Effects of Constant Temperature and Humidity on the Development of the Larvae and the Pupae of the Three Indian Species of Xenopsylla (Insecta: Siphonaptera)

M. Sharif

Phil. Trans. R. Soc. Lond. B 1949 **233**, 581-633
doi: 10.1098/rstb.1949.0004

EFFECTS OF CONSTANT TEMPERATURE AND HUMIDITY ON THE DEVELOPMENT OF THE LARVAE AND THE PUPAE OF THE THREE INDIAN SPECIES OF *XENOPSYLLA* (INSECTA: SIPHONAPTERA)

By M. SHARIF, D.Sc., Ph.D., *Assistant Director, Department of Entomology,*
*Haffkine Institute, Parel, Bombay (India)**

(*Communicated by D. Keilin, F.R.S.—Received* 25 *October* 1946—*Revised* 13 *September* 1948)

CONTENTS

I. INTRODUCTION

Our present knowledge of the bionomics of the Indian rat-fleas, *Xenopsylla cheopis* (Rothschild), *X. astia* Rothschild and *X. brasiliensis* (Baker), which are experimentally proved vectors of plague (Webster & Chitre 1930*b*, p. 422), though not equally effective, is incomplete (Hirst 1926, p. 201; Buxton 1938, p. 505); in view of this fact it was deemed necessary to ascertain the effects of different ecological factors on their development. The primary object of this study is to elucidate the causes governing the fluctuations in the flea numbers. The correlation between outbreaks of plague and the abundance and geographical distribution of these species of rat-fleas has been emphasized by many workers (see, for instance, Advisory Committee 1910, p. 568; Cragg 1921, p. 395; 1923,

---

* Now at Malaria Institute of Pakistan, Karachi.

Vol. 233. B. 607. (Price 15*s*.) [Published 29 June 1949

p. 961). In order to assess the wild populations of the fleas, several workers have made use of 'flea counts' of domestic rats; this method, as pointed out by Hirst (1926, p. 248) and Buxton (1938, pp. 505, 528), is hardly adequate for the purpose. Fleas do not always live on rats; a vast majority of them remain in the debris or litter of rat burrows, and a very small proportion of them visit their hosts in order to feed. There is not likely to be a fixed ratio between the number of fleas found on rats and those present in burrows (see Leeson 1936, p. 404; Buxton 1938, p. 528). In consequence, it is doubtful whether the flea counts have any significance. An accurate knowledge of the ecological factors that govern the fluctuations of flea populations is essential.

The present study is only concerned with the effects of *constant* temperature and humidity on the development of larvae and pupae of the three species of rat-fleas; as they are mostly inhabitants of the debris in a rat burrow, the prevailing micro-climatic conditions remain stable for a time sufficiently long to allow the insects to complete one or more generations. Constant temperatures are known to cause more rapid development than the variable temperatures in insects which live in equable climatic conditions, such as *Ephestia kuehniella* Zeller (see Ahmad 1937, p. 162); compare also Bacot's observations (1914, p. 538) that a sudden change in temperature 'near the date of spinning' cocoons, prolongs the period spent in the cocoon in certain species of flea.

II. MATERIAL AND TECHNIQUE

Flourishing stocks of the three species of Indian rat-fleas have been maintained since October 1938 at the Haffkine Institute by adopting a technique similar to that described by Leeson (1932, pp. 25–26), with a slight modification in the larval food, for which both blood and yeast were used. The original stocks of these fleas were obtained from the domestic rats of the City of Bombay.

The methods employed for conducting experiments were similar to those described in an earlier communication (Sharif 1937, pp. 225–226). Constant relative humidities (R.H.) were maintained by keeping appropriate mixtures of extra pure sulphuric acid (Merck) and distilled water at the bottom of desiccators in proportions stated by Shelford (1930, p. 252), and Buxton & Mellanby (1934, p. 174). Many of the experiments were conducted in incubators. A use was made of low-temperature incubators with forced circulation for temperatures lower than the room temperature; but for 0, 2, 10·5 and 17° C iron cupboards in the remote corners of low-temperature rooms were used.

In order to ascertain the effects of variations in temperature and humidity on the development of rat-flea larvae and pupae, all other factors, such as food, soil, darkness, etc., were kept uniform in the experiments. In most experiments unfed larvae under 24 hr. old were used. They were reared in batches in small pyrex glass tubes, having a diameter of $1\frac{1}{4}$ in. and a length of 2 in. Each tube was capped with a small piece of fine voile, kept in place by adhesive plaster to prevent the escape of adult fleas. To avoid delayed emergence of adults due to their unnecessary resting within cocoons, the tubes were shaken for a few minutes at every observation.

Most of my experiments were continuous, i.e. the recently hatched larvae continued at the same temperature and humidity up to adult emergence. In most cases cocoon formation was taken as a landmark between the completion of active larval life and adult emergence. This necessitated calculations for four developmental periods, viz. active larval life up to cocoon formation, resting larval and pupal life within cocoons, and combined larval and pupal life for the two sexes separately; for all the adult female fleas invariably emerged a few days before the first male. Experiments of each type were repeated in order to ensure the accuracy of results which were pooled. The means given are weighted averages. Most of the results were statistically evaluated, and were subjected to suitable tests of significance based on the formulae given by Fisher (1941) and Fisher & Yates (1943). The tests of significance used were the t and χ^2, using 'Yates's correction for continuity', except when the numbers were too few and the exact method of χ^2 was used. When tested in pairs for significance, a difference which is not statistically significant is indicated by the sign $-$, that which is significant at 5 % level is denoted by $+$, and that at 1 % level by $\times$; the latter is referred to as 'highly significant'. The signs in each column of the tables denote the level of significance between a particular value, against which a sign is inserted, and the one higher in the column with which it is connected by an arrow.

'Favourable' temperatures or humidities, as designated in this paper, are those at which successful development of about 100 % rat-flea larvae and pupae is possible, provided the other conditions are optimum. They should not be confused with 'optimum' temperatures or humidities, which give the quickest rate of development. The favourable temperatures comprise 'medial temperatures' (see Shelford 1930, p. 368), and adjacent ones at which, though growth rate is slower, there is no appreciable mortality. The favourable humidities include optimum and high humidities. Temperatures or humidities at which growth is possible even though slight are termed 'effective' or vital.

III. Historical survey

Most of the literature on the life history and the biology of fleas in general has already been reviewed by Sikes (1930, pp. 243–244) and Sharif (1937, pp. 226–228). As the three species of Indian rat-fleas were described for the first time in the beginning of the present century, our knowledge is comparatively recent; but owing to the discovery of their connexion with plague, a number of papers on their biology have been published. The earlier ones are by the Advisory Committee (1908, 1912) and Bacot (1914). Complications in interpreting their data arise from the fact that these workers presumed that all fleas found on the Indian rats were *Xenopsylla cheopis*; but they were really dealing with a mixture of *X. cheopis* and *X. astia*, and perhaps of *X. brasiliensis* also. In consequence, their observations on the bionomics cannot be considered applicable to *X. cheopis* alone. Moreover, both temperature and humidity fluctuated considerably during their experiments, and their methods of measurement and control of these factors were 'not so precise as those which are used nowadays' (Buxton 1938, p. 525).

Cragg & Swaminath (1923) carried out breeding experiments with *X. astia* at Agra under crudely controlled conditions of temperature and humidity. Hirst (1926, p. 211) reared the larvae of *X. astia* and *X. cheopis*, and Webster (1930, pp. 397–404) those of these two species and *X. brasiliensis* at the room temperature and humidity of Colombo and Bombay respectively. Hopkins (1935) and Edney (1945) bred the larvae of *X. cheopis* and

X. brasiliensis at a very few constant temperatures and humidities. Buxton (1938) made quantitative studies on the biology of *X. cheopis* under controlled conditions within a limited range of temperature and humidity in an ingenious 'synthetic mouse-hole'. Mellanby (1932*a*, p. 226) found that the large larvae of *X. cheopis* could survive an exposure of 24 hr. to a much higher temperature in moist than in dry air, and that there was a regular fall in their thermal death-points with the decrease of humidity. Subsequently, he (1933) defined the conditions of temperature and humidity under which this species can complete its metamorphosis.

In spite of the attempts made so far, our knowledge of the bionomics of Indian rat-fleas is imperfect and far from satisfactory. The larval diets used by almost all the workers for rearing these fleas have a poor and varied nutritive value, as is borne out by high and variable mortality rates in their experiments. The food factor might have influenced the results which are attributed solely to climatic conditions.

IV. Influence of debris or soil on the development of flea larvae

The three species of Indian rat-fleas pass their life intimately associated with debris; the eggs, larvae and pupae are always in debris, and even their adults spend a considerable portion of their life in it. Consequently, it is reasonable to suggest that the nature of debris or soil in a rat burrow influences the development of their early stages, just as it does in soil-inhabiting insects. Edney (1947, p. 394) actually demonstrated that the texture of sand, used as a matrix for the larval food, affected the development of *X. brasiliensis* materially. In order to ascertain the precise effects of temperature and humidity on the development of rat-flea larvae, it was found necessary to maintain standard and uniform soil conditions. The greatest difficulty, however, arises in controlling the biological content of the soil, as the number and variety of micro-organisms in soil are often great, and they may materially affect the developmental rate of flea larvae by increasing the nutritive value of their food (see Wigglesworth 1939, p. 286). For this reason an ignited and acid-washed sea sand was used in almost all the experiments.

