and Etges & Gresso, 1965) or a complete cessation (Pan, 1965 and Sturrock & Sturrock, 1970). In some cases oviposition was observed after a period, though at a lower level than in the uninfected controls (Etges & Gresso, 1965; Sturrock & Sturrock, 1970 and Meuleman, 1972).

Wright (1971) reviewed the published data to date. It can be concluded from this review that the complexity of the host-parasite relationship is such that the effect of the parasite on its host depends not only on the species of both, but also on the environmental conditions.

It would thus seem that the effect of Fasciola hepatica on its snail host Lymnaea truncatula is not, with any certainty, predictable from the effects of other trematode parasites on their snail hosts. Published data concerning the relationship between Fasciola hepatica and

Lymnaea truncatula is limited and in certain cases contradictory.

Hodasi (1972b) reports that Fasciola hepatica-infected Lymnaea truncatula in mass culture survived longer than did uninfected control snails, but that isolated individuals did not. Chowaniec (1961) reports that the overwhelming majority of infected snails die at the time of cercarial release or very soon after, and Styczynska (1956) reports that infected snails appear to be less able to survive drought conditions.

From her laboratory and field observations Roberts (1950) suggests that gigantism, as described by Rothschild (1941) in infected marine molluscs, may also occur in parasitised Lymnaea truncatula. Hodasi (1972b) records increased growth rates in infected snails, although his findings are complicated by the variation observed between mass-cultured and individually isolated snails. Nevertheless, in both cases the infected snails have an increased growth rate and ultimately reach a larger size (gigantism) than their respective control snails. Rondelaud & Vincent (1973) also report gigantism, but in this instance it is associated with a marked increase in the fragility of the snail shell.

Roberts (1950) found no evidence of the invasion of the snail's gonads by the parasite, nor of parasitic castration. However, Hodasi (1972b) provides ample evidence for the cessation of egg production, some invasion of the gonads and a striking degeneration of the 'ovotestis'. He postulates that the action of the parasite on the hepatopancreas (migration through and feeding on it) starves the ovotestis of some essential nutrient, or that the rediae liberate some chemical possessing

a sterilizing action. The cessation of egg production has also been observed by Jeans (personal communication). She reports that this is the result of the feeding of the rediae on the hermaphrodite gland. She postulates that what Hodasi observed was not the degeneration of the 'ovotestis' but the severed end of the duct leading from the hermaphrodite gland, which she found to degenerate as the gland is consumed by the parasite.

The aim of the studies described in this Chapter is to determine the effect of the parasite Fasciola hepatica on the survival, growth and reproduction of its snail host Lymnaea truncatula. The experiments were designed to investigate whether these effects are modified by the variation of an environmental parameter, i.e. temperature.

#### MATERIALS AND METHODS

The Biological Materials, Controlled Temperature Conditions are as described in Chapter 1 and the Infection Procedure, as described previously in Chapter 2.

#### The Determination of the Effect of Infection by Fasciola hepatica on the Survival, Growth and Reproduction of Lymnaea truncatula

90 adult snails of similar age and shell length were divided randomly into two equal groups of 45 snails. One group was used as the control, while in the other group each snail was exposed to infection by 5 miracidia. Both the experimental and control groups of 45 snails were then sub-divided into 3 sub-groups of 15 snails each. These 6 sub-groups were then maintained under the same conditions of temperature and light, and supplied with excess food and moisture.



The sub-groups were examined daily. When a snail died, its survival time (i.e. the time in days from infection to death) was calculated and recorded, together with its shell length (measured with a micrometer screw gauge), and its infective state was determined by dissection. (All potentially infective material was rendered safe by steam sterilization). The snail growth was monitored by periodic measurement of the snail's shell length. The egg masses produced by the snails in each sub-group were removed at regular intervals, counted, dissected to record their complement of eggs and then destroyed. The egg mass and egg production per snail was calculated at each time interval.

The above experiment was carried out at 4 constant temperature regimes ( $10^{\circ}$ ,  $16^{\circ}$ ,  $20^{\circ}$  and  $25^{\circ}\text{C}$ ), and a simulated Day/Night temperature regime (12 hours at  $20^{\circ}\text{C}$  and 12 hours at  $5^{\circ}\text{C}$ ).

## RESULTS

The postmortem dissection of the snails exposed to infection revealed that, in all but 18 snails in the simulated Day/Night temperature regime group, an infection did develop. Thus, where possible, this reduced infection must be taken into consideration when determining the effect of the parasite on the physiological processes of the snail.

### Survival

The Mean Survival Values, i.e. the time in days from the start of the experiment (the day of infection) to the day the snail died, for each group of snails, with their standard errors (Bailey, 1968) are given in Table 3:1. Only the snails in which an infection developed were used

to calculate the Mean Survival Value for snails maintained at the simulated Day/Night temperature regime. From the values given in Table 3:1, at each temperature, infection neither prolongs nor reduces the survival potential of an individual snail. The low value of the variance ratio ( $F = 0.001$ ) from an analysis of variance statistical test (Bailey, 1968) confirms the hypothesis that infection does not significantly affect the survival potential of the snail. The individual survival values are expressed as a series of histograms in Figures 3:1a and 3:1b. The approximate time at which cercariae first emerged from their snail host is indicated on these histograms. There does not appear to be a large increase in snail mortality corresponding to this onset of cercarial release.

#### Snail Growth

The mean shell lengths (a measure of snail size) from infection to approximately the 50% snail mortality level for each of the 5 temperature regimes are expressed with respect to time in Figure 3:2. Table 3:2 gives the mean snail shell length at death for the 5 temperature regimes and the percentage increase or decrease of infecteds over controls. Excluding the observations at  $10^{\circ}\text{C}$ , the effect of infection is to increase the overall growth rate of the snail resulting in a larger mean shell length at death (gigantism). At  $10^{\circ}\text{C}$ , the growth rate of the infected snails is slightly reduced, resulting in a smaller mean shell length at death.

#### Reproduction

The numbers of egg masses produced per snail, summed and plotted against time for each of the 5 temperature regimes, are expressed in Figure 3:3.

FIGURE 3:1a A Series of Histograms indicating the Time since Infection (in days) on which the Snails died.

- i) 10°C Controls
- ii) 10°C Infecteds
- iii) 16°C Controls
- iv) 16°C Infecteds
- v) 20°C Controls
- vi) 20°C Infecteds

The ↓ symbol indicates the onset of cercarial release.

Fig.3:1 a

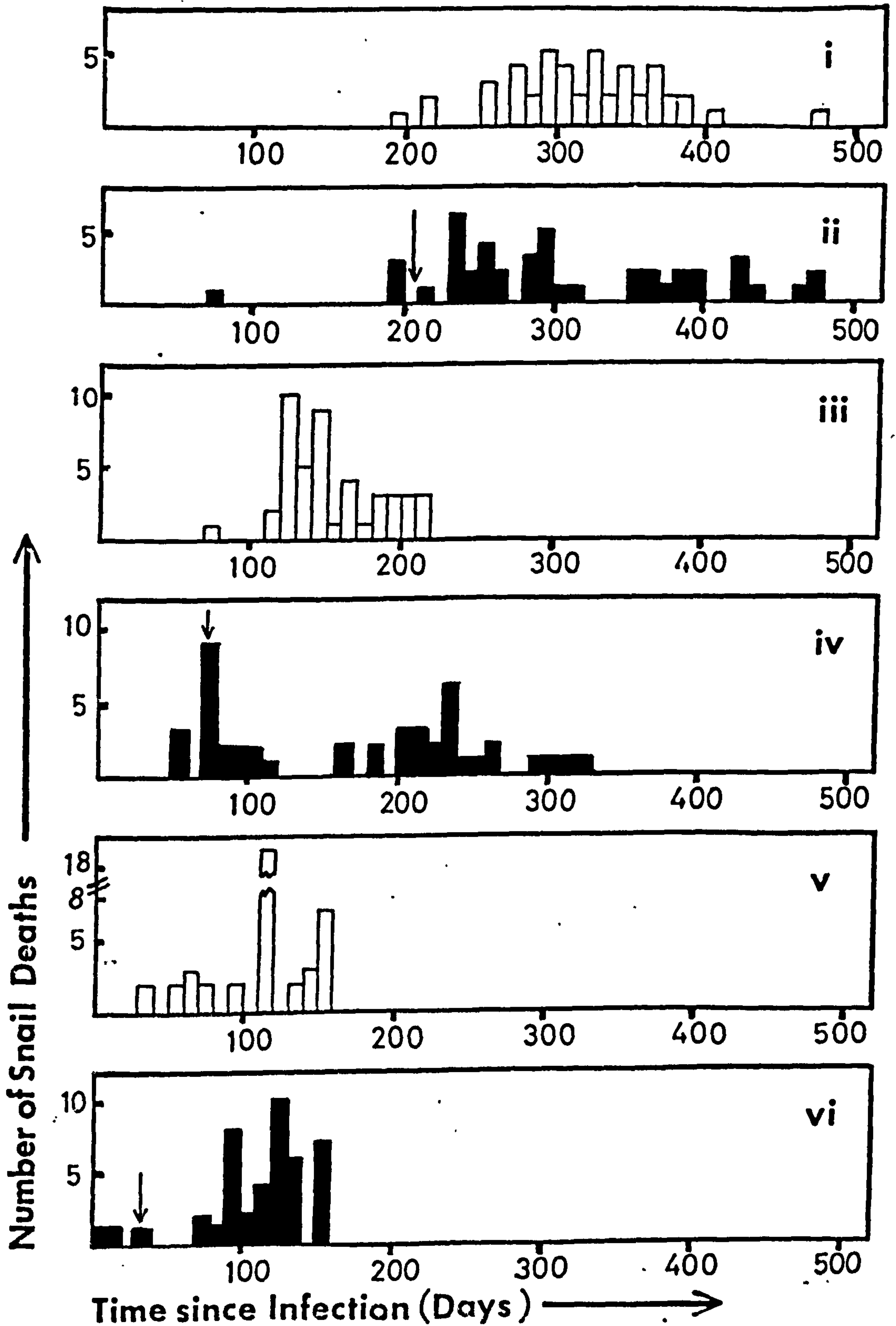


FIGURE 3:1b A Series of Histograms indicating the Time since Infection (in days) on which the Snails died.

- i) 25°C Controls
- ii) 25°C Infecteds
- iii) Day/Night Controls
- iv) Day/Night Infecteds

Fig.3:1 b

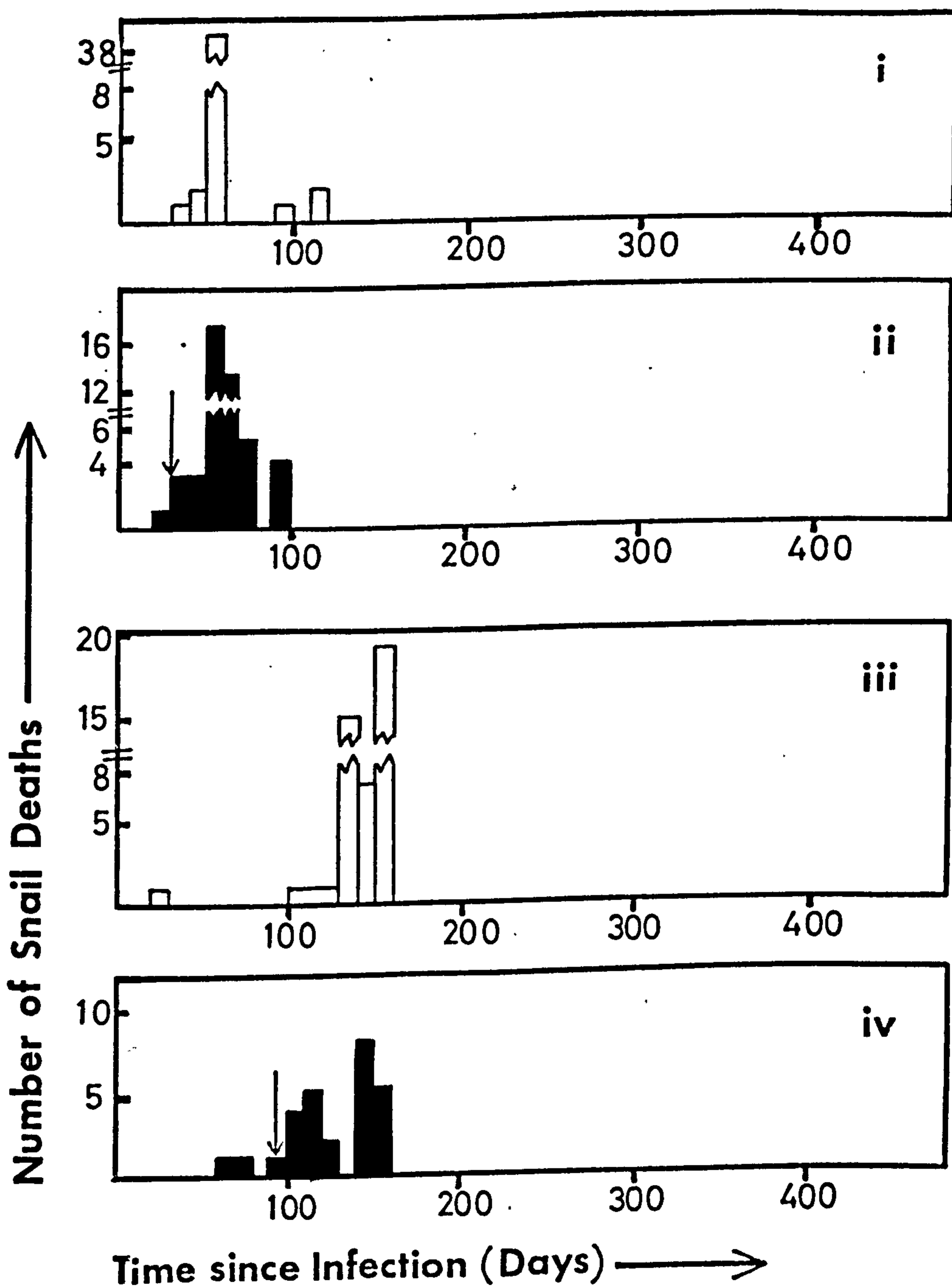


FIGURE 3:2 Plots of the Increase in Mean Shell Length (mm) against Time, days since Infection, for both Control and Infected Snails at 5 Temperature Regimes.

The solid lines - Controls  
The dotted lines - Infecteds

Fig.3:2

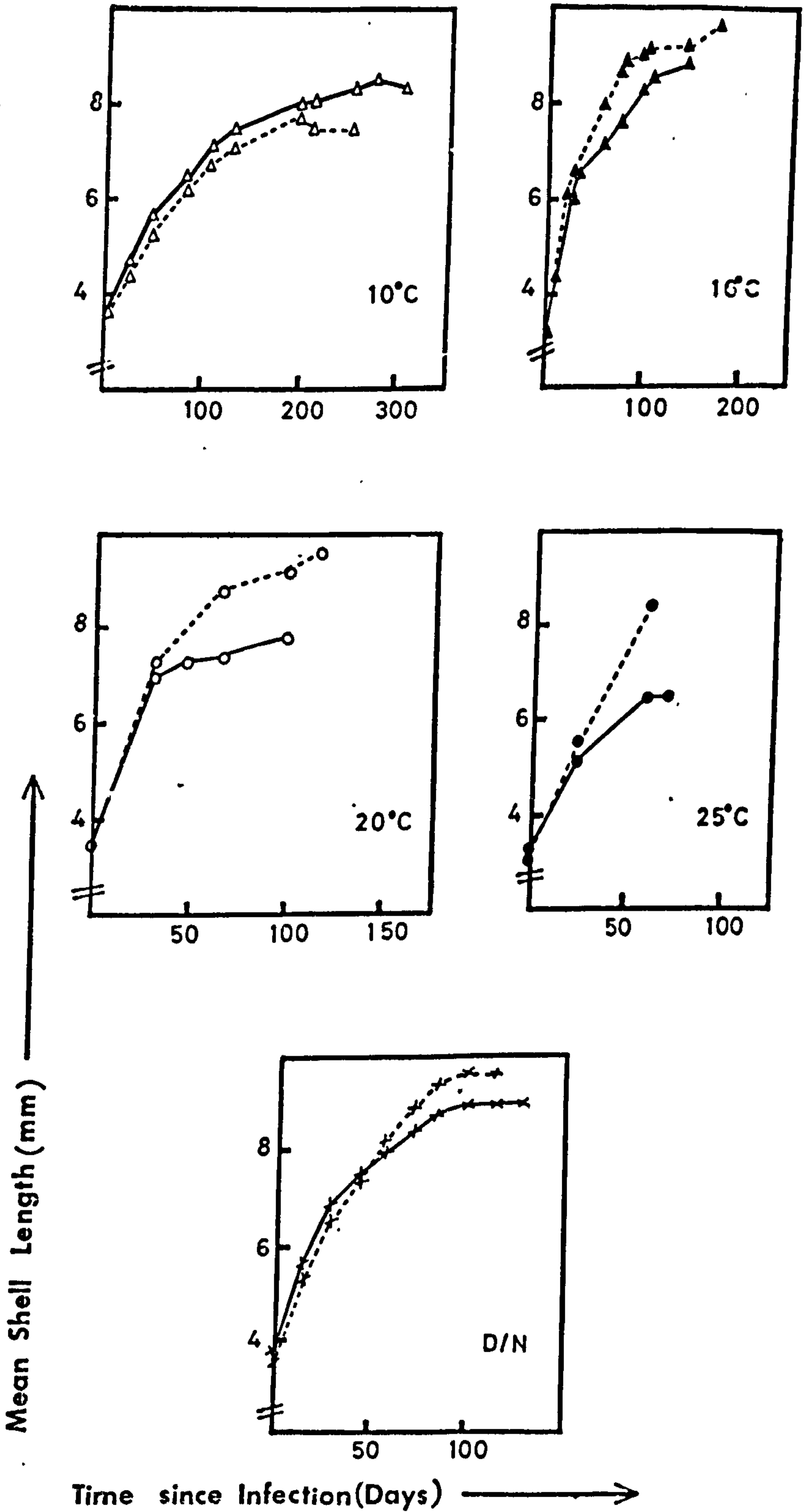




FIGURE 3:3 Plots of the Summation of the Number of Masses produced per Snail for both the Control and Infected Snails at 5 Temperature Regimcs.

The solid lines - Controls  
The dotted lines - Infecteds  
-□-□-□-□-□- - Estimated