Freshly discharged faeces of rat-flea larvae are sticky semi-solid drops, as the rectum of the larva is not provided with water-extracting rectal glands (Wigglesworth 1932, p. 142); these drops dry on exposure to air. In an experiment, when 100 fully fed third-instar larvae of *X. brasiliensis* were put to defaecate in a clean, empty tube at the favourable room temperature of 27 to 29° C and the optimum humidity of 80 %, the gummy faeces immobilized the larvae so that they died without pupation. It appears, therefore, that the faeces of flea larvae, when present in a sufficient quantity, can themselves prove detrimental to their successful development. It is possibly for this reason that the rearing of fleas failed, when no sand or debris was added to the flea-breeding receptacles by some of the previous workers (see, for instance, Advisory Committee 1908, p. 241).

In another experiment, fifty larvae of *X. brasiliensis*, after defaecation, were left in a clean tube to spin their cocoons without sand; they did not form individual cocoons, but spun a thin, large, web-like, silky covering of an irregular shape for most of them. This did not interfere with their successful development, as these larvae were exposed to an optimum humidity of 90 % at a favourable room temperature of 27 to 29° C. Sikes (1930, p. 244) actually found that in 'the absence of the sand, several larvae pupated without spinning cocoons'. The addition of sea sand or debris appears to be a necessity for the normal development of flea larvae and the formation of individual cocoons.

V. Changes undergone by the larval food at different humidities, and their bearing on flea breeding

As variation in the diet of flea larvae affects their developmental rate to a large extent (Advisory Committee 1908, p. 240), the use of a standardized food of known constitution, whose quantity and quality could be gauged, was considered necessary. A mixture of finely powdered dried horse blood and 'Yeast medicinal dry powder (Merck)' served as an ideal larval food for the three species of rat-fleas. At first 0·5 g. of each of these constituents were thoroughly mixed; then 5 g. of dry, pure sea sand were added and well stirred. When exposed to different humidities and temperatures, this artificial food underwent certain change which influenced the development of flea larvae materially; similar changes may occur in nature, and influence the multiplication of fleas.

Before placing rat-flea larvae in a tube, the moisture content of their food was brought into equilibrium with that of a particular atmospheric humidity to which the food was exposed for two or more days; it is known that the moisture of the food varies with that of the air (Sikes 1931, pp. 243–245). This enabled me to determine the exact effect of low humidities on flea breeding, as the food kept at the room humidity of Bombay, especially during the rainy months, took a long time to lose its moisture at low humidities. The neglect of this precaution vitiated the results, as flea larvae used to burrow deeply into the food and sand mixture, so that the humidity to which they were exposed was that of the mixture rather than that of the air above.

The changes undergone by the food varied with the degree of humidity, and they can be arranged easily in three categories: those occurred at (*a*) low effective humidities, (*b*) optimum humidities, and (*c*) high humidities.

(*a*) *Effects of low effective humidities*

At low effective humidities, especially 60 % R.H. and below, food containing larvae was altered in a characteristic way; the volume was reduced as the larvae consumed it, and throughout the exposure it remained dry, discrete and dull red. In some cases the food and sand mixture looked almost white, because the larvae had consumed the blood, though yeast was still present in a comparatively large quantity.

On exposure to 80 % R.H. at 17° C and to 60 % R.H. at 22° C the food decreased slightly in the tubes in which the larvae of *X. cheopis* and *X. brasiliensis* were reared; but in those that contained the larvae of *X. astia*, it was considerably reduced, especially the blood. Apparently, the moisture of air under these climatic conditions was not enough to maintain the normal water balance of the larvae of *X. astia* so that they consumed a large quantity of the food to gain additional water through metabolism; this perhaps shows that the water requirements of larvae of this species are the highest.

At 29° C and 60 % R.H. the food in the tubes in which the larvae of *X. cheopis* were reared was less reduced than it was in the tubes containing the larvae of *X. astia* and *X. brasiliensis*; this indicates that the water requirements of the larvae of the last two species were greater than those of *X. cheopis*. When subjected to 60 % R.H. with 32° C, the food became scarce in the tubes in which the larvae of *X. brasiliensis* were reared; but in those that had the larvae of the other two species its reduction was not so great. The water requirements of the larvae of *X. cheopis* being the lowest, the moisture content in the food under these conditions was enough to maintain their normal water balance; consequently, the consumption of food was less even though almost all of these larvae emerged successfully (table 23); on the other hand, a large number of the larvae of *X. astia* died early of desiccation at this humidity; in consequence, there was a less consumption of the food. A greater reduction of the food in the tubes, in which most of the larvae of *X. brasiliensis* were reared into adults, suggests that the water requirements of its larvae are somewhere between those of the larvae of the other two species.

Even a moisture content corresponding to 80 % R.H. at 36·5° C was not enough to supply the water requirements of the larvae of *X. astia*, because there was a perceptible reduction of the food in the tubes in which a few of its larvae (table 23) were reared into adults; whilst in those containing the larvae of the other two species the volume of food actually increased, as their larvae did not develop successfully at this humidity and 36 or 36·5° C (tables 29, 32). At 36·5° C, which is near the upper effective limit for the larvae of *X. astia*, the loss of water through evaporation must indeed be very great (see Wigglesworth 1939, p. 356); consequently, more food was consumed by the larvae of this species in order to maintain their water balance even at so high a humidity as 80 % R.H.

There was a reduction of food in the tubes, containing the larvae of *X. cheopis* and *X. brasiliensis*, when kept at 13° C even with 90 % R.H.; possibly the larvae ate more food owing to their very prolonged larval life.

It appears, therefore, that at a number of temperatures combined with low humidity the larvae consume much food. It seems probable that they do this in order to secure more metabolic water, which they require to compensate the excessive evaporation at low humidities. This has been demonstrated by Fraenkel & Blewett (1944, p. 136) in the case of larvae of the moth *Ephestia kuehniella*, and the beetles *Tribolium confusum* Duval and *Dermestes vulpinus* Fabricius. A greater consumption of blood than yeast, however, indicates that rat-flea larvae require protein for the production of water of metabolism, because the former is richer in protein which, as pointed out by Mellanby (1932*b*, p. 425), is the chief source of metabolic water.

(*b*) *Effects of optimum humidities*

Changes undergone by the food at the optimum humidities of 80 and 90 % were not so much influenced by temperature variations as by the water vapour of the air. The food increased in volume slightly at 80 % R.H. with 22° C or above, doubtless because some of the food materials are hygroscopic; but it remained dry enough to fall grain by grain, and its colour varied from light red to red. At 90 % R.H. at 17° C or higher temperatures the food increased considerably in volume, and quickly became deep red.

When the food and sand mixture was not disturbed for a few days, a crust formed on its surface at the favourable humidities. Crust formation was of two types: one appeared at the optimum humidities of 80 and 90 % with temperatures lower than 32° C; the other was formed at these humidities with this temperature or above, and also at the high humidities of 97·5 and 100 % even with lower temperatures. The former type was thin, the upper layer of food particles and larval excrement expanding and adhering to sand particles to form a crust, but the underlying food and sand particles remained loose. This thin crust did not prevent the successful development of rat-flea larvae, perhaps because it did not interfere much with the aeration of food and sand mixture. The second type was thick and the mixture gradually formed a compact mass; swelling of the food particles blocked the spaces between the sand particles, which hindered or altogether stopped the aeration of the mass. A recently formed mass was brittle; but later increased stickiness and the growth of a fungus on the surface of the mixture, held the sand particles in a firm mass, that would not break even if the tube was violently shaken. If larvae and pupae within or without cocoons were trapped in such a mass, they died.

If the food and sand mixture was disturbed daily by shaking the tube, a crust and a mass were not formed. The food particles, however, became slightly sticky, and clung to one another to form a few minute brittle lumps at 90 % R.H., though not at 80 % R.H. The food never became putrid and soggy at both these humidities, and the sand particles always remained discrete. The development of flea larvae and pupae proceeded without much interference at these humidities; consequently, they are considered optimum. Even at 94·8 % R.H. and 27° C the food did not become soggy.

(*c*) *Effects of high humidities*

At the high humidities of 97·5 and 100 % the food increased very quickly in volume. It formed a brittle mass with sand, having crust on the surface, even after one or two days' undisturbed exposure, and this subsequently became more firm and compact, particularly at higher temperatures. In order to prevent mortality amongst rat-flea larvae due to this cause, it was found necessary to expose the mixture to high humidities for two or more days before putting in the larvae. If this precaution was not observed the mortality amongst larvae was very high. This was seen in experiments with the larvae of *Xenopsylla astia* (table 1) at 38° C, which were placed in a freshly prepared mixture, in which many actually died within a day. The survivors did not grow, and all of them died in a significantly shorter time than those that had not suffered injury as the result of crust formation (tables 1, 31).

The subsequent development of larvae was dependent on the degree of damage attributable to crust formation. If it was slight, as in the case of *X. astia* (table 1), exposed to

TABLE 1. SHOWING THE INHIBITORY EFFECT OF CRUST FORMATION ON THE GROWTH OF RECENTLY HATCHED LARVAE OF THE THREE SPECIES OF *XENOPSYLLA* AT DIFFERENT HUMIDITIES AND TEMPERATURES

species	temp. (° C)	R.H. (%)	days in which crust formed	no. of larvae used	larvae died due to crust formation		no. of larvae showed subsequent growth	remarks
					no.	Sig. χ^2 test		
astia	38·0	80·0	1	99	*76	↑	0	all larvae died in 1–10 (1·79 ± 0·18) days in first instar
astia	38·0	90·0	1	66	*42	– ↑	0	all larvae died in 1–4 (2·09 ± 0·18) days in first instar
astia	38·0	97·5	1	66	*47	– –	0	all larvae died in 1–5 (1·70 ± 0·15) days in first instar
cheopis	13·0	100	26	80	73	↑ │	7	7 larvae died in first and second instars in 51–130 (89·00 ± 10·69) days
brasiliensis	27·0	100	4	38	36	– ↑	2	2 larvae died in second and third instars in 8 days
astia	27·0	100	3	76	73	– – ↑	3	2 larvae died in first instar and 1 reached third instar
astia	36·5	100	3	40	25	× × ×	15	4 larvae formed cocoons

* Larvae died within a day.