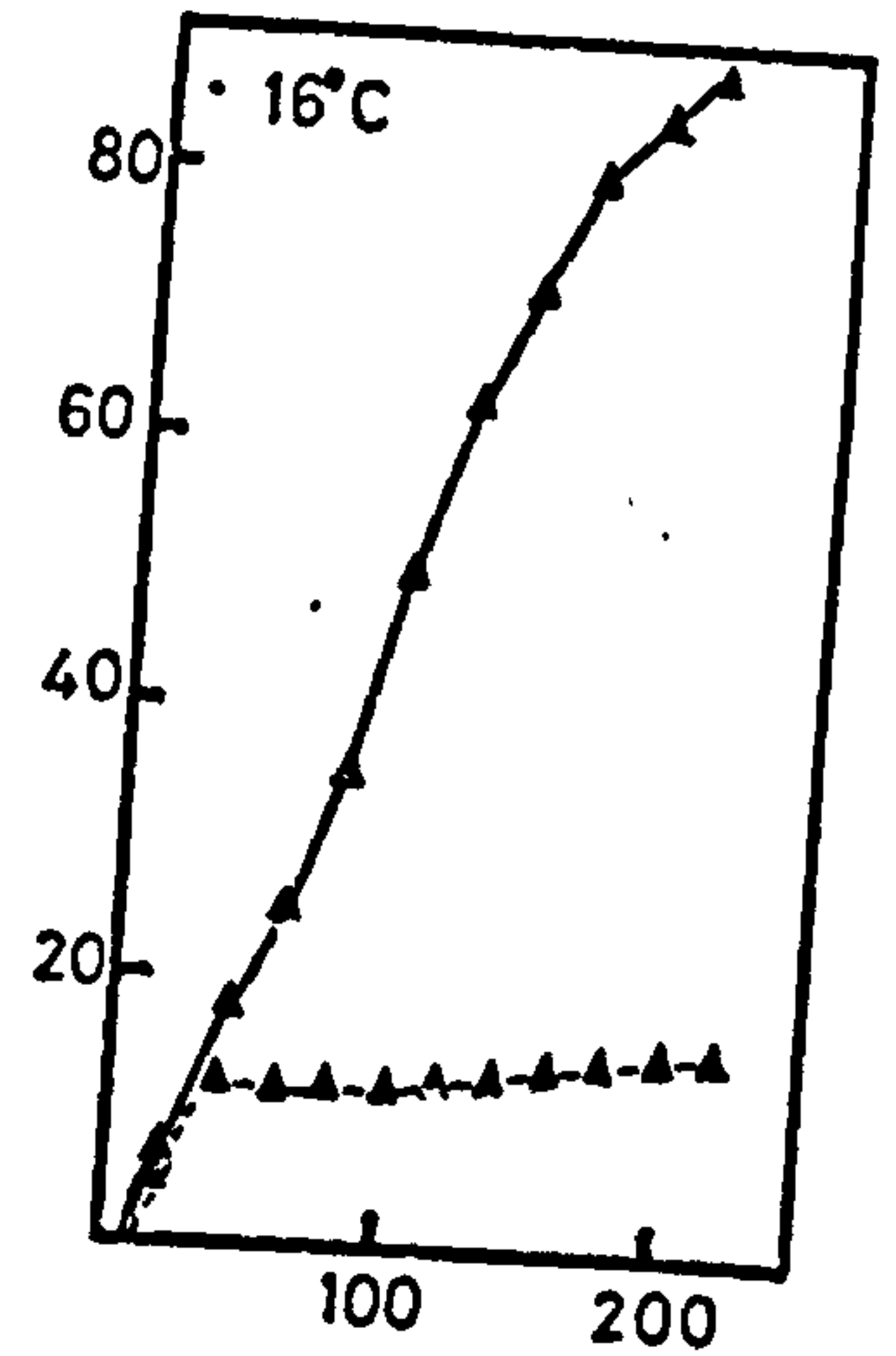
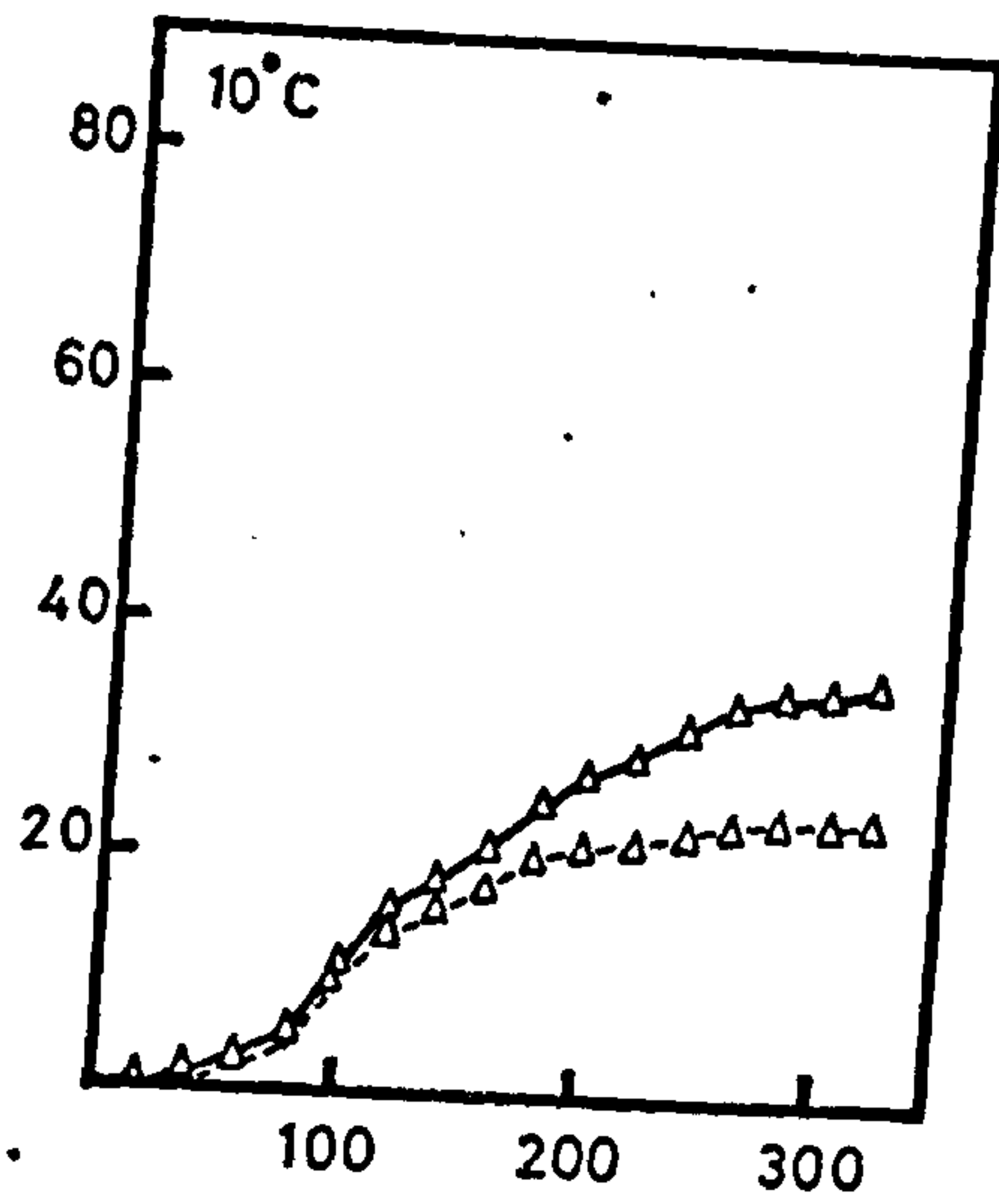
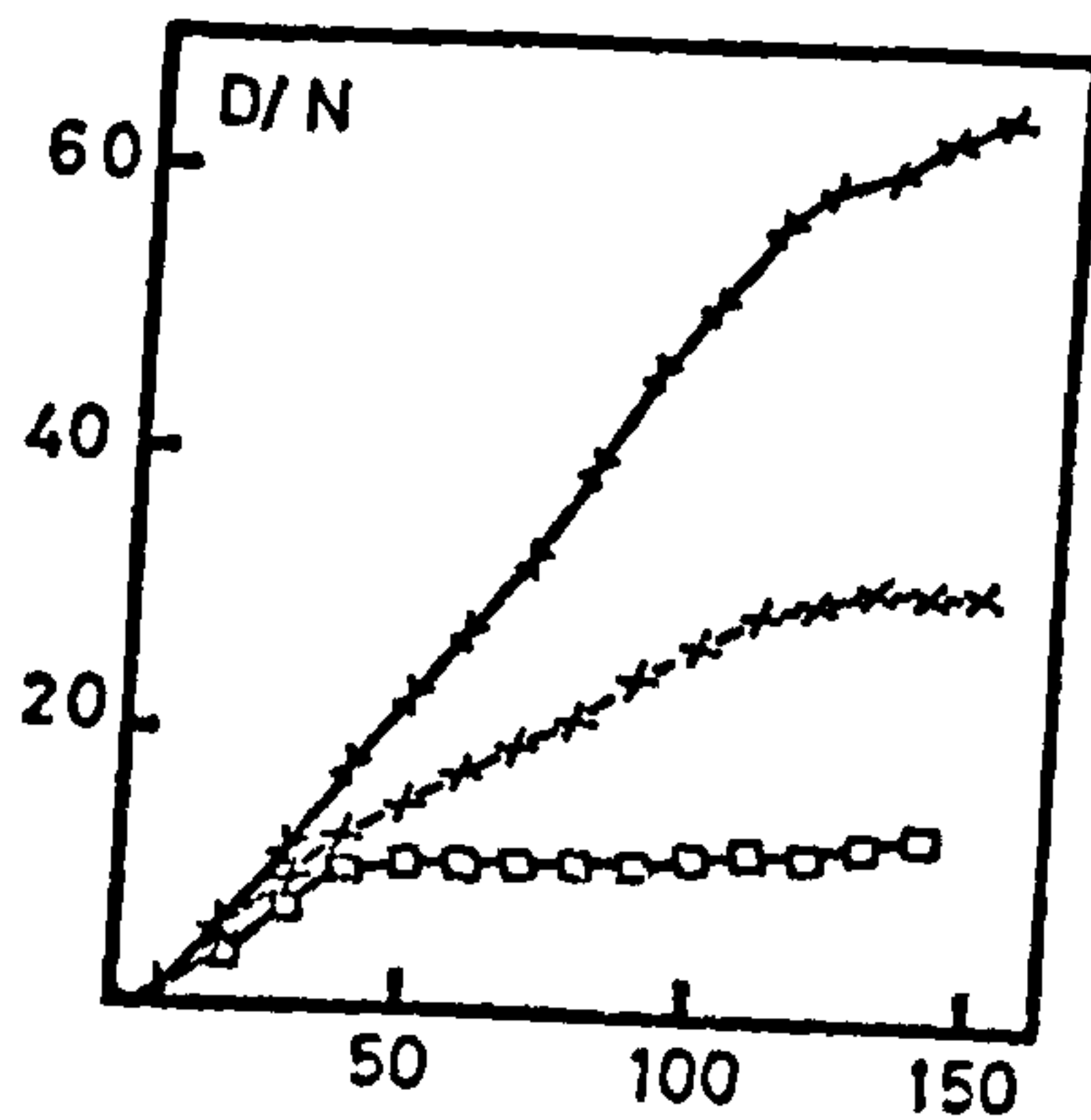
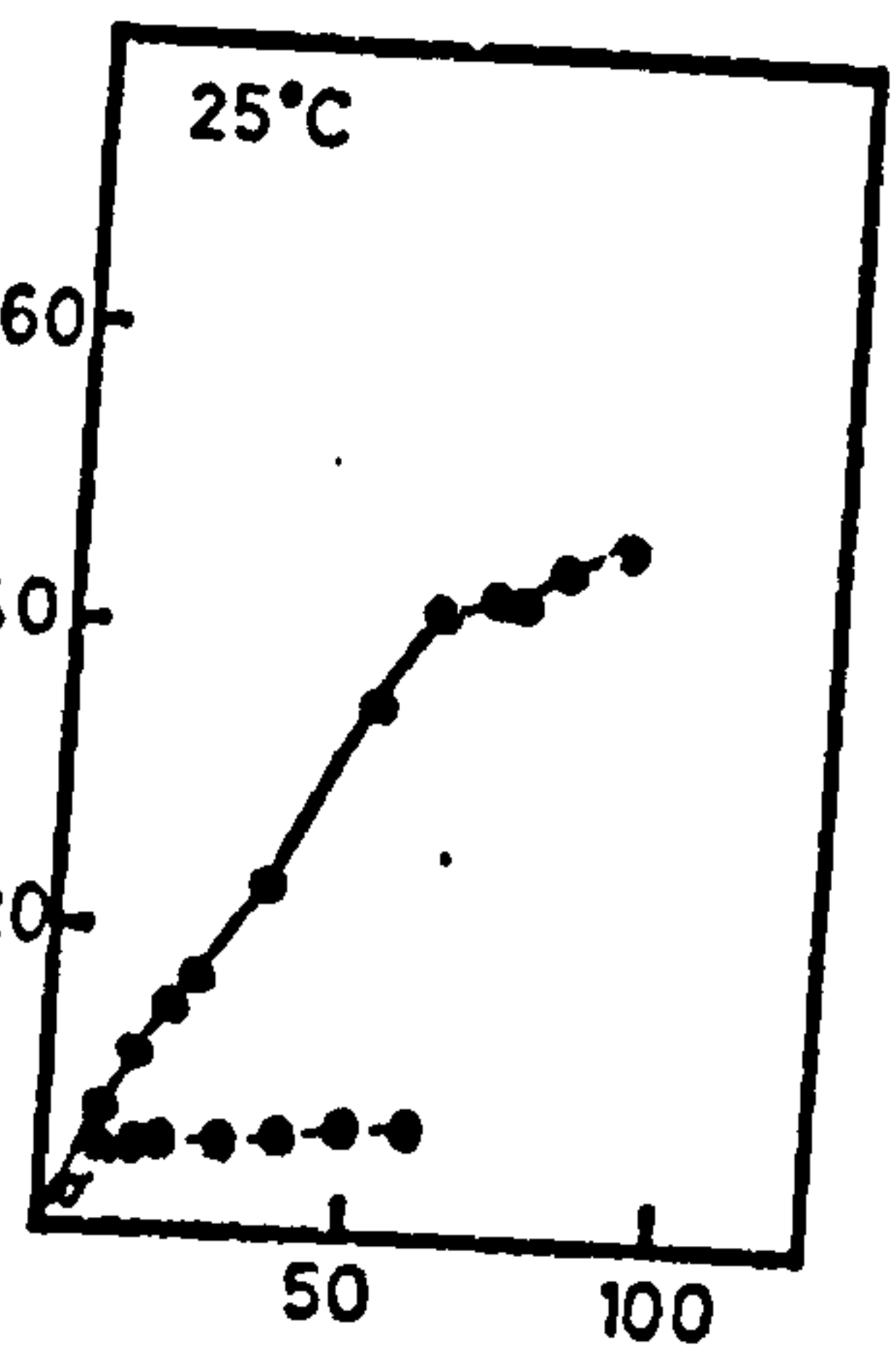
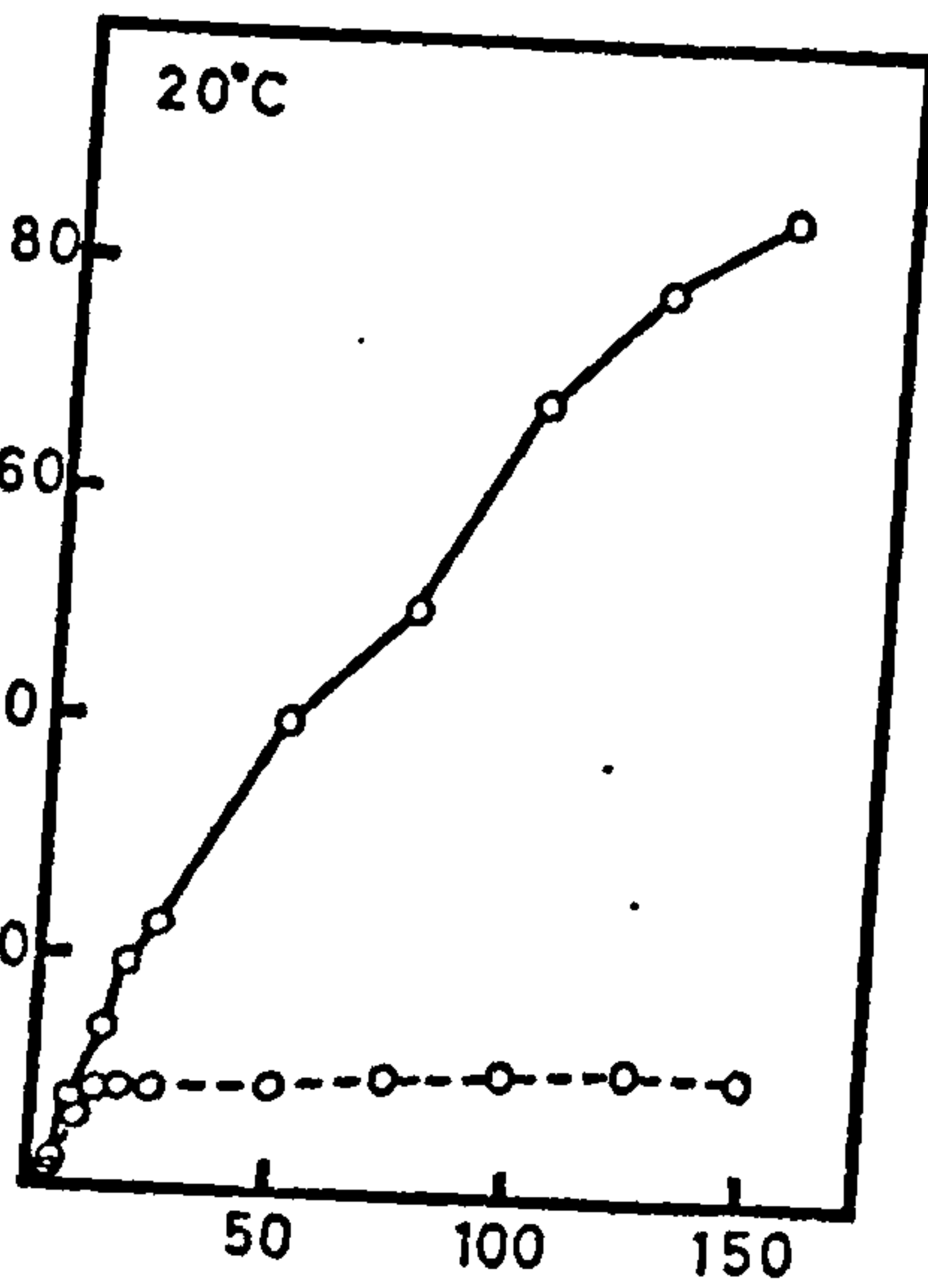


Fig. 3:3

Summed Number of Masses Produced per Snail



Time since Infection (Days)

TABLE 3:1

Temperature Regime °C	Mean Survival Values and their Standard Errors (Bailey, 1968)	
Constat	Controls	Infecteds
10	317.7 (8.0)	305.6 (13.1)
16	154.2 (4.9)	170.2 (12.3)
20	110.0 (5.2)	113.4 ( 2.7)
25	67.8 (1.8)	62.0 ( 2.2)
Day/Night		
20/5	138.8 (4.4)	129.7 ( 1.5)

The Mean Survival Values with their Standard Errors for the Infected and Control Snails at the 5 Temperature Regimes.

TABLE 3:2

Temperature Regime °C	Mean Shell Length at Death, with Standard Errors (mm)		Percentage Increase of Infecteds over Controls
	Controls	Infecteds	
Constant			
10	8.505 ± .208	7.564 ± .226	-ve 11.06%
16	8.892 ± .095	9.305 ± .197	+ve 4.65%
20	7.769 ± .092	9.163 ± .265	+ve 17.94%
25	6.478 ± .156	7.813 ± .229	+ve 20.61%
Day/Night			
20/5	8.817 ± .157	9.647 ± .259	+ve 9.41%

The Mean Shell Lengths at Death and the Percentage Increase in Size of the Infecteds over the Controls at the Specific Temperature Regimes.

and the numbers of eggs produced per snail in Figure 3:4. Estimated plots of the numbers of egg masses and the numbers of eggs produced for the simulated Day/Night regime are included; these were obtained by subtracting from the values observed, the 'expected' egg mass and egg productions (calculated from the control group) for the uninfected snails in the group.

From Figures 3:3 and 3:4 the reproduction of the snails exposed to infection continues as for uninfected controls for a period, and is then considerably reduced or totally inhibited. The approximate times (in days) from infection to the onset of the reduction of reproduction, for the 5 temperature regimes, are given in Table 3:3. Included in this table are the mean minimum developmental times of the larval stages of the parasite at the same 5 temperature regimes (Chapter 2, Table 2:3), and the duration of normal reproduction expressed as a proportion of the relevant minimum development time. From the values in Table 3:3 the duration of normal reproduction in infected snails can be seen to be directly dependent on temperature, as was the development rate of the parasite.

## DISCUSSION

The potential survival time of the snail Lymnaea truncatula parasitised by Fasciola hepatica has been shown to be neither prolonged nor reduced in comparison with the survival time of uninfected control snails, when the temperature is varied between 10° and 25°C and the snails maintained under conditions of excess food and moisture. However, under adverse conditions (e.g. reduced moisture and exposure to molluscicides), there may be some reduction in the potential survival time.

FIGURE 3:4 Plots of the Summation of the Number of Eggs Produced per Snail for both the Control and Infected Snails at 5 Temperature Regimes.

The solid lines - Controls  
The dotted lines - Infecteds  
□-□-□-□ - Estimated

Fig. 3:4

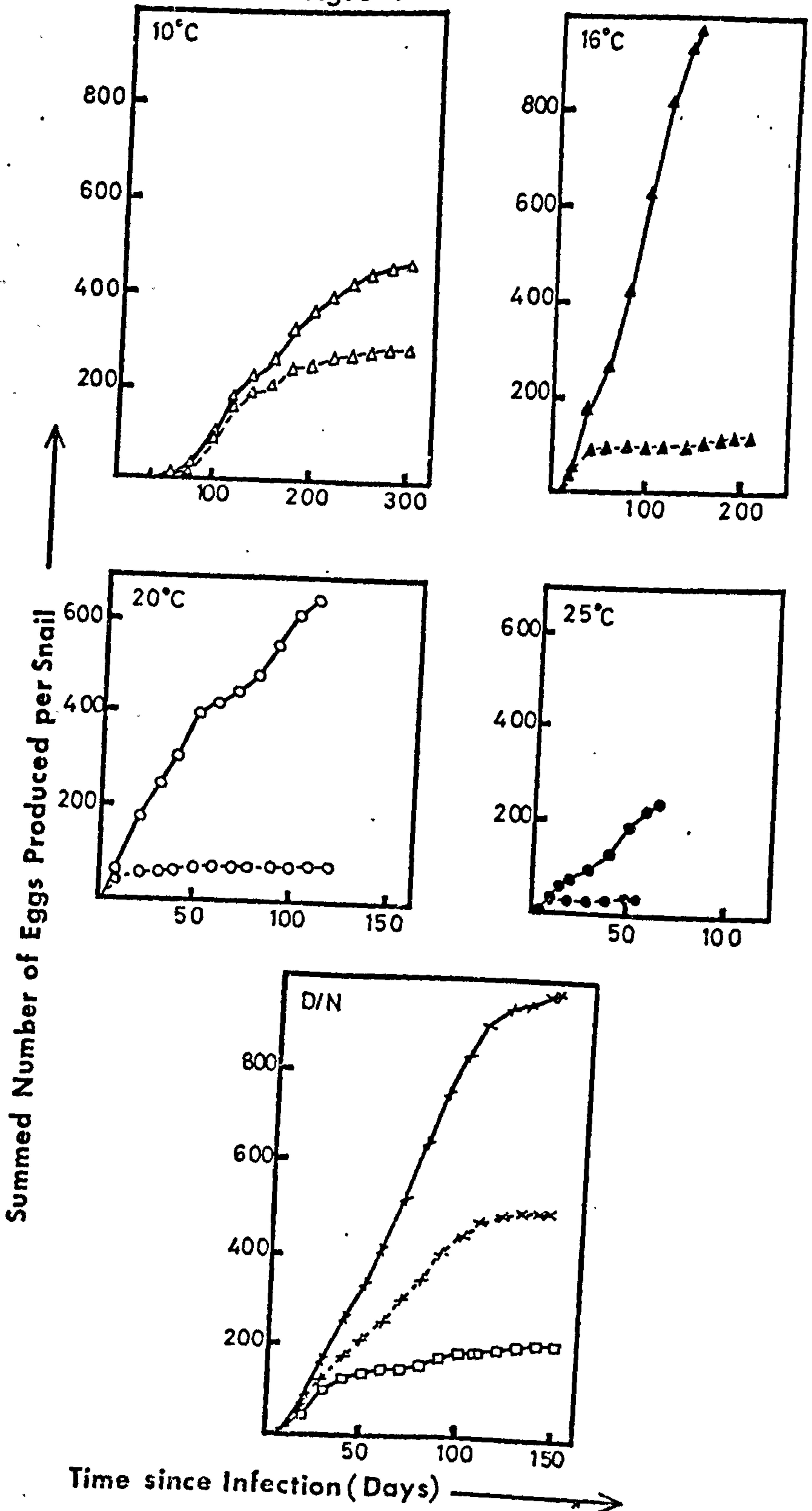


TABLE 3:3

Temperature Regime (°C)	Duration of Normal Reproduction in Infected Snails (Days)	Mean Minimum Development Time of the Larval Stage of the Parasite (Days)	Duration of Normal Reproduction expressed as a Proportion of the Minimum Development Time of the Parasite
Constant			
10	125	208	0.601
16	30	71	0.423
20	13	38	0.342
25	10	30	0.333
Day/Night			
20/5	35	89	0.394

The approximate Duration of Normal Reproduction by the Infected Snails, the Mean Minimum Development Time of the Larval Stage of the Parasite and the Duration of Normal Reproduction expressed as a Proportion of the Minimum Development Time, at the 5 Temperature Regimes.



The mass mortality of infected snails at or very soon after the onset of cercarial release, as reported by Chowaniec (1961) was not observed (Figures 3:1a and 3:1b).

From the postmortem dissection of the infected snails it has been shown that it is the death of the snail and not the exhaustion of the redial stage which terminates the metacercarial production. Thus, the parasite's best interest is served by not causing the premature death of its host.

The measurements taken of snail shell length showed that the effect of infection was to induce gigantism in all but those snails maintained at the constant temperature regime of 10°C, (Figure 3:2 and Table 3:2). These observations of increased growth rates resulting in an increased mean shell length at death (gigantism) are in agreement with the findings of Roberts (1950), Hodasi (1972b) and Rondelaud & Vincent (1973). No marked decrease in the strength of the shell of infected snails was observed, contrary to the observations of Rondelaud & Vincent (1973).

Within the temperature range 16° to 25°C the degree of gigantism, calculated as the percentage increase in the mean shell length at death of the infected snails over that of the uninfected control snails, increased with temperature (Table 3:2). At 10°C the growth rate of infected snails was reduced and the mean shell length at death was 11.06% smaller than for the uninfected control snails.

The result of infection on the reproduction of mature snails is either a considerable reduction or total inhibition of both the egg masses

and the numbers of eggs produced. The reduction is a delayed action, i.e. there is a period of normal reproduction (virtually identical to the reproduction of uninfected control snails) prior to the reduction of reproduction (Figures 3:3 and 3:4). The duration of this normal reproduction appears to depend on the temperature at which the snails were maintained (Table 3:3). This observation of a period of normal reproduction will only apply if the snails are mature when they are exposed to infection; if immature then the resulting number of eggs produced will most probably be dependent on the degree of sexual maturity of the snail at the time of infection.

The duration of normal reproduction in snails infected when mature, if expressed as a proportion of the minimum development time of the larval stages of the parasite, indicates a direct correlation between the onset of the reduction in the reproduction and the development of the parasite, for all but the 10°C constant temperature regime. From my own observations on the growth of the larval stages within the host at various constant temperature regimes (see Appendix 1), the most mature individual parasites, at a time corresponding to approximately 0.35 of their development, will be rediae which are migrating through and feeding on the snail's tissue. The onset of reduced reproduction thus appears to correspond to the stage in the development of the parasite where the rediae are migrating through and feeding on the snail's tissue. This is in full agreement with the findings of Hodasi (1972b) and Jeans (personal communication), that it is the action of the rediae which causes the reduction or total cessation of reproduction.

Hodasi (1972b) postulates that the rediae, by migrating through and feeding on the hepatopancreas, starve the gonads of some essential

nutrient or that the rediae liberate a chemical which possesses a sterilizing action, (cf. McClelland & Bourns, 1969). However, Jeans (personal communication) from her observations of the hermaphrodite gland situated within the coils of the hepatopancreas, believes that this gland is destroyed directly by the rediae which feed upon it.

If the assumption is made that the rediae produce a chemical which exerts an inhibitory effect on the snail's gonads, then it would seem probable to expect that while rediae are present, reproduction will be inhibited. From the postmortem dissection of the infected snails which revealed the presence of rediae, and the observed resumption of egg production in those groups of snails in which it was initially inhibited (at 16°, 20° and 25°C, see Figure 3:4), the presence of rediae does not induce the total inhibition of reproduction. Furthermore, in snails maintained at 10°C in which rediae were present, eggs were produced at a constant rate; albeit at a lower rate than from the uninfected controls.

If the assumption is made that whilst in the hepatopancreas the rediae are non-specific feeders and that they will eat the hermaphrodite gland only if they contact it, then the resumption of egg production would seem probable, since the mollusc tissue is known to have remarkable powers of regeneration (Rees, 1934). The continuous low rate of egg production observed at 10°C would also seem probable since the parasite burden, estimated from the metacercarial production rate (Chapter 2, Table 2:6), is low, and thus with fewer rediae there is less chance of the hermaphrodite gland being totally consumed. This low parasite burden also explains the apparent extension of the normal reproductive period at 10°C (Table 3:3), since fewer rediae will take longer to have

an appreciable effect on the hermaphrodite gland and thus on reproduction.

The reduction in the reproductive potential of the snail host as a direct result of the presence of the parasite is of little importance to the survival of the snail population under natural conditions: it has been found that: 1) the infection rate in the field is low - a mean value of 5% (estimated from Ollerenshaw (1974)) and 2) the reproductive capacity of uninfected snails is very high; one snail in the course of two generations is able to give rise to 25,000 individuals in 12 weeks (Ollerenshaw, 1971b).

The parasitization of Lymnaea truncatula by Fasciola hepatica results in castration and gigantism. McClelland & Bourns (1969), from investigations with Lymnaea stagnalis infected by Trichobilharzia ocellata, suggest that these observed phenomena are cause and effect; castration sparing the snail the double burden of both egg and parasite production and since in nutritional terms it is more 'expensive' to produce eggs, the resulting effect on the snail is gigantism. This resulting effect may also be explained if it is postulated that the gonads limit the somatic growth of the snail (e.g. by hormone production). Unfortunately, experimental work on the hormonal production of snail gonadal tissues and on the endocrine control of snail growth is extremely limited (Boer & Joosse: 1975). Wesenberg-Lund (1934) was not able to induce gigantism in Littorina littorea by the destruction of their gonads using X-rays. Nevertheless, from Figure 3:2 and Table 3:3, the increase in the length of the infected snails becomes apparent at or just after the reduction in reproduction. Thus, in whatever manner the castration of the snail induces gigantism, it would seem that the

castration and gigantism observed here are cause and effect.

The induction of gigantism is a direct advantage to the survival of the parasite population, for it enables an increased exploitation of the reproductive potential, especially of multiple infections, since as described in Chapter 2 it is the shell size that limits the metacercarial production rate.

The modification by temperature of the effect of Fasciola hepatica on Lymnaea truncatula is complex, since both the parasite and the snail have been shown to respond to variation in temperature (Chapters 1 & 2). However, from the investigations described in this Chapter, the overriding modifying action of temperature is the determination of the development and the multiplication of the parasite. For example, at 10°C the parasite developed at a slow rate and the multiplication factor was low; thus there were fewer individual parasites and their action on the gonads was not sufficient to reduce the reproduction and induce gigantism. At 20°C, however, the development rate and the multiplication factor were both high; the gonads were damaged and gigantism resulted.

In conclusion, the parasitization of Lymnaea truncatula by Fasciola hepatica, under laboratory conditions of excess food and moisture, does not affect the survival potential of the snail, but castration which induces gigantism does occur. Without further investigations it is not possible to determine the precise way in which the parasite castration induces gigantism. The effects of parasitization of the snail by the larval fluke under drought (or even other than optimal moisture) conditions or with less than excess food have not been investigated.