100 % R.H. at 36·5° C, some larvae grew, and a few even spun cocoons within a period not statistically different from that required at the same temperature with an optimum humidity of 90 % (table 24); subsequently even these died owing to sogginess of the food. In contrast, if the hardship undergone by the larvae was severe, as at 100 % R.H. with 13 and 27° C (table 1), most larvae of the three species died owing to crust formation; and the differences between deaths and those of *X. astia* at 36·5° C were highly significant. The few survivors grew slowly, and died without completing their larval life even at the favourable temperature of 27° C.

Even when the food and sand mixture was frequently disturbed, at high humidities the food particles soon adhered to form small lumps, whose size and number gradually increased with the length of exposure. After some time the lumps became gummy, and sand particles stuck to them to form clods which were of a dark greenish colour and putrid; later they became more and more wet, till they were literally soaked with water. Pupae or larvae within or without cocoons entrapped into such soggy clods, were killed off. The presence of soggy clods even proved detrimental to the emerged adults, as in a few cases they got stuck to the clods and died. Owing to clod formation the presence of cocoons could not be detected, as they became involved in the clods. The changes undergone by the food occurred more rapidly at 100 than at 97·5 % R.H., and more rapidly at high temperatures; thus when exposed to 100 % R.H., the mixture became soggy and dark greenish in 158 days at 13° C and 25 days at 36·5° C.

Almost 100 % success in rearing rat-flea larvae was obtained at high humidities by shaking tubes daily, even when all the above-mentioned changes occurred in the food and sand mixture. This shows that the putrefying changes have no intrinsically harmful effect on the development of their larvae and pupae. In some cases the excessive moistening of the mixture resulted in a highly significant prolongation of the larval life of the three species (table 24), which may be due to the inhibition of normal metabolic activities of their larvae owing to lack of aeration for short periods, brought about by irregular disturbance of the mixture. Death, however, occurred at high humidities at any stage, if the mixture was not disturbed often; this may have been due to suffocation, or to mechanical entanglement of the insects, in crusts and clods. Failure in rearing fleas under excessively moist conditions has been experienced by earlier workers (see, for instance, Advisory Committee 1908, p. 241; Buxton 1938, p. 514).

In view of the success obtained, under experimental conditions, in rearing rat-fleas at high humidities, it is suggested that a high moisture content is not itself inimical to the development of the larvae or pupae; it will only exercise harmful effects in combination with the food, as will be explained later (see p. 593), or because the food and sand mixture becomes crusted. This is corroborated by my field observations made in connexion with a plague survey in Sholapur and Dharwar districts of the Bombay Province, where I had several occasions to examine the burrow contents of both wild and domestic rodents. It was found that the debris in the beds of different species of rodents varied considerably as regards its organic content.

I observed that the rodent *Millardia meltada* (Gray), which burrows in the flat open fields, does not harbour fleas. *Gunomys kok* (Gray) lives in raised banks round fields, and is poorly infested with fleas which are mostly picked up during its wanderings. During the four rainy months from June to September their burrows are very often subject to inundation, and the soil in them remains supersaturated with water for a very long time. The debris in the burrows of both these wild rodents is very rich in organic material owing to the presence of stored food; consequently, crust formation on the debris and its wetness prevent flea breeding in their burrows for at least four rainy months in a year; this, I think, accounts for the almost complete absence of fleas on both these rodents throughout the year. In contrast, *G. kok* in Bombay Island, which has begun to live in the residential premises and cannot store harvested crops in its burrow, harbours more than twice as many fleas as *Rattus rattus rufescens* (Gray), the Indian house-rat (Webster & Chitre 1930*a*, p. 340).

The wild rodent, *Tatera indica* (Hardwicke), found in the same areas as the above two species, is always heavily infested with *Xenopsylla astia*, and its burrows were often found to contain several hundreds of fleas. This Indian gerbille usually burrows in elevated barren lands, especially the mounds. There is no storage of food in its burrows; but they contain leaves, stems and roots of grasses with moderate fungus growth. Owing to their location, the burrows of *Tatera indica* are not inundated even during heavy rains; consequently, moisture in them never reaches a stage when in combination with organic debris it would reduce flea breeding.

In the case of *Rattus r. rufescens*, damage to the early stages of fleas due to high humidities in combination with debris is unlikely for two reasons: first, the debris in the bed of this house-rat is poor in organic material; secondly, owing to the location of its burrows inside the houses, the presence of excessive moisture can only be rare. Nevertheless, in the burrows of this rat, flea infestation was not heavy.

In view of these field observations, it is suggested that inundation of a rodent burrow with a view to prevention of flea breeding, as suggested by some workers (see, for instance, Bishopp 1931, p. 13; Ewing 1931, p. 367), will give indifferent results. In the case of a domestic rat burrow occasional flooding might even help to increase its flea population; but a continuous flooding for a number of days may have a deterrent effect; but even that is doubtful, as excessive moisture can only exercise a harmful effect on flea breeding in combination with debris rich in organic material.

VI. Influence of fungus on the rearing of flea larvae

It is often asserted that fungus growth prevents the breeding of fleas (see, for instance, Advisory Committee 1912, p. 314; Hirst 1926, p. 210; Buxton 1938, p. 514). In my experiments the growth of some air-borne fungi only interfered with flea breeding indirectly by holding food and sand particles together so as to form a compact mass (see p. 587); otherwise it was not harmful. In an experiment on rearing *Xenopsylla cheopis* (table 23) at 32° C and 90 % R.H., the recently hatched larvae were placed on the surface of a compact mass of food and sand mixture with abundant fungus growth. All the larvae developed into adults, even though most of them remained near the surface owing to firmness of the mass. Six days after putting the larvae in the tubes there was not much fungus growth left, and many of the cocoons were seen on the surface. As almost all the mixture remained in the form of a compact mass, the larvae could only have fed on the mould, and on such blood and yeast as they could scrape from the surface. Even in other experiments on flea breeding the mould used to disappear after placing the larvae in the tubes; possibly they had eaten it.

The species of the fungi were not determined; possibly more than one species grew on the food. Fungus growth was often observed on undisturbed food and sand mixture exposed to optimum and high humidities. It also occurred on dead larvae and pupae at these humidities; indeed, its presence on immobile stages made it easy to distinguish the dead from the live ones.

VII. Behaviour of flea larvae

When disturbed, rat-flea larvae feign death for a few seconds. All the larval instars of *X. cheopis* and *X. astia* and the young larvae of *X. brasiliensis* coiled in the manner of a watch-spring, the last mentioned remaining in that condition for a longer time. In contrast, the fully fed third-instar larvae of *X. brasiliensis* straightened themselves so stiffly that they almost looked dead. Larvae used to burrow into the food and sand mixture right down to the bottom of the tube, and their tracks were in the form of tunnels.

Rat-flea larvae bred at low humidities were weak, emaciated and sluggish, having a dull and dry appearance; even in the defaecated state they looked definitely smaller than those kept at the favourable humidities. At humidities of 60% or lower food, especially blood, adhered to the body surface and hairs of the larvae and naked pupae. At the optimum humidity of 80% at 17 and 32° C, or 90% at 13 and 35° C, some food particles adhered. But at favourable humidities with medial temperatures the growing larvae were robust and active, without any adherence of food particles.

VIII. Cocoons

I have treated the cocoon as a definite stage, because the metamorphic changes start in the resting larva and are continued till adult formation; also because the exact pupation period within cocoons cannot be ascertained without disturbing the normal course of development.

Temperature variations materially affect the developmental rate of the stages within cocoons (table 24); but in the case of humidity only variations at the lower limit of the tolerable did so (see p. 602). The effects of both factors, however, were influenced appreciably by previous treatment, as is pointed out by Bacot (1914, p. 648). In my own work I find that stages within the cocoons spun at favourable temperatures and humidities were less adversely affected, when subsequently subjected to the unfavourable conditions of temperature and humidity, than those which had been exposed to conditions of temperature and humidity which were barely tolerable. The unfavourable effect of a high temperature is demonstrated by the experiments which compare the recently hatched larvae and the cocoons of *X. brasiliensis* (tables 25, 27) at 35° C with 90% R.H. In this connexion the water content of a spinning larva probably plays an important part; this is borne out by the experiments comparing the recently hatched larvae and the cocoons of *X. astia* (tables 4, 28) exposed to 35° C with 60% R.H. These experiments show that the resting larvae within the cocoons spun at the optimum humidity of 80 or 90% and favourable temperature had enough reserve of water to permit their subsequent development even at the unfavourably low humidity of 60%.

I can confirm that female larvae have consistently shorter larval life than males, as has been observed by Edney (1947, p. 394) in *X. brasiliensis*.

(*a*) *Cocoon formation*

At favourable temperatures and humidities cocoon formation in rat-fleas is a normal occurrence; but it can be prevented by repeated disturbance of the larvae after they have cleared their guts, as was done in the experiments with *X. cheopis* and *X. brasiliensis*, marked with an asterisk (table 23), at 17° C with 80 and 90% R.H. In most of my experiments larvae and cocoons were kept throughout at the same temperature and humidity, and were seldom disturbed; consequently, the proportion of cocoons formed was very high, and there were few false or empty cocoons. The formation of cocoons depends on the metabolic activity of larvae; at the extreme temperatures of 13 and 36·5° C almost all the larvae of *X. cheopis* and *X. brasiliensis* failed to spin cocoons (tables 13, 29); at the low humidity of 50% with 22 and 27° C (tables 5, 23), many larvae do not spin, perhaps because they cannot afford to lose the water necessary for silk production.

TABLE 2. SHOWING THE MEASUREMENTS OF COCOONS OF THE THREE SPECIES OF *XENOPSYLLA* SPUN BY LARVAE BRED AT DIFFERENT TEMPERATURES AND HUMIDITIES

				length (mm.)			breadth (mm.)		
species	temp. (° C)	R.H. (%)	no. of cocoons	range	mean	Sig. *t* test	range	mean	Sig. *t* test
cheopis	27	50·0	16	2·00–2·28	2·108	↑	1·40–1·60	1·513	↑
cheopis	27	94·8	26	2·68–3·20	2·903	× ↑	1·40–1·84	1·668	× ↑
astia	27	90·0	7	2·80–3·80	3·137	× + ↑	1·52–1·92	1·663	× − ↑
cheopis	35	60·0	4	2·00–2·16	2·090	− × × ↑	1·24–1·40	1·350	× × × ↑
cheopis	35	80·0	19	1·92–2·48	2·318	× × × + ↑	1·28–1·60	1·434	× × × − ↑
cheopis	35	90·0	20	2·12–2·68	2·380	× × × × − ↑	1·04–1·64	1·356	× × × − − ↑
brasiliensis	35	80·0	9	1·88–2·08	1·960	× × × + × × ↑	1·32–1·52	1·440	+ × × − − − ↑
brasiliensis	35	90·0	24	1·84–2·40	2·146	− × × − × × × ↑	1·12–1·52	1·325	× × × − × − × ↑
astia	35	90·0	30	2·32–2·92	2·661	× × × × × × × ×	1·40–1·56	1·473	+ × × × − × − ×

Bacot (1914, p. 609) observed that both the size and the texture of cocoons vary with the size of particles of sand or debris. In my experiments sand particles of uniform size were used, which eliminates variation due to this cause. The size and the texture of cocoons, however, depended on the degree of moisture content of air. Cocoons spun at the low humidities of 50 and 60% were baggy with loose meshes, and were significantly smaller than those formed at the higher humidities (table 2); they were soft, and would change shape when pressed. The spinning of silken webbing at these low effective humidities was much less, perhaps because its greater production would require a larger consumption of water, and the larvae at these humidities could not afford much loss of water through secretion of silk.

The cocoons (table 2), spun at 80% R.H., are of medium size and soft; their walls are thin with moderately close meshes, in which silken threads are quite distinct. Those formed at 90% R.H. are hard, having dense wall with meshes very close, so as to give a papery appearance; they do not differ statistically in dimensions from those formed at 80% R.H. The cocoons spun at 94·8, 97·5 and 100% R.H. have a thick, compact, papery wall composed of a large quantity of webbing, and are comparatively large.* Apparently, the thickness of the cocoon wall depends on the state of the metabolic activities of the larva. This is also borne out by the fact that at 80% R.H. with 35° C the cocoons of *X. brasiliensis* had loose meshes, whilst those of *X. astia* had moderately close meshes, as the larval activity was less in the former species than in the latter at this temperature, which is very near the upper fatal limit of *X. brasiliensis* (see p. 625). Even the cocoons of *X. cheopis* formed at 90% R.H. with 35° C had meshes closer than those of *X. brasiliensis*, and those of *X. astia* had a thick papery lining.

On the whole, the cocoons of *X. astia* (table 2) were statistically the biggest and those of *X. brasiliensis* the smallest, when spun at the same humidity. The cocoons of *X. brasiliensis* were more or less spherical, and those of the other two species were elongated oval.

As regards the mode of cocoon formation in fleas, I (Sharif 1937, p. 230) differed from Bacot (1914, p. 473). Actually the flea larva first secretes a few silken threads which are woven by it into a very soft, collapsible and loose string-bag-like covering of an irregular form, and sand particles adhere to it owing to the stickiness of the freshly formed silken threads. Further addition of gummy silken webbing to the inside of the wall of such a cocoon gives it a typical compact oval shape. The cocoons spun against the wall of a tube have no sand particles in the surface which is against the glass; this shows that flea larvae do not collect sand particles at the time of cocoon formation, as is suggested by Bacot. A recently spun cocoon was always large owing to the loose adherence of sand particles; but some of the loosely attached sand particles fell off due to shaking. This also indicates that the adherence of sand particles is purely accidental, and that they are only attached to its outer surface, as has been mentioned by Hirst (1926, p. 202).

(*b*) *Influence of organic material in the cocoon wall on the development of stages within cocoons*

Those cocoons with larval food in their meshes did not undergo any change at low and optimum humidities. But at the high humidities of 97·5 and 100% they became mouldy and soggy; finally, such cocoons became disorganized, and the dead insects within were so disintegrated that it was difficult to distinguish them. On subsequent exposure to dry air, the sticky cocoons became very hard. On the other hand, even at high humidities cocoons devoid of food remained normal.

In order to ascertain the exact role of different humidities on the growth of stages within cocoons, I studied two types of cocoons, one to two days old; all of them contained resting larvae of *X. cheopis* (table 26), *X. brasiliensis* (table 27) and *X. astia* (table 28), and were subjected to 35° C. In one type the larvae, after defaecation, were allowed to spin cocoons in the usual mixture of food and sand; in the other, cocoons were formed in pure acid-washed sand. At the high humidities of 97·5 and 100% marked differences were noted in the development of the larvae within cocoons of the two categories; the differences were manifested either by the formation of advanced pupae, or by adult emergence. At 100% R.H. there was total failure in rearing larvae of the three species within those cocoons which included particles of food; more than that, there were no apparent signs of growth

---

* Actual measurements of cocoons formed at 97·5 and 100% R.H. are not included in the table, as they, along with others, were unfortunately lost.

in *X. brasiliensis* and *X. cheopis*; though in *X. astia* some of the cocoons contained advanced pupae. But within the cocoons of the three species devoid of food the development of the larvae proceeded without any adverse effect. Apparently, then, high humidities in themselves do not prevent the development of stages within cocoons; but in combination with finely powdered food, the particles of which expand and block the interspaces in their webbing, high humidity has resulted in death. The fact that adults emerged even from some of the putrid cocoons of *X. astia* at 97·5 % R.H., shows that as long as the access of air into cocoons was possible the growth of the larvae and the pupae within proceeded.

The presence or absence of food in the cocoon wall did not affect the development of stages within the cocoons of the three species (tables 26 to 28) at an optimum humidity of 90 %. Similarly, at a low humidity of 60 % there was no significant difference in the development of stages within the two types of cocoons of *X. astia*; but in the case of *X. brasiliensis* there was a significantly better growth of the larvae within cocoons containing food, as is indicated by the formation of the advanced pupae.

(*c*) *Function of the cocoon*

Though the presence of the cocoon is not very effective, yet, at certain critical borderline conditions, it definitely prevents fatal desiccation; at low effective humidities only those larvae and pupae within the cocoons (table 23, all species) completed their development successfully. This is also borne out by the absence of any material difference between the percentages of advanced pupae formed within the cocoons of the three species (table 14), when exposed to a low humidity of 60 % and to higher humidities at 13° C. A greater loss of water from the naked resting larvae than from the larvae within the cocoons of the pine sawfly has been demonstrated by Ullyett (1936, pp. 201–204). Even naked pupae, which are less sensitive to loss of water than the larvae (table 23), did not develop successfully at low effective humidities; though growth proceeded in some of them, as they contained fully formed adults.

Similarly, at the temperatures of 13 and 35° C larvae and pupae within the cocoons of the three species (tables 13, 14, 23, 25, 26, 27, 28) showed better growth than those without cocoons; there was a slight success in the breeding of stages within the cocoons of the three species at 13° C and of *X. cheopis* and *X. brasiliensis* at 35° C, but none at all in the naked resting larvae, many of which actually died without pupation. Evidently, then, the cocoon affords some protection against these extreme effective temperatures.

I cannot agree with Bacot (1914, p. 534) that flea larvae 'spin cocoons before the onset of climatic conditions, which would be fatal to them in the free state'; it is known that under favourable climatic conditions cocoons are usually formed; also the protective value of the cocoon is limited, because at critical borderline temperatures and very low effective humidities the insects in cocoons are often killed (tables 14, 23, 25, 26, 28, 30). According to Bacot (1914, p. 474), the removal of pupae from their cocoons is not fatal. I, however, found that naked larvae and pupae can only develop successfully under favourable conditions of temperature and humidity.

IX. Pupal and adult emergence

In the life cycle of rat-fleas the transitional period, in which the pupa leaves the larval skin or the adult emerges from the pupal skin, appeared to be most vulnerable, as moulting and emergence at low effective humidities and critical borderline temperatures was more adversely affected than growth.

Some larvae and pupae of *X. cheopis* (tables 29, 30) grew at 36·5° C, but failed to undergo metamorphosis. Similarly, almost all the larvae of *X. brasiliensis* (table 25) at 80 and 90% R.H. with 35° C, and many larvae of both these species (table 13) at 90 and 100% R.H. with 13° C, which reached the resting stage, failed to pupate. The failure in the emergence may be attributed to a lack of sufficient water. On the other hand, some larvae within the cocoons of both these species (table 14), spun at an optimum humidity of 90% and a favourable room temperature, pupated on subsequent exposure at 13° C even with 80% R.H.; possibly water stored at the favourable humidity and temperature took a long time to evaporate, and this permitted pupation. The effect of the subsequent loss of water was, however, felt at the time of adult emergence, as only a few adults emerged from the pupal skins.

The fact that a fairly high percentage of the larvae within the cocoons of *X. cheopis* (table 26) failed to pupate at 35° C with 60% R.H., and that the proportions of advanced pupae formed and adults emerged at this low humidity were significantly lower than at higher humidities, shows that the 60% R.H. is harmful to metamorphosis. Moreover, both the proportions of naked resting larvae that pupated, and of those that gave rise to adults in *X. cheopis* and *X. brasiliensis* (table 23) at 17° C with 60% R.H. were significantly lower than with 80% R.H. Even the proportions of adults which emerged from naked pupae of both the species at 90% R.H. were significantly higher than at 80% R.H.