However, from Chapter 1 Section B it has been shown that under drought conditions the snail becomes dormant - active feeding and reproduction cease: the latter would result in conservation of stored nutrients. In infected snails maintained at temperatures within the range 10° to 25°C it is likely that the parasite would continue to feed on the snail's tissue, depleting the stored nutrients and thus probably reducing the snail's survival potential.

## CONCLUSION

The results obtained from the investigations here described have demonstrated the importance of temperature as a controlling influence on the life processes of both the snail Lymnaea truncatula and the intramolluscan stage of the parasite Fasciola hepatica. Many of the effects of environmental temperature have here been described mathematically, with those concerning rates of biological processes conforming to an exponential relationship, as expected (Schmidt-Nielsen: 1975).

The temperature ranges over which the snail and the parasite are active (i.e. develop, grow and, where relevant, reproduce), have been found to be different, especially at the lower end of the range. The estimated lower temperature limits are as follows:

Parasite : Egg	:	9.5°C
Parasite : Intramolluscan Phase	:	7.6°C
Snail : Egg	:	7.0°C
Snail : Young and Adult	:	5.0°C

With respect to the study of fascioliasis, these findings are of prime importance, for the snail can be seen to have an advantage over the parasite because of its ability to exploit more of the potential time available for growth, development and reproduction. It is possible to quantify this advantage by utilizing data collected (Smith: personal communication) of the weekly mean maximum and minimum temperature readings at the soil air interface over two years (1973/4 and 1974/5) at two confirmed snail and parasite habitats (one in the Lake District and one in Wales); the mean percentage of the time exploitable by each of the two snail and parasite stages over the two years at each site are as follows:

Stage	% Thornythwaite: Lake District	% Hendy: Wales
Parasite : Egg	46%	52%
Parasite : Intramolluscan	60%	71%
Snail : Egg	63%	78%
Snail : Young & Adults	86%	92%

From the investigations concerning soil moisture the importance of the moisture content of the snail's microhabitat has been demonstrated.

This finding will modify the exploitable time available to the snail and the parasite: however, there will still be an advantage to the snail over the parasite, as the times when the snail is active, but not the parasite, will be during the spring, autumn and part of the winter period when the microhabitats will be adequately supplied with free water (Smith: personal communication).

The effects of temperature and miracidial exposure dose on the production of metacercariae were also investigated. The results obtained indicate that the temperature not only controls this factor by directly affecting the development of the parasite, but also by various effects on the snail host, e.g. determining its survival time - a vital point as the duration of the production of metacercariae is limited by the survival of the snail, not the exhaustion of the redial stage. Miracidial exposure doses of above two per snail have been found not to give rise to increased metacercarial production; the limiting factor being the size of the snail host.

The effect of the parasite on the snail was investigated and at least within the temperature range 16° - 25°C gigantism was demonstrated.



The investigations described in this thesis have clarified and quantified certain aspects of the biology of the parasite and the snail.

However, as with many biological investigations, questions were posed which indicate requirement for further work. For example:

- 1) Determination of the effect of soil moisture content on the uninfected and infected snails, especially at temperatures other than 20°C, and possibly at a varying but controlled temperature.
- 2) The effect of intermediate temperatures (13°, 18° & 22°C) on the snail and the intramolluscan stages of the parasite should be investigated.
- 3) Experiments are needed to elucidate the action of the parasite which induces gigantism, including a possible clarification of the hormonal control of snail growth.

## A study of the effect of temperature on the growth of *Fasciola hepatica* in *Lymnaea truncatula*

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### SUMMARY

The growth rates of the intramolluscan stages of the parasite *Fasciola hepatica* were determined at three temperatures 16°, 20° and 25 °C. A graphical relationship of growth rate with respect to temperature was produced and a growth array was calculated from this. Meteorological data were summarized in the form of a temperature array. These arrays were then used in conjunction with a simple equation to simulate parasite growth within the snail host. A digital computer was employed to solve iteratively the simulation equation, initially by checking its application against experimentally determined results. It was then employed with the meteorological data to simulate growth with respect to environmental temperatures and the results obtained were compared with those derived from field collections. Estimates were also made of the duration of the shortest life-cycle under field conditions.

### INTRODUCTION

Temperature is important in determining the rate of development of the intramolluscan stages of digenetic trematodes and hence in the timing of events in their life-cycle (Smyth, 1966; Wright, 1971). Thus in the case of *Fasciola hepatica* the annual variation in environmental temperatures of southern Britain apparently results in a short 'summer infection' and a long 'winter infection' of the snail host (Ollerenshaw, 1959). It is not clear from the literature whether these short summer and long winter infections represent two complete life-cycles of the parasite per annum. The lower temperatures prevailing in northern Britain and Norway allow the maturation of only one infection in snails per year, whilst in Iceland the disease (fascioliasis) is absent in spite of the presence of the intermediate and final hosts (Ollerenshaw, 1971*b*).

This pattern of temperature effects in Northern Europe can be seen to influence the growth of other organisms and has been intensively investigated in relation to plants. In the more recent investigations of plant growth (de Wit, Brouwer & Penning de Vries, 1969; Patefield & Austin, 1971), growth has been determined in the laboratory under a range of defined conditions. A digital computer has then been used to simulate growth by combining these laboratory data with environmental determinations of the parameters affecting growth. The prediction of growth

rate, resulting from this simulation, can then be compared with the actual plant growth which occurred under field conditions.

We have used similar methods to investigate the effects of temperature on parasite development. In this paper we describe experiments designed to determine the growth rate of the intramolluscan stages of *F. hepatica* over a range of constant temperatures. This information is then used in conjunction with meteorological data on temperature as the basis for a simulation of parasite development. The results of this simulation are then compared with data on actual parasite development under field conditions (Ollerenshaw, 1959).

## MATERIALS AND METHODS

### *Biological material*

Parasite eggs were teased from the uteri of flukes maintained in laboratory white rats infected 3–4 months previously. After washing, the eggs were incubated in the dark at 25 °C for 12 days. Mass hatching of the miracidia was effected by the exposure of the developed eggs to light. The snail host was maintained on cultures of the blue-green alga *Oscillatoria* grown on mud slopes under artificial light.

### *Determination of parasite growth*

The growth rate of the parasite was determined at 16°, 20° and 25 °C. All the snails used in an individual experiment were infected from the same batch of miracidia, each snail being exposed to five miracidia in a solid watch-glass containing aerated tap water. The snails were then maintained in groups of not more than 15, on the algal cultures with continual conditions of excess food and moisture, in order to eliminate the possible effects of starvation or desiccation (Kendall, 1953).

Dry weight was used to estimate parasite growth. No attempt was made to measure growth until the appearance of the first rediae. The dry-weight determinations were carried out at 3- to 4-day intervals until after the point of cercarial release. At each time interval, five snails were gently crushed in balanced salt solution (Pullin, 1971) and the largest and most mature redia from each snail was quickly transferred to a single preweighed aluminium foil disk. The disk was then dried for 24 h at 105 °C and reweighed on a Cahn Microbalance to an accuracy of  $\pm 0.2 \mu\text{g}$ .

### *The simulation of growth*

The simulation of parasite growth using environmental temperature is based on the equation:

$$\log_e w_{(t+n)} = \log_e a_t + bn,$$

where  $w_{(t+n)}$  = dry weight at time  $t+n$ ;  $a_t$  = dry weight at time  $t$ ;  $b$  = the growth rate over a given temperature range; and  $n$  = the time increment, in days.

For each of the three constant temperatures the measurements of the dry weights of the rediae were transformed to natural logarithms and plotted against time; only the values up to the onset of cercarial shedding were used. The best straight

line for each was fitted using linear regression. Theoretically the intercept on the  $y$  axis should be the same for each line as the initial weight of the parasite on the day of infection will be the same at each temperature. Confidence limits were calculated for each of the intercept values. Since they were found not to be significantly different from each other, the mean intercept was taken as the initial value. New regression lines were then calculated so as to pass through this intercept. The slopes of these gave the relative growth rate of the parasite at the specific temperatures.

The three relative growth rates thus obtained plus a zero value at 10 °C (no growth occurs at or below this temperature (Wright, 1971; Ollerenshaw, 1971a)) were plotted against temperature and linear regression was used to give the best straight line. The five values of  $b$  (the growth array) to be used in a computer simulation were then taken as the mid-points of the temperature ranges: 0-9.99, 10-14.99, 15-19.99, 20-24.99 and 25-29.99 °C.

The data on environmental temperature were obtained from the Meteorological Office, Bracknell, for Manchester Airport in the form of mean hourly temperature values for the year 1969. These data were converted to the proportion of each day ( $n$ , the time increment) that the temperature was within the ranges listed above. We thus have five values of  $n$  for each day of the calendar year (the temperature array) and the value of  $n$  can vary between 0 and 1.

The simulation can be first employed to check the validity of the growth equation in predicting the duration of parasite development. The computer is used to iterate the growth equation between an initial value of  $a$  ( $a_{\min}$ ) and a final value of  $w$  ( $w_{\max}$ ) at each of the constant temperatures 16°, 20° and 25 °C. For the iteration two sets of values can be substituted for  $b$ ; the growth rate as experimentally determined, and the growth rate read from the regression line of experimental growth rate against temperature. The value of  $n$  was taken as unity at each of the three selected temperatures.

Provided that these simulations produce realistic estimates of development at the three selected temperatures, the computer can be used to solve the equation between  $a_{\min}$  and  $w_{\max}$  for every day of the year on which the temperature rose above 10 °C. This is based on the fact that infection of snails will not occur below 10 °C and does not occur during the winter months, restricting the development of the parasite outside its host to the period May to October (Ollerenshaw, 1971b). Thus, using the growth array to provide values of  $b$  and the temperature array to provide values of  $n$ , the simulation will produce a development time (days) between  $a_{\min}$  and  $w_{\max}$  for each day of the year on which an iteration was started. This necessitates allowing the iteration to proceed for a period greater than one year.

The value of the fixed regression intercept on the  $y$  axis is  $a_{\min}$  and assumes that radial growth starts immediately after infection of the snail by the miracidium.

The mean of the maximum weights attained by rediae in the three growth experiments is  $w_{\max}$ .

Table 1. *The redial dry-weight determinations obtained over a range of temperatures*

Temperature					
16 °C		20 °C		25 °C	
Days post-infection	Dry weight ( $\mu$ g)	Days post-infection	Dry weight ( $\mu$ g)	Days post-infection	Dry weight ( $\mu$ g)
32	18.2	24	32.5	15	22.8
35	29.0	27	34.8	19	35.0
38	35.9	30	39.0	22	43.4
41	47.7	33	41.5	26	58.6
44	36.2	36	72.2	30	115.7
47	86.0	39	114.4	33	31.5
50	110.9	42	77.3	36	32.9
53	114.0	45	74.3	—	—
56	115.4	—	—	—	—
59	64.6	—	—	—	—

## RESULTS

The redial dry-weight data obtained at each temperature are given in Table 1. The results demonstrate the relationship between temperature and rate of parasite development. The times for each temperature at which maximum redial weight is reached are given in Table 3 and compared with data from Ollerenshaw (1971a). It should be noted that the experimentally determined development times are only accurate to  $\pm 1\frac{1}{2}$  days due to the interval between successive readings. The sharp fall in redial dry weight immediately following these times is due to the escape of cercariae from the rediae. The rediae retain their normal dimensions, the space formerly occupied by cercariae now being filled with fluid. It should be noted that the development times (Table 3) represent the minimum possible period from infection of the snail to the maturation of the first redia. The maximum weights attained by rediae at each temperature are very similar.

The regression lines of the growth data against time, initially with the separate intercepts and with the fixed intercept are illustrated in Fig. 1a and 1b respectively. The regression line of the relative growth rates against temperature is illustrated in Fig. 1c. The growth array obtained from this last regression is given in Table 2. The temperature array data are not shown owing to their bulk, but they can be calculated from the standard meteorological statistics, obtainable from the Meteorological Office, Bracknell.