It seems that the proper water content of the larva or the pupa is an essential condition for the pupal or the adult emergence. If an insect lacks sufficient water either as the result of excessive evaporation or of low metabolic activity at the extreme effective temperature, there is always a failure in the emergence of the next stage; the explanation may well be that the moulting fluid secreted below the cuticle of the previous stage dries up, so that the next stage cannot effect an escape. This has been demonstrated in the case of emergence of the adult blowfly by Evans (1935, p. 296).

X. Effects of humidity on the growth of flea larvae and pupae

The water requirements of larvae of the three species of rat-fleas are fairly high, for they cannot be maintained at lower than 50% R.H., at which they died of dehydration without any signs of growth (table 4). A fairly wide range of optimum and high humidities, at the same temperature, did not influence their active larval and combined larval and pupal durations (figures 1 to 4), although in many cases slight but significant differences were observed at different favourable humidities (table 24).

The humidity range for the development of rat-flea larvae, being dependent on their water requirements, varies in different species; it was slightly wider in *X. cheopis* (figures 1, 2) but much narrower in *X. astia* (figure 4) than in *X. brasiliensis* (figure 3). This fact contradicts the assumption of Hirst (1926, p. 250) that '*X. cheopis* is somewhat less adapted to survive extremes of dryness than *X. astia*'. The range of effective humidities for these larvae also varied with the temperature; it was wide at 27° C, and gradually narrowed, as a temperature departed from it on either side; at 38° C the larvae of *X. astia* (tables 4, 31) only grew with 80 and 90% R.H. This should not happen, if a particular saturation deficiency is essential for their growth. I cannot agree with Buxton (1938, p. 527) that 'at higher temperatures the larvae will be found to tolerate drier air because they develop more quickly, and are therefore exposed for a shorter time'. At higher temperatures the rate of evaporation increases 'even when the saturation deficiency remains unchanged' (see Wigglesworth 1939, p. 356). Consequently, one would expect that the effective range of humidity would be narrowed at higher temperatures. Mellanby (1935, p. 329) also observed that 'at certain middle "optimum" temperatures, a low humidity is less unfavourable than is the same (relative) humidity at either a higher or a lower temperature'.

The different humidities influenced the development of larvae in such a way that the adults varied appreciably in size. The adults of *X. cheopis* and *X. brasiliensis* (table 3), reared at 50% R.H., were much smaller than those at higher humidities. Similarly, the adults of the three species, reared at 60% R.H., were statistically smaller than those at the higher humidities, especially 80 and 90% R.H.

The largest adults of the three species (table 3) were those bred at the optimum humidities of 80 and 90%. No consistently significant* differences between the dimensions of adults reared at these humidities existed. In most cases the adults of the three species bred at 97·5% R.H. were smaller than those at the optimum humidities; but 33% of the sets of measurements in *X. cheopis*, 50% in *X. brasiliensis* and 25% in *X. astia* at this humidity were not statistically different from those at optimum humidities.

The adults reared at low humidities, especially those close to the lethal limit, appeared less sclerotized than those at the favourable humidities, and were of light brown colour. I cannot agree with Bacot (1914, p. 473) in that 'the very small males of *X. cheopis*...might have one instar less' than normal, or with Webster (1930, p. 401) in that the 'naked pupae might result in dwarf fleas'. Small and less sclerotized adults are due to malnutrition in the larval stage; because, in the absence of sufficient moisture in the food, a part of the digested food is utilized by the larvae for producing additional metabolic water to compensate the increased evaporation at low humidities, which results in small pupae and adults. (For certain other insects see Fraenkel & Blewett 1944, p. 134.)

The adults (table 3), reared at the optimum humidities of 80 and 90%, were significantly much smaller in *X. brasiliensis* than in the other species; but they were statistically of the same size in the other two species, when reared at 22° C. The adults of *X. cheopis* bred at 32° C were, however, significantly longer than those of *X. astia*; but the males of the latter species were broader than those of the former.

In view of the different effects of various humidities on the development of rat-flea larvae, they have been arranged into four categories: (*a*) fatal humidities, (*b*) low effective humidities, (*c*) optimum humidities, and (*d*) high humidities.

---

* The discrepancy in the significant differences between the length and breadth measurements of both sexes at the same humidity is perhaps due to their irregular contraction as the result of killing them in rectified spirit. Weight would have been a better measure for assessing the effects of different humidities on rearing adults.

TABLE 3. SHOWING THE MEASUREMENTS OF ADULTS OF THE THREE SPECIES OF *XENOPSYLLA* REARED FROM LARVAE AT DIFFERENT TEMPERATURES AND HUMIDITIES

species	temp. (° C)	R.H. (%)	no. of ♀♀	length of ♀♀ (mm.) range	length of ♀♀ (mm.) mean	Sig. *t* test	breadth of ♀♀ (mm.) range	breadth of ♀♀ (mm.) mean	Sig. *t* test	no. of ♂♂	length of ♂♂ (mm.) range	length of ♂♂ (mm.) mean	Sig. *t* test	breadth of ♂♂ (mm.) range	breadth of ♂♂ (mm.) mean	Sig. *t* test
cheopis	22	50·0	6	1·36–1·72	1·587	↑	0·68–0·76	0·733	↑	5	1·40–1·56	1·480	↑	0·60–0·72	0·656	↑
cheopis	22	60·0	78	1·36–1·96	1·767	× ↑	0·72–1·00	0·873	× ↑	89	1·44–2·00	1·807	× ↑	0·64–0·88	0·778	× ↑
cheopis	22	80·0	109	1·68–2·24	1·950	× × ↑	0·84–1·08	0·952	× × ↑	97	1·80–2·12	1·974	× × ↑	0·72–1·04	0·864	× × ↑
cheopis	22	90·0	105	1·72–2·16	1·944	× × − ↑ ↑	0·84–1·08	0·970	× × × ↑ ↑	61	1·84–2·16	1·988	× × − ↑ ↑	0·72–1·00	0·848	× × − ↑ ↑
cheopis	22	97·5	96	1·68–2·00	1·874	× × × ×	0·84–1·08	0·953	× × − +	76	1·80–2·16	1·969	× × − −	0·76–0·96	0·845	× × × −
brasiliensis	22	50·0	1	1·48	1·480	↑	0·56	0·560	↑	0	—	—		—	—	
brasiliensis	22	60·0	80	1·36–1·80	1·633	− ↑	0·72–0·96	0·855	× ↑	59	1·40–1·76	1·605	↑	0·68–0·84	0·759	↑
brasiliensis	22	80·0	71	1·48–1·92	1·704	× × ↑	0·80–1·00	0·882	× × ↑	65	1·56–1·84	1·678	× ↑	0·72–0·88	0·795	× ↑
brasiliensis	22	90·0	39	1·60–1·80	1·697	× × − ↑ × ↑	0·80–0·92	0·872	× + − ↑ × ↑	43	1·52–1·84	1·733	× × ↑ × ↑	0·64–0·84	0·737	+ × ↑ × ↑
brasiliensis	22	97·5	36	1·48–1·84	1·702	× × − −	0·76–0·96	0·861	× − + −	24	1·56–1·80	1·690	× − +	0·64–0·76	0·712	× × +
astia	22	60·0	56	1·40–1·92	1·754	↑	0·76–1·00	0·889	↑	31	1·64–2·00	1·840	↑	0·68–0·88	0·769	↑
astia	22	80·0	48	1·76–2·16	1·967	× ↑	0·92–1·16	0·994	× ↑	46	1·80–2·12	1·960	× ↑	0·76–0·96	0·851	× ↑
astia	22	90·0	68	1·64–2·20	1·944	× − ↑ − ×	0·84–1·08	0·972	× + ↑ − ×	61	1·88–2·24	2·005	× × ↑ − ×	0·76–1·04	0·864	× − ↑ − ×
astia	22	97·5	73	1·64–2·04	1·897	× × ×	0·84–1·04	0·958	× × −	55	1·72–2·20	1·960	× − ×	0·72–0·92	0·833	× + ×
brasiliensis	27	50·0	24	1·24–1·64	1·482	↑	0·72–0·84	0·765	↑	17	1·16–1·64	1·454	↑	0·56–0·72	0·633	↑
brasiliensis	27	90·0	25	1·56–1·88	1·715	×	0·68–0·92	0·810	×	27	1·56–1·88	1·742	×	0·64–0·80	0·714	×
cheopis	32	60·0	50	1·60–2·08	1·820	↑	0·76–0·96	0·853	↑	42	1·48–1·92	1·706	↑	0·68–0·84	0·769	↑
cheopis	32	80·0	39	1·80–2·20	1·990	× ↑ ↑	0·84–1·00	0·910	× ↑ ↑	39	1·80–2·16	1·994	× ↑ ↑	0·72–0·84	0·779	− ↑ ↑
cheopis	32	97·5	81	1·72–2·12	1·883	× ×	0·80–1·00	0·879	× ×	76	1·64–2·04	1·866	× ×	0·68–0·96	0·801	× +
brasiliensis	32	60·0	28	1·48–1·72	1·621	↑	0·72–0·92	0·820	↑	46	1·40–1·72	1·614	↑	0·60–0·80	0·707	↑
brasiliensis	32	80·0	50	1·52–1·88	1·689	× ↑ × ↑	0·80–0·96	0·890	× ↑ + ↑	42	1·56–1·84	1·685	× ↑ × ↑	0·68–0·80	0·746	× ↑ × ↑
brasilensis	32	97·5	27	1·52–1·80	1·641	− ×	0·72–0·92	0·844	+ ×	27	1·52–1·76	1·658	+ −	0·68–0·88	0·733	+ −
astia	32	60·0	28	1·48–1·88	1·759	↑	0·68–0·92	0·813	↑	28	1·44–1·88	1·699	↑	0·64–0·80	0·713	↑
astia	32	80·0	37	1·60–2·08	1·866	× ↑ × ×	0·80–0·96	0·914	× ↑ − ×	33	1·64–2·08	1·870	× ↑ × ×	0·64–0·96	0·851	× ↑ × ×
astia	32	97·5	68	1·60–2·04	1·800	− ×	0·72–0·96	0·871	× ×	44	1·68–2·00	1·865	× −	0·68–1·00	0·796	× ×

(*a*) *Fatal humidities*

Recently hatched larvae shrank even on a day's subjection to fatal humidities; almost all of them died in the first larval instar. The effects of different fatal humidities at various temperatures are set forth in table 4.

At 13° C a relative humidity of 60% was fatal to the larvae of *X. cheopis* and *X. brasiliensis*, for all of them died in the first larval instar. At 17° C 50% R.H. proved fatal to the larvae of both the species, and more than half of them were killed in 2 days. The larvae of *X. brasiliensis* were more susceptible to dryness than those of *X. cheopis*; the difference in length of life is highly significant under these climatic conditions. Even at 60% R.H. with 17° C nearly all the larvae of *X. astia* failed to grow, though some of them lived for a very long time. Only one larva, not included for calculation of the mean survival duration, completed its larval life in 63 days, and died as an early pupa; this shows that this humidity is not far below the critical borderline limit for its larva at this temperature. There are, evidently, specific differences in the susceptibility of the larvae to desiccation, *X. cheopis* being slightly less and *X. astia* considerably more susceptible than *X. brasiliensis*.

A very large amount of facts on the limits of temperature and humidity which are fatal to the larvae of the three species is set out in table 4. The facts for these species are interpreted in figures 1, 3 and 4. At the different temperatures tried, 40% R.H. was rapidly fatal to the larvae of all the species; similarly, 45% R.H. was fatal. Mellanby (1933, p. 198) also found that the larvae of *X. cheopis* 'died very soon' at 40% R.H. or below and 18 to 35° C. Even 50% R.H. proved fatal to the larvae of *X. astia*, though they lived for a few days at 27° C without showing any signs of growth. Half the larvae of *X. cheopis* and *X. brasiliensis* died within a day at 50% R.H. with 32° C, and only a few that lived for more than 15 days died in the second larval instar.

At 35 and 38° C 60% R.H. was fatal to the larvae of *X. astia*. Most of them died within a day at 35° C; only a few grew and died in the second larval instar. All the larvae died within a day at this humidity with 38° C, and the time in which they died was significantly

TABLE 4. SHOWING THE EFFECTS OF FATAL HUMIDITIES WITH EFFECTIVE TEMPERATURES ON THE RECENTLY HATCHED LARVAE OF THE THREE SPECIES OF *XENOPSYLLA*

species	temp. (° C)	R.H. (%)	no. of larvae used	days in which all the larvae died		Sig. *t* test	remarks
				range	mean		
cheopis	13	60	250	3–68	6·52	↑	245 larvae died in 3–13 (5·49 ± 0·18) days
brasiliensis	13	60	192	3–51	4·73	×	190 larvae died in 3–13 (4·29 ± 0·10) days
cheopis	17	50	200	2–5	2·75	↑	81 larvae alive after 2 days
brasiliensis	17	50	110	1–4	2·23	× ↑	
astia	17	60	176	1–30	5·47	× ×	1 larva formed naked pupa
cheopis	22	45	105	1–3	1·79	↑	27 larvae died in 1 day
brasiliensis	22	45	20	1–3	1·65	– ↑	3 larvae alive after 2 days
astia	22	50	44	1	1·00	× ×	
cheopis	27	40	105	1–2	1·16	↑ ↑	
astia	27	40	30	1	1·00	+ ↑	
cheopis	27	45	105	1–3	1·20	– + ↑	
astia	27	50	135	2–7	2·87	× × ×	89 larvae died within 2 days
cheopis	32	40	110	1	1·00	↑ ×	
brasiliensis	32	40	108	1	1·00	– ↑	
cheopis	32	50	178	1–29	4·13	× × ↑	6} larvae died in second instar
brasiliensis	32	50	85	1–22	4·32	× × – ↑	3}
astia	32	50	151	1	1·00	– – × ×	
astia	35	60	89	1–8	1·54	↑	5 larvae died in second instar
cheopis	37	60	64	1	1·00	+ ↑	
astia	38	60	100	1	1·00	× –	

shorter than with 35° C. This shows that with the rise of temperature the desiccating influence of a humidity is enhanced, which is also borne out by the larvae of *X. cheopis* when kept at 40% R.H. with 27 and 32° C. The fact that all the larvae of *X. cheopis* died within a day at 60% R.H. and 37° C indicates its fatal drying effect at this temperature.

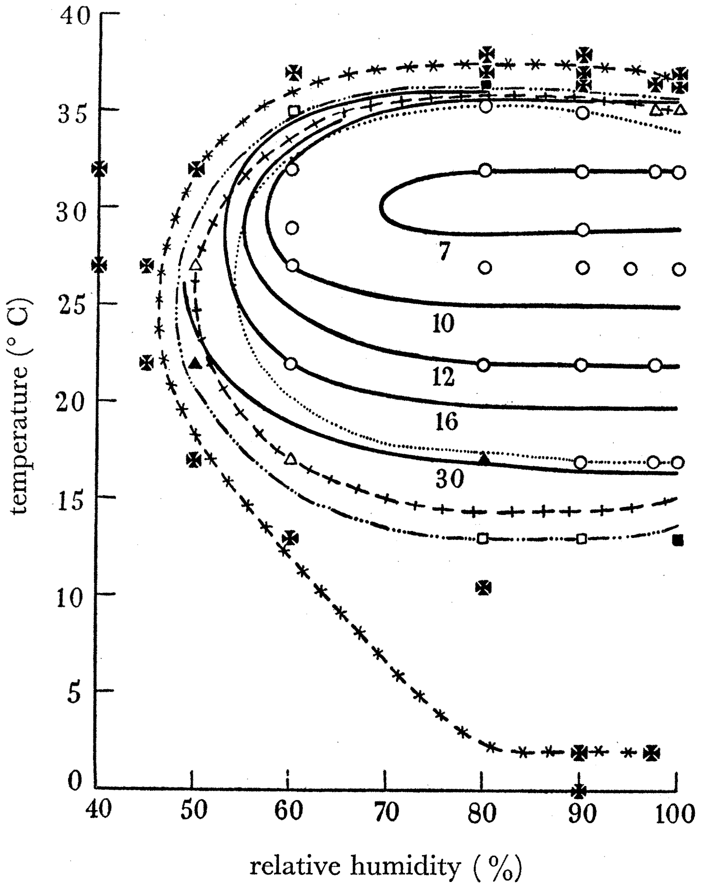

FIGURE 1. The influence of various combinations of temperature and humidity on the larval development of *X. cheopis.* ○ denotes that about 90 %, △ about 50 % and ▲ about 33 % of the larvae spun cocoons; □ signifies that about 33 % and ■ about 10 % of the larvae only reached the naked resting larval or pupal stage, and ✠ most of the larvae died with a little or no growth. Continuous lines indicate equal development curves (figures near the curves represent the average number of days in which larvae spun cocoons or completed active larval life). Dotted line denotes that about 90 %, and a line of dash-with-bar that about 50 % of the larvae spun cocoons; a line of dash-with-three-dots signifies that only about 33 % of larvae reached the resting larval or pupal stage; dash-with-cross line that almost all died within an average of 5 days. Data in tables 4, 7, 8, 13, 23, 24, 25, 29, 32.

The behaviour of rat-flea larvae towards unfavourable humidity is a good indication of its degree of remoteness from the lowest effective humidity. Whenever all the larvae die within a day, the humidity is far below the lowest vital limit; but when some of them live for a number of days even without showing any sign of growth, the humidity is very close to that limit. There were, however, some individual variations in humidity tolerance.

It is clear then that the critical humidity for flea larvae varies with the temperature; it approximates to 45% R.H. at 22 and 27° C, 50% R.H. at 17 and 32° C and 60% R.H. at

13 and 35° C for *X. cheopis* and *X. brasiliensis*, and 50 % R.H. at 22 to 32° C and 60 % R.H. at 17, 35 and 38° C for *X. astia*. The 5-day larval mortality curves of the three species (figures 1, 3, 4) even depict this trend. They also show that the critical relative humidity varies according to the species; it was slightly lower in *X. cheopis* and considerably higher in *X. astia* than in *X. brasiliensis*, which agrees with the specific differences in the minimum water requirements of their larvae (see p. 586).