The results of the simulation of parasite development at constant temperatures are illustrated in Table 3 and compared with our own experimental data and that of Ollerenshaw (1971a). Within the limits of accuracy of the techniques involved, the experimental determinations of development time by ourselves and Ollerenshaw are fairly similar. Our estimates are longer at the high temperature (25 °C) and apparently shorter at the low temperature (16 °C). Using the calculated growth rates, the simulation of growth at constant temperatures produces development

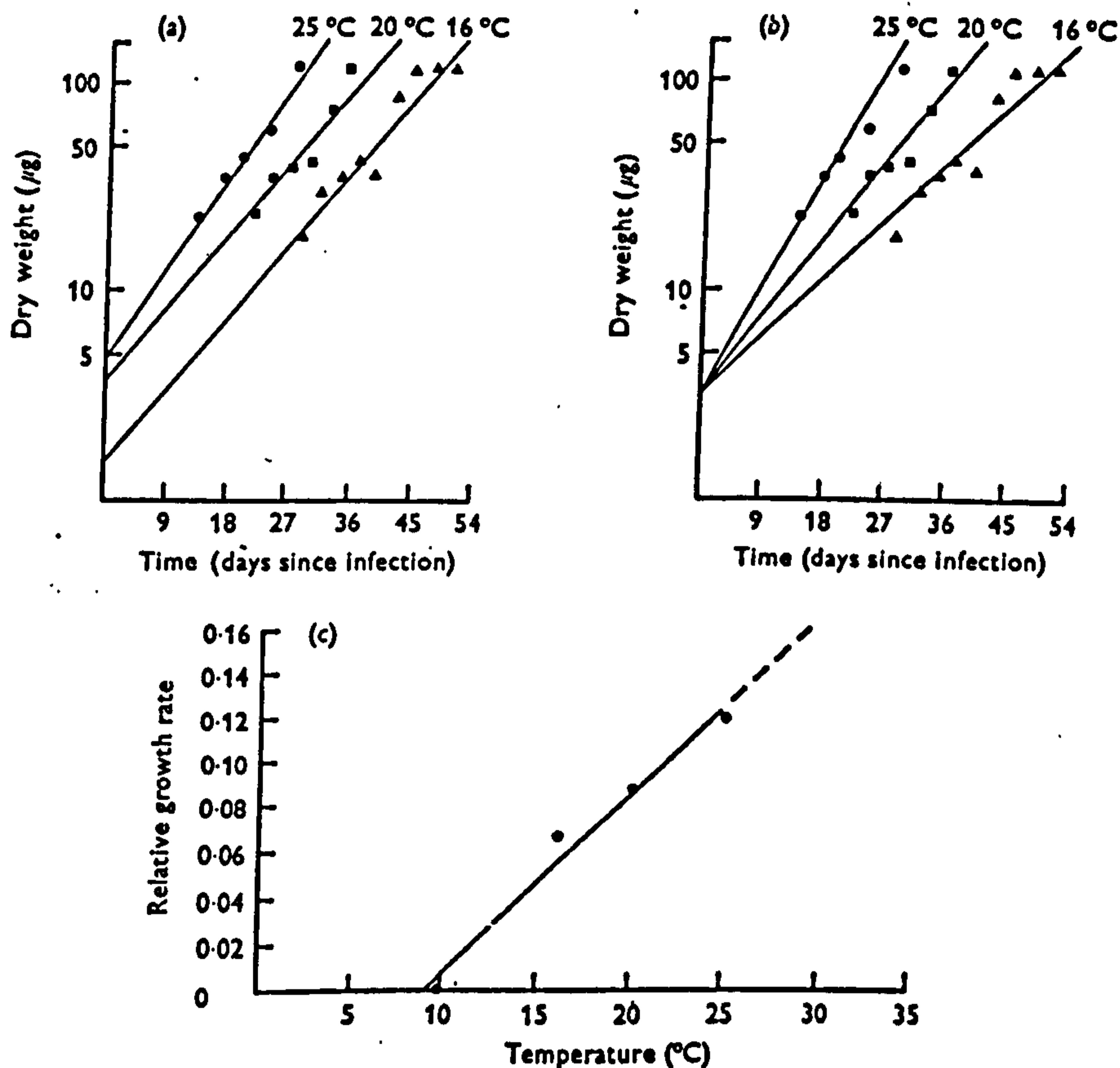


Fig. 1. (a) Plots of dry weight of the parasite against the days since infection, with their separate intercepts. (b) Plots of the same data as (a) but with a fixed intercept. (c) The relationship of the relative growth rate and temperature  $^{\circ}\text{C}$ .

times in close agreement with our experimental data. Simulations using growth rate values read from the regression line of experimental growth rates on temperature, gave close correlation at 20° and 25 °C, but produced a somewhat longer estimate than expected at 16 °C.

The result of the simulation of parasite growth with respect to variation in environmental temperatures is illustrated in Figs. 2 and 3. The histogram in Fig. 2 shows the number of times per month that a simulated infection reached maturity irrespective of when that infection was started. Since the restriction was placed that infection only occurred from May to October, the peak in August to October of the first year represents the short summer development of the parasite. The peak from May to July of the second year represents the long winter development which was started in the previous autumn. In Fig. 3 the duration of an infection (in days) is plotted against the time of the year at which the infection occurred. It can be seen that the shortest larval development occurs in infections starting in late June and July. The longest predicted larval development occurs in infections

Table 2. *The growth array*

Temperature range (°C)	Relative growth rate
25-29.99	0.1450
20-24.99	0.1055
15-19.99	0.0660
10-14.99	0.0262
0- 9.99	Zero

Table 3. *A comparison of the times to maximum redial weight of the infections*

(Time in days to maximum redial weight.)

Temperature (°C)	Ollerenshaw (1971a)	Our own experiments	Simulation growth rate from experiment	Simulation growth rate from growth array
10	—	—	—	—
15	82	—	—	—
16	—	56	56	68
17.5	53	—	—	—
20	40	39	42	43
22.5	34	—	—	—
25	25	30	30	29
27	22	—	—	—

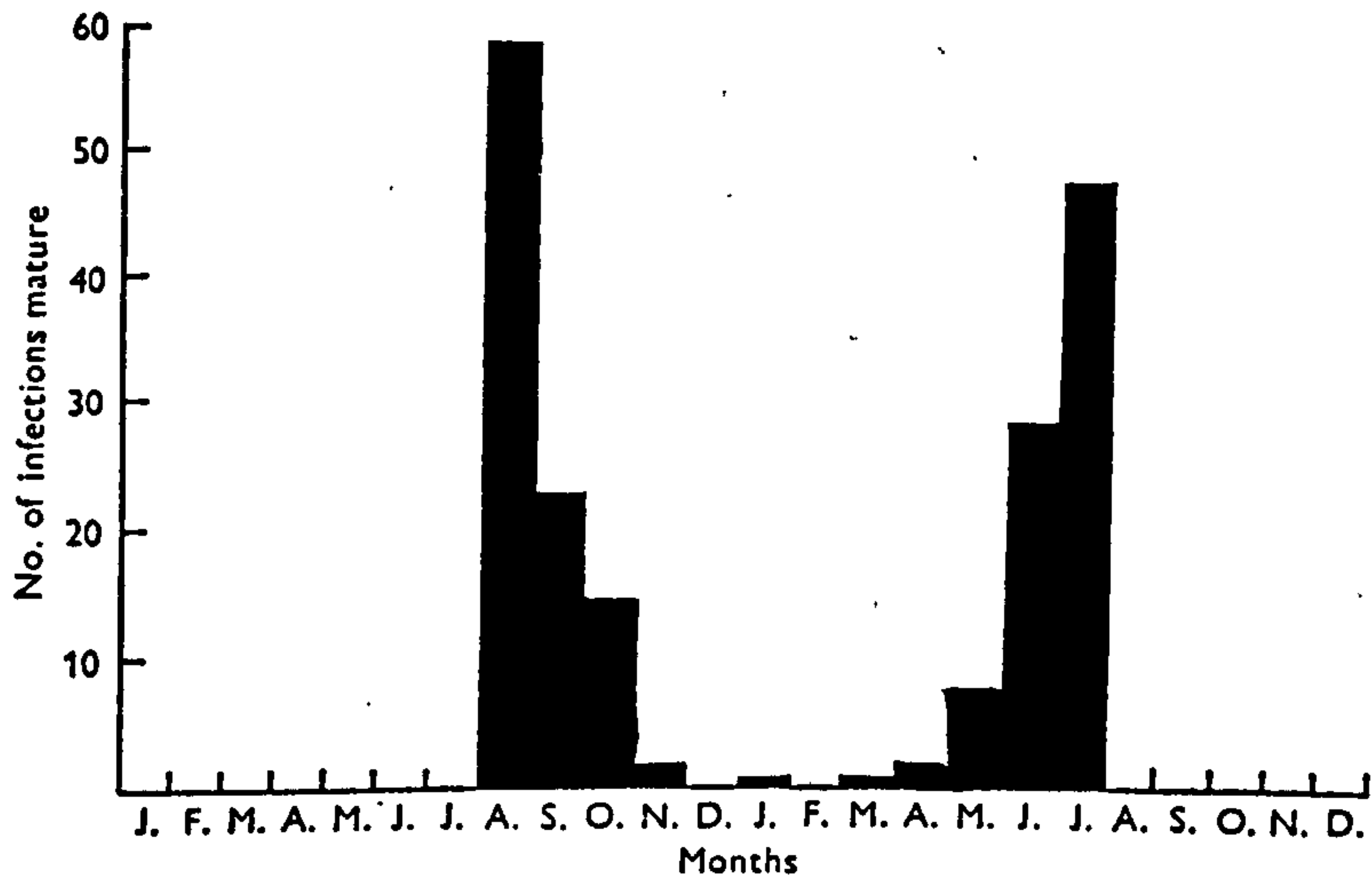


Fig. 2. The number of infections that mature per month as predicted by the simulation.

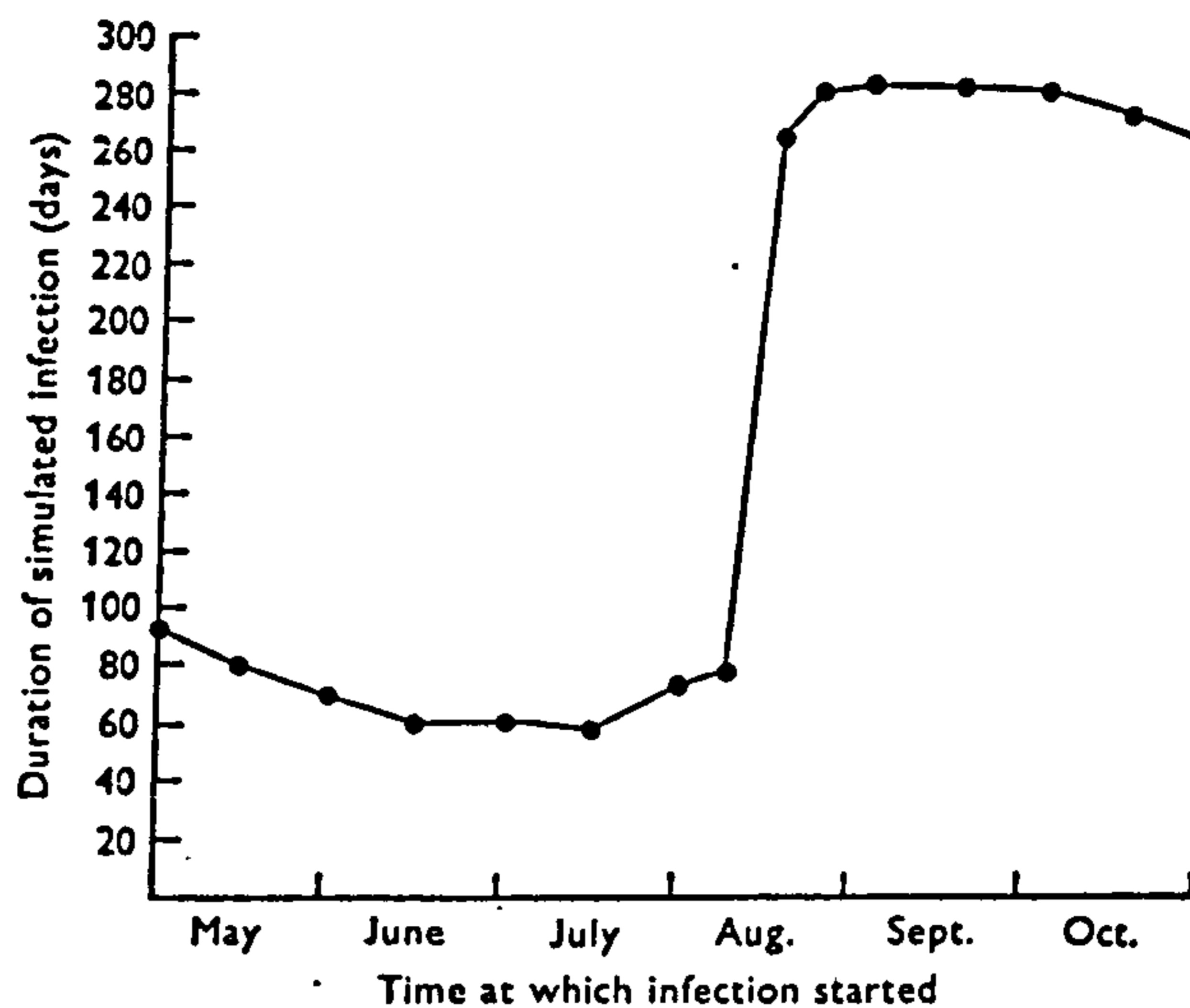


Fig. 3. A plot of the duration of simulated infections (in days) against the time at which they started.

starting in mid-August through to late October. The graph also clearly shows the switch-over point in mid-August from the short summer to the long winter development.

#### DISCUSSION

When the natural logarithm of the parasite dry weight is plotted against time (Fig. 1a) the intercepts are not the same; our use of a fixed intercept regression to rectify this could produce some distortion in the final lines. It can in fact be seen that it is only the 16 °C line which is considerably affected. In addition to this, the development time from our experiment at 16 °C was much shorter than the value estimated from Ollerenshaw (1971a). The difference here is partly counteracted in the simulation by using the values of relative growth rate taken from the regression line Fig. 1c. The development time at 16 °C calculated from this line can be seen (Table 3) to give a value much nearer to that expected from Ollerenshaw (1971a).

The development of the parasite within the snail consists of two separate stages, namely the sporocyst and redia. It has not been possible to determine the growth of sporocysts since they are minute and almost impossible to remove from snail tissue. We have, therefore, made a reasonable assumption that redial growth is initiated on the first day after the infection of the snail by the parasite. It should be noted that the weight range over which redial growth was measured accounts for approximately 85% of the increase in dry weight of the redia during its development within the snail. At low temperatures it is possible that a mixture of daughter rediae and cercariae will be produced by mother rediae (Dinnik & Dinnik, 1964). We have no evidence to suggest that daughter redial production affects the rate of cercarial maturation. We have, therefore, taken no account of this in the simulation.



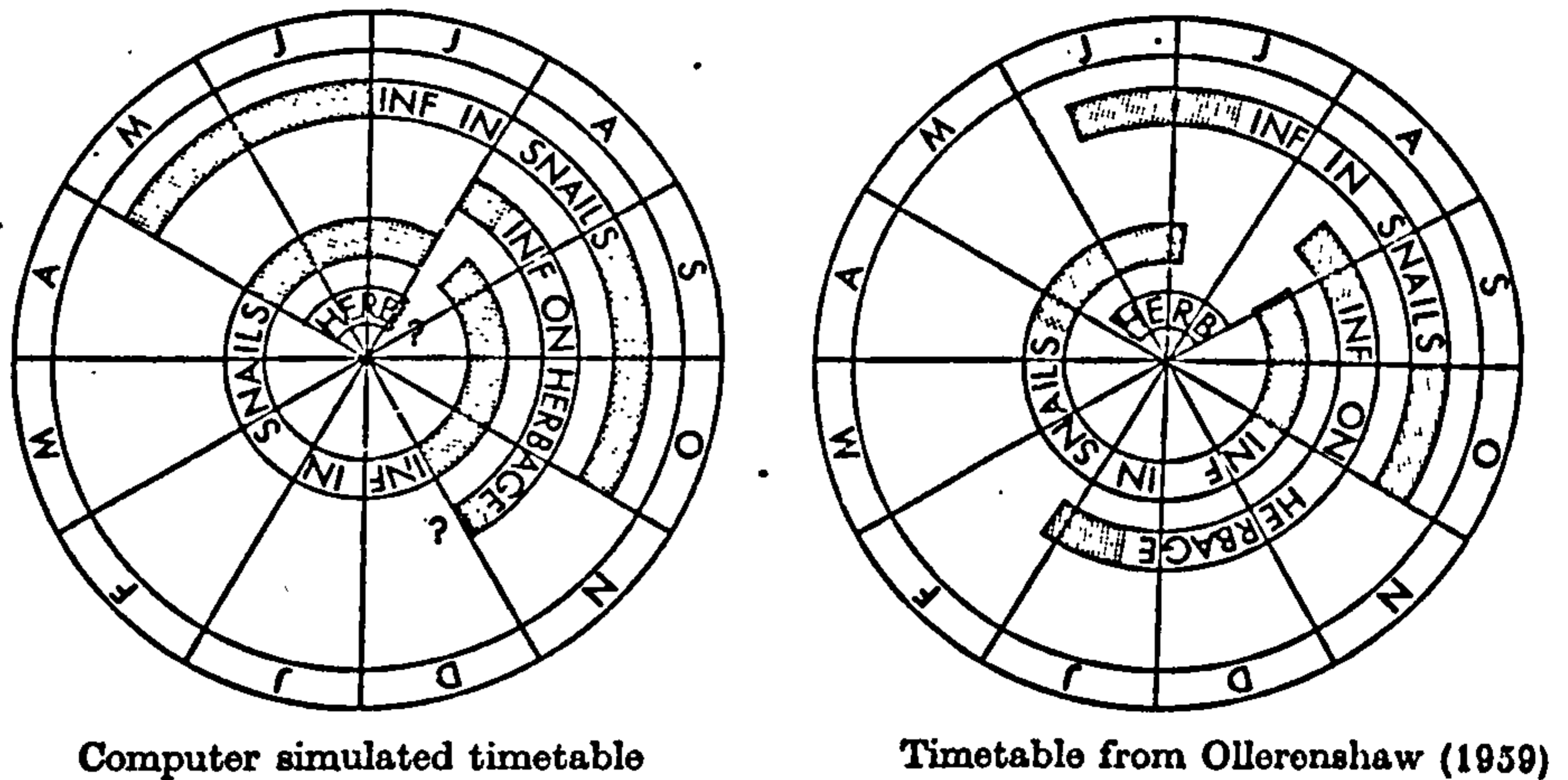


Fig. 4. The seasonal time-tables. The stippled arcs show the presence of the parasite. The two ? symbols indicate that these arcs may extend further, depending on the survival of the cysts on the herbage.

The results of the simulation illustrated in Fig. 2 can be represented as a seasonal timetable, based on the method used by Ollerenshaw (1959). This is compared with a modified version of his timetable which was compiled from field data (Fig. 4). These diagrams show the period during which the parasite is carried within the snails and when it is on the herbage during a cycle of 12 months. The summer and winter infections, represented by the two peaks in Fig. 2, are shown as the two outer and two inner arcs of Fig. 4, respectively. The infection within the snail represents the sporocyst and redial stages, whilst the infection on the herbage represents the cercarial and metacercarial stages.

If we presuppose the availability of fully developed parasite eggs during the warmer months (May to October), then the simulated cycle can be compared with field data. In the simulation, infection starts in the snail at the beginning of May, however, infection is not detected in the field until mid-June. This discrepancy might well be explained by the failure of fieldworkers to detect sporocysts or early rediae in the snails. The latest time at which an infection can occur in the field and mature in the same year varies from mid-August to early September depending on climatic conditions (Ollerenshaw, 1971*a*). In the simulation, which is based upon temperature data for 1969, the switch to an overwintering infection occurs in mid-August. The last snail infections occur at the end of October and mature in the following July; infections may not occur this late in the field.

In the simulation, cercarial shedding by the summer infections starts at the beginning of August and the largest number of infections matured during that month. Ollerenshaw's timetable suggests that cercarial shedding does not occur in the field until mid-August. In the case of the winter infection there is good agreement between the simulation and the field data, both of which indicate cercarial shedding in early May. This close agreement between the simulation and the field data makes it highly likely that temperature is the primary factor influencing the rate of development of the parasite.

The results of the simulation can be used to make a tentative estimate of the duration of the complete parasite life-cycle in the field, allowing a period of 3 weeks for egg development (Rowcliffe & Ollerenshaw, 1960) and of 7 weeks for the maturation of the fluke within its mammalian host (Pantelouris, 1965). We can thus determine whether the short summer and the long winter infections described by Ollerenshaw (1959) represent two life-cycles of the parasite per annum. The simulation predicts that a snail infected in mid-May will shed cercariae at the beginning of August. Assuming immediate infection of the mammalian host, parasite eggs will not be ready to hatch before mid-October. A snail infected at this time will then shed cercariae towards the end of the following July. Parasite eggs resulting from another immediate infection of the mammalian host will then not be ready to hatch and infect their snail intermediate host until mid-October. This makes a minimum total of 17 months for two complete life-cycles of the parasite. In most cases two complete cycles will take considerably longer. It therefore seems more correct to say that in southern Britain there are two overlapping periods of cercarial shedding, resulting from the short summer and long winter infections, rather than two complete life-cycles of the parasite per annum.

In order to simulate growth, a series of temperature ranges of 5 °C extent were selected and a relative growth rate for each range was determined (the growth array). The proportion of each day that the temperature was within each of the given ranges was calculated (the temperature array). The selection of 5 °C ranges was arbitrary, but the multiplication of the growth values by the appropriate values in the temperature array in the computer simulation produced estimates of growth in agreement with field data. This is sufficiently encouraging to suggest that this method could be used to investigate temperature-related growth of any parasite stage, e.g. the egg. In addition, the technique might be expanded to produce a model of the whole life-cycle by the inclusion of other important parameters affecting growth, e.g. soil moisture. This we are now attempting to do in the case of *F. hepatica*.

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#### REFERENCES

- DINNIK, J. A. & DINNIK, N. N. (1964). The influence of temperature on the succession of redial and cercarial generations of *Fasciola gigantica* in a snail host. *Parasitology* 54, 59-65.
- KENDALL, S. B. (1953). The life history of *Limnaea truncatula* under laboratory conditions. *Journal of Helminthology* 27, 17-28.
- OLLERENSHAW, C. B. (1959). The ecology of the liver fluke *Fasciola hepatica*. *Veterinary Record* 71, 957-63.
- OLLERENSHAW, C. B. (1971a). Some observations on the epidemiology of fascioliasis in relation to the timing of molluscicide applications in the control of the disease. *Veterinary Record* 88, 152-64.
- OLLERENSHAW, C. B. (1971b). Forecasting liver fluke disease in England and Wales 1958-1968 with a comment on the influence of climate on the incidence of disease in some other countries. *Veterinary Medical Review* no. 2/3, 289-312.

- PANTELOURIS, E. M. (1965). *The Common Liver Fluke*. Oxford: Pergamon Press.
- PATEFIELD, W. M. & AUSTIN, R. B. (1971). A model for the simulation of growth of *Beta vulgaris* (L). *Annals of Botany* 35, 1227-50.
- PULLIN, R. S. V. (1971). Composition of the haemolymph of *Lymnaea truncatula*, the snail host of *Fasciola hepatica*. *Comparative Biochemistry and Physiology* 40 A, 617-26.
- ROWCLIFFE, S. A. & OLLERENSHAW, C. B. (1960). Observations on the bionomics of the egg of *Fasciola hepatica*. *Annals of Tropical Medicine and Parasitology* 54, 172-81.
- SMYTH, J. D. (1966). *The Physiology of Trematodes*. Edinburgh and London: Oliver and Boyd.
- WIT, C. T. DE, BROUWER, R. & PENNING DE VRIES, F. W. T. (1969). A dynamic model of plant and crop growth. In *Potential Crop Production* (ed. P. F. Wareing and J. P. Cooper). London: Heinemann.
- WRIGHT, C. A. (1971). Ecology of life cycles. In *Flukes and Snails*. London: Allen and Unwin Ltd.

## REFERENCES

- BAILEY, J.L. (1931). Some Data on Growth, Longevity and Fecundity in Lymnaea columella. Say: Biologia Generalis 7 : 407.- 428.
- BAILEY, N.T.J. (1968). Statistical Methods in Biology. London : English Universities Press.
- BARBOSA, F. COELHO, M. & DOBBIN, J.. (1954). Qualidades de vetor dos hospederios de Schistosoma mansoni no Brazil. II Duracao da infestacao e eliminacao de cercarias em Australorbis glabrata. Publicacoes Avulsas Brazil III, 7, 79 - 92.
- BELLE, E.A. (1959). The effect of microenvironment on the free-living stages of Bunostomum trigonocephalum. Canadian Journal of Zoology 37, 289 - 298.
- BOER, H.H. & JOOSSE, J. (1975). Endocrinology, cpt. 6. In Pulmonates Vol. 1. Ed. Fretter, V. & Peake, J. London : Academic Press.
- BORAY, J.C. (1963). The ecology of Fasciola hepatica with particular reference to its intermediate host in Australia. Proceedings of the 17th World Veterinary Congress, Hanover. 1, 709 - 715.
- BORAY, J.C. & ENIGK, K. (1964). Laboratory studies on the survival and infectivity of Fasciola hepatica and Fasciola gigantica metacercariae. Zeitschrift fur tropenmedizin und parasitologie. 15, 324 - 331.
- BOUILLON, J. (1956). Influence of Temperature on the Histological Evolution of the Ovotestis of Cepea nemoralis. L. Nature, London. 177, 142 - 143.

BRUMPT, E. (1941). Observations biologiques diverses concernant Planorbis (Australorbis) glabratus, hôte intermédiaire de Schistosoma mansoni. Annals of Parasitology. 18: 9 - 45.

CHOWANIEC, W. (1961). Influence of environment on the development of the liver fluke and the problem of super-invasion and re-invasion in the intermediate host. Acta Parasitologica Polonica. IX, 463 - 479.

CHU, K.J., SABBAGHIAN, H. & MASSOUD, J. (1966). Host-parasite relationship of Bulinus truncatus and Schistosoma haematobium in Iran.

2) The effect of exposure dose of miracidia on the biology of the snail host and the development of the parasites. Bulletin of the World Health Organisation. 34, 121 - 130.