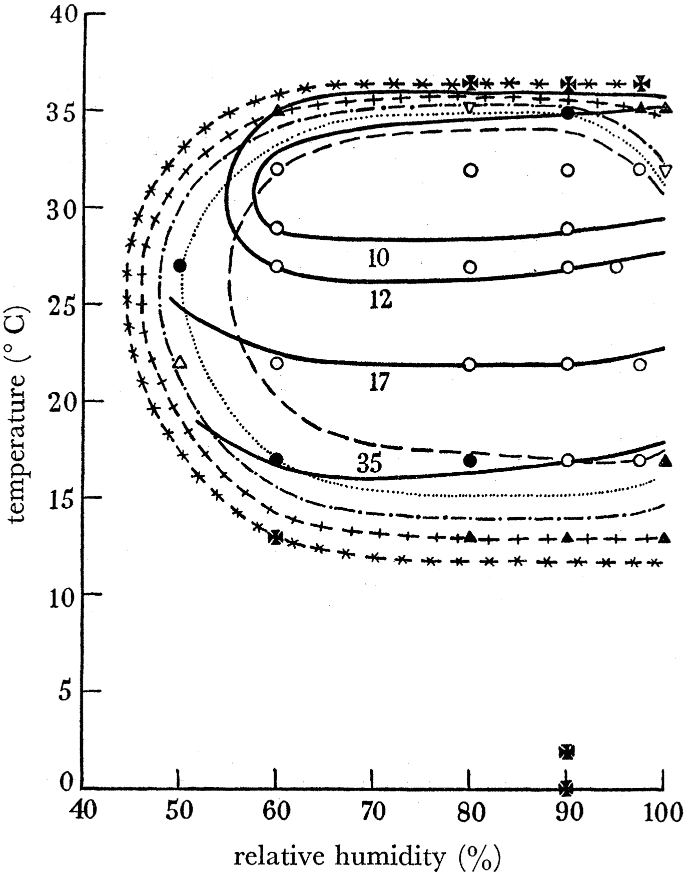

FIGURE 2. The influence of various combinations of temperature and humidity on the development of stages within the cocoons of *X. cheopis*. ○ denotes that about 90 % of the resting larvae within and without cocoons emerged as adults; ● that about 50 % of the total resting larvae and 90 % of those within cocoons, △ 17 % of the total and about 50 % of those within cocoons, and ▽ about 50 % and ▲ less than 10 % of the larvae within cocoons emerged as adults; ✠ signifies that no larvae within cocoons were reared into adults. Continuous lines indicate equal development curves (figures near the curves represent the average cocoon life in days). Dash line denotes that about 90 % of the total resting larvae within or without cocoons became adults; dotted line that about 50 % of the total and about 90 % of those within cocoons became adults; dash-with-one-dot line signifies emergence of adults from about 50 %, and dash-with-bar line from less than 10 % of the cocoons; dash-with-cross line shows failure of emergence of adults from the cocoons. Data in tables 11, 12, 14, 23, 24, 26, 30.

There is a considerable discrepancy between the critical limit of humidity for the larva of *X. cheopis* observed by myself (figure 1) and by Bacot (1914, pp. 454, 516, tables XX, XXV); my figure is slightly below 50 % R.H. against his 60 to 65 % R.H. for the same temperature range. This may be partly due to his defective control of humidity; but, I believe, it is chiefly due to the poor larval food given by him. It is likely that in nature the limit of critical humidities for the larvae of three species of rat-fleas may be higher than those mentioned above, as the larval food available in a rat burrow may be poorer in its nutritive value than the ideal food offered by me.

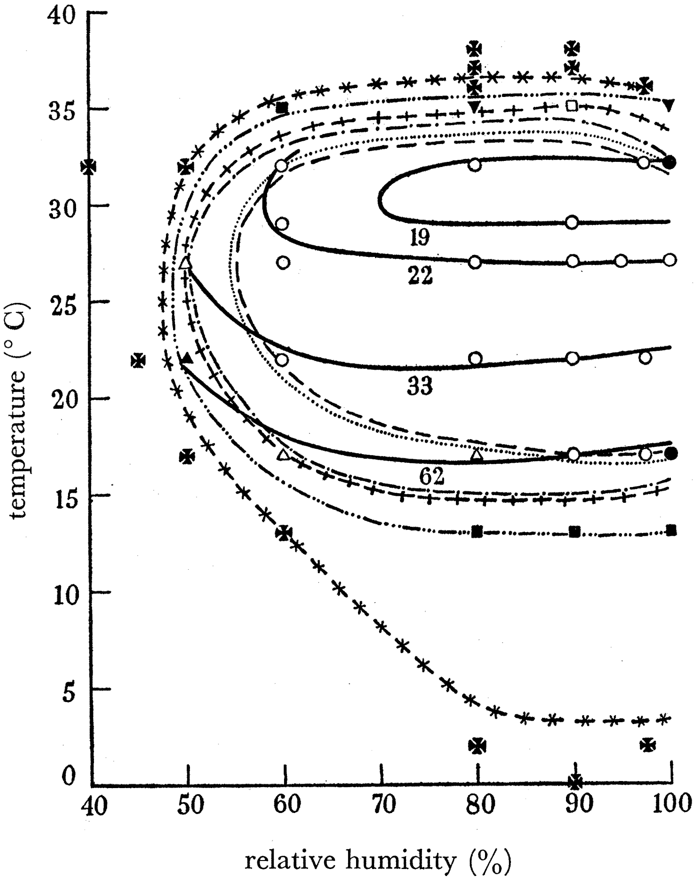

Figure 3. The influence of various combinations of temperature and humidity on the combined larval and pupal development of *X. brasiliensis.* ○ denotes that about 90 %, △ about 50 % and ▲ 4 % of the larvae spun cocoons and emerged into adults, and ● about 90 % of the larvae spun cocoons and about 50 % of them emerged into adults; □ and ▼ signify that 77 % and about 50 % of the larvae reached the resting stage and 52 % and about 20 % of them respectively spun cocoons without any adult emergence; ■ indicates that only about 10 % of the larvae reached the naked resting larval or pupal stage; ✠ most of the larvae died with a little or no growth. Continuous lines denote equal development curves (figures near the curves represent the average combined active larval and cocoon life in days of the emerged adults). Dash line signifies that about 90 %, and dash-with-one-dot line about 50 % of the larvae grew into adults; dotted line that about 90 % and dash-with-bar line about 50 % of the larvae spun cocoons; dash-with-3-dots line that only about 10 % of the larvae reached the resting larval or pupal stage; dash-with-cross line that almost all the larvae died within an average of 5 days. Data in tables 4, 5, 7, 8, 13, 23, 24, 25, 32.

(*b*) *Low effective humidities*

Low effective humidities influenced adversely the development of rat-flea larvae in two different ways: first, their growth rate was appreciably retarded; secondly, the mortality rate was very variable mostly owing to variation in cocoon formation, as the naked resting larvae and pupae failed to develop into adults (see p. 593). Retardation in their growth was due to the insufficiency of moisture both in the food and air at low humidities, which interfered with their normal metabolic activities, possibly in the way explained by Fraenkel & Blewett (1943, p. 468) in the case of larvae of stored-products insect pests. According to them, under the dry condition of food, 'the water necessary for building up tissue sand maintaining life is provided by the metabolism' and 'this excessive strain on metabolism in supplying water is one of the causes which slows down growth'. The developmental rate of rat-flea larvae at low humidities varied according to the degree of dryness; the effective humidity was very near its critical limit at 50 % R.H. and 22 and 27° C for *X. cheopis* and *X. brasiliensis* (tables 5, 23, 24): under these conditions the growth was considerably retarded, and the mortality was high and variable.

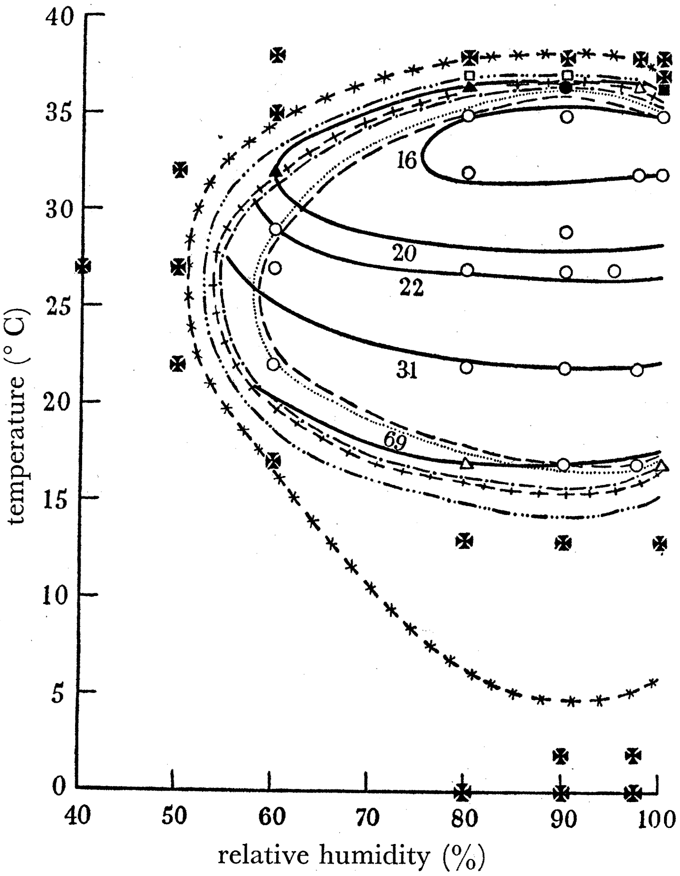

FIGURE 4. The influence of various combinations of temperature and humidity on the combined larval and pupal development of *X. astia*. Explanation as in figure 3, except that ▲ denotes that less than 33 % of the larvae spun cocoons and emerged into adults, □ and ■ about 50 and 25 % of the larvae reached the resting stage and 33 and 10 % of them respectively spun cocoons without any adult emergence, and dash-with-3-dots line about 50 % of the larvae only reached the resting larval stage. Data in tables 4, 7, 8, 13, 23, 24, 31.

Table 6 shows the marked drying effect of 50 % R.H. on the recently hatched larvae of *X. cheopis*, when they were not fed. In order to ascertain the exact effect of a low humidity on the breeding of larvae, the food and sand mixture should have been brought into equilibrium with the atmospheric humidity as it was at 50 % R.H. with 22° C (table 23), owing to 7 or 17 days' previous exposure to these climatic conditions, when a few larvae of *X. cheopis* were reared into adults. If, however, the mixture was dried and then exposed to the same conditions for only 2 days, there was practically no success in breeding of the larvae of *X. cheopis* and *X. brasiliensis* (table 5); though a few of them grew, and one larva of the latter species became adult after 57 days. When the recently hatched larvae of both the species were exposed to these climatic conditions with the mixture that was previously exposed for 3 days to 30% R.H. at 6° C, all died in 3 days. Evidently, it is the humidity in the spaces in the food and sand mixture, which can only be influenced by the moisture content of the food, that must presumably affect the larvae. It seems that the moisture in the food governs the breeding of rat-flea larvae. They always burrowed deep into the mixture, and only the moisture content of the food both as a diet and solid surroundings could affect them and not the atmospheric humidity above. Thus in nature the moisture of debris is more important for flea breeding than the burrow humidity, as has been suggested by Ingram (1927, p. 235).