COELHO, M.V. (1954). Acao das formas larvarias de Schistosoma mansoni sobre a reproducao de Australorbis glabratus. Publicacoes Avuls. Inst. Aggen Magalhaes. Brazil. 3: 39 - 54.

CROFTON, H.D. (1965). Ecology and Biological Plasticity of Sheep Nematodes. I. The effect of temperature on the hatching of eggs of some sheep nematodes. Cornell Veterinaria 1965. Volume 55, pp 242 - 250.

DE JESUS, Z. (1935). Lymnaea philippinensis, an intermediate host of Fasciola hepatica in the Philippines, with some observations on the bionomics of the parasite. The Philippine Journal of Science 58, 299 - 317.

DeWITT, R.M. (1954). Reproductive Capacity in a Pulmonate Snail Physa gyrina. Say: American Naturalist 88: 159 - 164.

- DINNIK, J.A. & DINNIK, N.N. (1959). Effect of the seasonal variations of temperature on the development of Fasciola gigantica eggs in the Kenya Highlands. Bulletin of Epizootic Diseases of Africa 7, 357 - 369.
- ETGES, F.J. & GRESSO, W. (1965). Effect of Schistosoma mansoni infection upon fecundity in Australorbis flabratus. The Journal of Parasitology 51: 757 - 760.
- GRAINGER, J.N.R. (1959). The effect of constant and varying temperatures on the developing eggs of Rana temporaria L. "Zoologischer Anzeiger" Bd. 163, Heft 9/10, 265 - 277.
- HARRIS, R.E. & CHARLESTON, W.A.G. (1971). Control of fascioliasis - some theoretical and practical considerations. New Zealand Veterinary Journal 19, 4, 65 - 72.
- HEPPLESTON, P.B. (1972). Life History and Population Fluctuations of Lymnaea truncatula, the snail vector of Fascioliasis. Journal of Applied Ecology 9. 235 - 248.
- HEYNEMAN, D. & UMATHEVY, T. (1968). Interaction of trematodes by predation within natural double infections in the host snail Indoplanorbis exastus. Nature (London) 217, 283 - 285.
- HODASI, J.K. (1971). Thesis. PhD. University of Bristol.
- HODASI, J.K. (1972a). The output of cercariae of Fasciola hepatica by Lymnaea truncatula and the distribution of metacercariae on grass. Parasitology 64, 53 - 60.
- HODASI, J.K.M. (1972b). The effects of Fasciola hepatica on Lymnaea truncatula. Parasitology 65: 359 - 369.

- HODASI, J.K.M. (1976). The Effects of Low Temperature on Lymnaea truncatula. Zeitschrift fur Parasitenkund 48. 34. 281 - 286.
- HOPE CAWDREY, M.J. GETTINBY, G. & GRAINGER, J.N.R. (1978). Mathematical models for predicting the prevalence of liver-fluke disease and its control from biological and meteorological data. In World Meteorological Organisation Technical Note No. 159 "Weather and Parasitic Animal Disease".
- KENDALL, S.B. (1949a). Bionomics of Lymnaea truncatula and the parthenitae of Fasciola hepatica under drought conditions. Journal of Helminthology 23, 57 - 68.
- KENDALL, S.B. (1949b). Nutritional factors affecting the rate of development of Fasciola hepatica in Lymnaea truncatula. Journal of Helminthology 23, 179 - 190.
- KENDALL, S.B. (1950). Snail Hosts of Fasciola hepatica in Britain. Journal of Helminthology 24,  $\frac{1}{2}$ , 63 - 74.
- KENDALL, S.B. & McCULLOUGH, F.S. (1951). The emergence of the cercariae of Fasciola hepatica from the snail Lymnaea truncatula. Journal of Helminthology 25, 77 - 92.
- KENDALL, S.B. (1953). The Life-History of Lymnaea truncatula under Laboratory Conditions. Journal of Helminthology. XXVII.  $\frac{1}{2}$ . 17 - 28.
- KRULL, W.H. (1941). The number of cercariae of Fasciola hepatica developing in snails infected with a single miracidium. Proceedings of the Helminthological Society, Washington, U.S.A. 8, 55 - 58.
- LAWSON, J.R. (1977). The Biology of the cercariae and early Schistosomulum of Schistosoma mansoni. D.Phil. University of York 1977.

- MASSOUD, J. (1974). The effect of variation in miracidial exposure dose on laboratory infections of Ornithobilharzia turkestanicum in Lymnaea gedrosiana. Journal of Helminthology 48, 139 - 144.
- McCLELLAND, G. & BOURNS, T.K.R. (1968). Effects of Trichobilharzia ocellata on growth, reproduction and survival of Lymnaea stagnalis. Experimental Parasitology. 24: 137 - 146.
- McCRAW, B.M. (1970). Aspects of the Growth of the Snail Lymnaea palustris (Muller). Malacologia 1970, (10) 2: 399 - 415.
- McDONALD, S.C. (1973). Activity Patterns of Lymnaea stagnalis (L) in relation to temperature conditions: A Preliminary Study. Malacologia 1973: 14: 395 - 396.
- MEHL, S. (1934). Die lebensbedingungen der leberegel-schnecke (Galba truncatula Muller). Arb. Bayer Landesanstalt Pfl.Bau. 10.
- MEULEMAN, E.A. (1972). Host-parasite inter-relationships between the freshwater pulmonate Biomphalaria pfeifferia and the trematode Schistosoma mansoni. Netherlands Journal of Zoology. 22: 355 - 427.
- MICHELSON, E.H. (1961). The Effects of Temperature on Growth and Reproduction of Australorbis glabratus. American Journal of Hygiene: 73: 66 - 74.
- NICE, N.G. & WILSON, R.A. (1974). A study of the effect of temperature on the growth of Fasciola hepatica in Lymnaea truncatula. Parasitology 68, 47 - 56.



- OLIVIER, L. BARBOSA, F.S. & COELHO, M.V. (1954). The influence of infection with Schistosoma mansoni on survival of Australorbis glabratus. Publicacoes Avuls. Inst. Aggen Magalhaes Brazil. 3: 63 - 71.
- OLLERENSHAW, C.B. (1967). Some observations on the epidemiology and of fascioliasis in Wales. Second International Liver Fluke Colloquium, Wageningen, Holland. 103 - 125.
- OLLERENSHAW, C.B. (1971a). Forecasting Liver fluke disease in England and Wales 1958 - 1968 with a comment on the influence of climate on the incidence of disease in some other countries. Veterinary Medical Review No. 2/3, 289 - 312.
- OLLERENSHAW, C.B. (1971b). Some observations on the epidemiology of fascioliasis in relation to the timing of molluscicide applications in the control of the disease. The Veterinary Record 88, 152 - 164.
- OLLERENSHAW, C.B. (1974). Forecasting liver fluke disease. Symposia of the British Society for Parasitology. 12: 33 - 52.
- OLSEN, O.W. (1944). Bionomics of the Lymnaeid snail, Stagnicola bulimoides techella, the intermediate host of the liver fluke in Southern Texas. Journal of Agricultural Research: 69, 389 - 493.
- PAN, C. (1963). Generalized and focal tissue responses in the snail, Australorbis glabratus infected with Schistosoma mansoni. Annals of the New York Academy of Science. 113. 475 - 485.
- PAN, C. (1965). Studies on the host-parasite relationship between Schistosoma mansoni and the snail Australorbis glabratus. American Journal of Tropical Medicine and Hygiene. 14. 931 - 976.

- PAULINI, E. (1958). Bilharziasis control by application of molluscicides. Bulletin of the World Health Organisation 18: 975 - 988.
- PERLOWAGORA-SZUMLEWICZ, A. (1968). The reaction of Australorbis glabratus (Biomphalaria glabrata) to infection with Schistosoma mansoni. Rev. Inst. Med. Trop. Sao Paulo. 10: 219 - 288.
- PETERS, B.G. (1938). Habitats of Lymnaea truncatula in England and Wales during dry seasons. Journal of Helminthology 16: 213 - 260.
- REES, G.F. (1934). Cercariae patellae (Lebour 1911) and its effect on the digestive gland and gonads of Patella vulgata. Proceedings of the Zoological Society (London) 1934: 45 - 53.
- ROBERTS, E.W. (1950). Studies on the life cycle of Fasciola hepatica and of its snail host Lymnaea truncatula in the field and under controlled conditions in the laboratory. Annals of Tropical Medicine and Parasitology 44, 187 - 206.
- RONDELAUD, D. & VINCENT, M. (1973). Les effets du parasitisme sur la croissance de Galba truncatula Muller en Limousin. Societe de Biologie de Poitiers, Paris. 167: 736 - 738.
- ROTHSCHILD, M. (1941). The effect of trematode parasites on the growth of Littorina neritoides. Journal of the Marine Biological Association. 25: 69 - 80.
- ROWCLIFFE, S.A. & OLLERENSHAW, C.B. (1960). Observations on the bio-nomics of the egg of Fasciola hepatica. Annals of Tropical Medicine and Parasitology 54, 172 - 181.
- RUSSELL, E.W. (1973). Soil Conditions and Plant Growth. Longmans: 10th Edition.

- SCHMIDT-NIELSEN, K. (1975). *Animal Physiology: Adaptation and Environment*. London: Cambridge University Press.
- SCHOFIELD, R.K. (1935). The pF of Water in Soil. *Transcriptions of the 3rd International Congress of Soil Science*. 2: 37 - 48.
- SMITH, G. (1978). A Field and Laboratory Study of the Epidemiology of Fascioliasis. D.Phil. Thesis: University of York 1978.
- SOKAL, R.R. & ROHLF, F.J. (1969). Biometry: The Principles and Practice of Statistics in Biological Research. W.H. Freeman & Co.: San Francisco, U.S.A.
- STIREWALT, M.A. (1954). Effect of snail maintenance temperature on the development of Schistosoma mansoni. Experimental Parasitology 3, 504 - 516.
- STURROCK, B.M. (1966). The influence of infection with Schistosoma mansoni on the growth rate and reproduction of Biomphalaria pfeifferi. Annals of Tropical Medicine and Parasitology. 60. 187 - 197.
- STURROCK, B.M. & STURROCK, R.F. (1970). Laboratory studies of the host-parasite relationship of Schistosoma mansoni and Biomphalaria glabrata from St. Lucia, West Indies. Annals of Tropical Medicine and Parasitology. 64, 3. 357 - 363.
- STYCZYNSKA, E. (1956). The influence of the dessication of Galba truncatula O.F. Mull. on the development and surviving of the development stages of Fasciola hepatica L. Wiadomosci Parazytologiczne 2. 261 - 262.

- SWALES, W.E. (1935). The life cycle of Fascioloides magna (Bassi, 1875) the large liver fluke of ruminants in Canada. Canadian Journal of Research 12, 177 - 215.
- TAYLOR, E.L. & MOZLEY, A. (1948). A culture method for Lymnaea truncatula. Nature, London, 161: 894.
- TAYLOR, M. (1922). Water Snails and Liver Flukes. Nature, London, 110: 701.
- THOMAS, A.P. (1883). The life history of the liver fluke. Quarterly Journal of Micro Science, 23: 99 - 133.
- VAN DER STEEN (1967). The Influence of Environmental Factors on the oviposition of Lymnaea stagnalis (L) under laboratory conditions. Archives Neerlandaises de Zoologie Tome XVII: 4: 403 - 468.
- VAUGHN, C.M. (1953). Effect of Temperature on Hatching and Growth of Lymnaea stagnalis appressa. Say: American Midland Naturalist 1953 Volume 44. 214 - 228.
- WALTON, C.L. (1918). Liver Rot of Sheep and Bionomics of Lymnaea truncatula in the Aberystwyth Area. Parasitology. 10. 232 - 266.
- WALTON, C.L. & JONES, W.N. (1926). Further observations on the Life-history of Lymnaea truncatula. Parasitology 18: 144 - 147.
- WALTON, C.L. & WRIGHT, W.R. (1926). Hydrogen-ion Concentration and the distribution of Lymnaea truncatula and Lymnaea peregra with a note bearing on mosquitoes. Parasitology 18, 363 - 369.

WESENBERG-LUND, C. (1934). Contributions to the development of the trematode Digenea. Kungl. Danske Vidensk. Selsk. Skifter. Naturw. Math. Afd. Raekke. g: 1 - 223.

WILSON, R.A. & DRASKAU, T. (1976). The Stimulation of Daughter Redia Production during the Larval Development of Fasciola hepatica. Parasitology, 72: 245 - 257.

WILSON, R.A. & TAYLOR, S.L. (1978). The effect of variations in host and parasite density on the level of parasitization of Lymnaea truncatula by Fasciola hepatica. In Parasitology 76, 91 - 98.

WRIGHT, C.A. (1971). The pathogenesis of Helminths in the Mollusca. Helminthological Abstracts 35: 207 - 224.

YONG, R.N. & WARKENTIN, B.P. (1975). Soil Properties and Behaviour. Elsevier Scientific Publication Co., New York.