TABLE 5. SHOWING THE EFFECT OF A MODERATELY LOW MOISTURE CONTENT OF THE FOOD ON THE GROWTH OF RECENTLY HATCHED LARVAE OF THE TWO SPECIES OF *XENOPSYLLA* AT A RELATIVE HUMIDITY OF 50% WITH A TEMPERATURE OF 22±0·5° C

species	no. of larvae used	larvae died in first instar		days in which larvae died in first instar		Sig. *t* test	no. of larvae pupated without cocoon formation	no. of larvae spun cocoons and duration of their active larval life		no. of adults reared	remarks
		no.	Sig. χ^2 test	range	mean			larvae	days		
brasiliensis	25	21	↑	2–3	2·29	↑	3	1	31	1	1 E.P. and 2 A.P. died
cheopis	35	34	–	1–3	1·91	+	0	0	—	0	1 larva died as prepupa

Bacot (1914, p. 546) stated that the degree of humidity had no 'marked influence on the length of rest in the cocoon' of *X. cheopis*. This was corroborated by Edney (1945, p. 408) for *X. brasiliensis*. I found that at moderately low humidities the cocoon period of the three species was not affected adversely, as the excessive loss of water from the insect was prevented by the cocoon (see p. 593); but it was definitely retarded at a very low effective humidity, as is borne out by a significant prolongation of the cocoon life of *X. cheopis* (table 24) at 50% R.H. with 22 and 27° C. The cocoon period was only affected adversely, if the larvae had previously suffered hardship for want of sufficient moisture in the food and air, as was the case in the first experiment with *X. cheopis* at 27° C. The interval between the first and the last cocoon formation was considerably prolonged, especially at very low humidities; the same was observed with adult emergence from cocoons, and delay usually occurred towards the end at the time of male emergence.

TABLE 6. SHOWING THE EFFECTS OF DIFFERENT HUMIDITIES ON THE VIABILITY OF THE UNFED RECENTLY HATCHED LARVAE OF *XENOPSYLLA CHEOPIS* AT A TEMPERATURE OF 22±0·5° C

R.H. (%)	no. of larvae used	days in which all the larvae died		Sig. *t* test
		range	mean	
50·0	35	2– 3	2·11	↑
60·0	20	3– 7	3·85	× ↑
80·0	35	3–11	4·89	× – ↑
97·5	35	3– 8	4·03	× – –

At 13° C 80% R.H. had a drying effect on the larvae of the three species (table 13). In *X. astia* they died in a much shorter time than at 90% R.H. In *X. cheopis* and *X. brasiliensis* there was no pupation at 80% R.H., and it was rare at 90% R.H.

The desiccating influence of 60% R.H. at 17° C had a serious effect on the larvae of *X. cheopis* and *X. brasiliensis* (tables 23, 24), as their growth rate was significantly retarded. This humidity affected the larvae of *X. brasiliensis* more adversely than those of *X. cheopis*. Even 80% R.H. at 17° C exerted a little drying effect on the larvae of both species, as is borne out by significantly lower proportions of adults emerged than at 90% R.H. The behaviour of naked resting larvae and pupae of *X. astia* at 80% R.H. was similar to that of the other species at 60% R.H., as some of its naked resting larvae failed to pupate, and none of its naked pupae were reared into adults; the larval life of this species was significantly longer than at 90% R.H.

At 22° C 50% R.H. is very near the critical limit for the larvae of *X. cheopis* and *X. brasiliensis*, as their development was greatly influenced by initial, slight variations in the humidity in the spaces in the food and sand mixture (see pp. 601, 602). When the food was conditioned to this humidity, many larvae of *X. cheopis* (tables 23, 24) died in first instar; only a few were reared into small weak adults, whose larval life was more than double that at 60% R.H. Even the latter humidity had a marked retarding effect on the growth of the larvae of *X. cheopis* and *X. brasiliensis*. The unfavourable desiccating influence of 60% R.H. and 22° C on the larvae of *X. astia* is indicated by a highly significant retardation in their growth and an appreciable mortality in the larvae and the naked pupae; this resulted in a highly significant reduction in the proportion of cocoon formation and adult emergence. Though some of the naked resting larvae pupated, no adults emerged. Even 80% R.H. had a slight desiccating influence on *X. astia* larvae, as their development was significantly longer than at 90% R.H.

One notes that the drying effect of 50% R.H. on the larvae of *X. cheopis* and *X. brasiliensis* (tables 23, 24) was less at 27 than at 22° C; this humidity is fairly close to the critical limit of these species, as the growth rate was also greatly influenced by the degree of moisture in the food and sand mixture. In the first experiment with *X. cheopis* at 50% R.H. and 27° C the mixture was comparatively dry, as it had been brought into equilibrium with the air; these conditions produced a high larval mortality. Larvae that were strong enough to adapt themselves to this very low humidity dragged on, lived longer and grew into adults. In the second experiment the mixture had been exposed to 80% R.H. for 3 days, and its initial moisture was comparatively high; consequently, there was a significantly higher proportion of development at 50% R.H. with 27° C. The fact that larval and cocoon life was much longer in the first experiment than in the second (the differences are highly significant) indicates that the developmental rate is influenced greatly by the loss of water which rat-flea larvae suffer in their early life, especially within the first few days. Similarly, in the first experiment with *X. brasiliensis* at 50% R.H. with 27° C, having a low initial moisture in the food, there was a significantly poor success in development, when compared with the second experiment in which the initial moisture in the food was a little higher. Death occurred in all the developmental stages from a first larval instar to an advanced pupa in both these experiments; this shows that 50% R.H. has a uniformly adverse effect on all stages of *X. brasiliensis*. The combined larval and pupal life of both species at this humidity was considerably longer than at higher humidities. Even at 60% R.H. highly significant retardation in the growth rate of all three species occurred when compared with higher humidities, though there was almost 100% success in cocoon formation and adult emergence.

At 29° C the retarding effect of 60% R.H. on the larval development of the three species (tables 23, 24) was not materially different from that at 27° C. In *X. astia*, there was, however, a lower proportion of cocoon formation or adult emergence than at 90% R.H. and the same temperature. The difference was highly significant statistically and was due to early death of some larvae, which did not occur in the other two species. The desiccating influence of 60% R.H. was not so marked at 27 and 29° C in the three species as it was at other temperatures, a point of interest.

At 32° C the desiccating influence of 60% R.H. was again indicated in the three species (tables 23, 24) by a significant retardation in their growth; also by the successful development which only occurred in larvae that remained within cocoons. This is more marked in *X. brasiliensis* than in *X. cheopis*. There were a few lagging larvae of *X. brasiliensis* that lived more than 18 days. The drying effect of 60% R.H. with 32° C on the larvae of *X. astia* was most pronounced, for many of them died in the first instar. This shows that this humidity is very near the lower vital limit of this larva; this is also borne out by the fact that the initial moisture of the food affected the proportion of successful development. When the initial exposure of the food, which had been previously kept at 80% R.H. for a few days, to 60% R.H. was for only 1 or 2 days, as in the first experiment, a number of the larvae grew into adults. In contrast, if the food was first brought into equilibrium with this low humidity, as in the second experiment, many larvae died within 4 days; only a few of them bred successfully. The proportions of cocoon formation and adult emergence in both the experiments with *X. astia* were significantly lower than those at higher humidities.

At 35° C 60% R.H. exerted a great desiccating influence on the recently hatched larvae of *X. cheopis* and *X. brasiliensis* (table 25); growth, though possible in some of them, was not enough to permit their successful development. Most of the larvae of *X. brasiliensis* died in the first instar; only a very few completed their active larval life, and these died without cocoon formation and pupation. The growth of this larva at this humidity was significantly poorer than at higher humidities. On the other hand, most of the larvae of *X. cheopis* grew at 60% R.H., and died in different developmental stages; a few of them even spun cocoons, and only those that remained within cocoons pupated. The growth was not simultaneous, as all the three larval instars were found after 16 days. The development of the larvae of *X. cheopis* at this humidity was significantly better than that of *X. brasiliensis*.

According to Mellanby (1932*a*, p. 226, figure 4), all large larvae of *X. cheopis* died within 24 hr. at 60% R.H. with 34° C. This is probably due to the fact that he had exposed larvae without food. Subsequently, he (1933, p. 198) observed even that at 70% R.H. and 35° C the larvae of this species did not develop successfully. The fact that the larvae of this species survived in my experiments under almost identical conditions of temperature and humidity is possibly due to the better nutritive conditions.

Though 80% R.H. improved the larval developmental rate of the three species a good deal at 35° C, yet it exercised a desiccating influence. Owing to a great adverse effect of this temperature on the larvae of *X. brasiliensis* (table 25), the drying effect of this humidity was most marked, as the larvae died in all instars; many of them reached the resting stage, and some even spun cocoons, but none pupated. The proportion of cocoon formation was significantly lower and the active larval life longer than at 90% R.H. The adverse effect of 80% R.H. was less on the larvae of *X. cheopis* (tables 23, 24) at 35° C; most of them spun cocoons, and a number of them were reared into adults. The growth, however, was simultaneous at this humidity. No adults emerged from the naked pupae; even death occurred in different stages of development within cocoons. The active larval life was statistically longer at 80% R.H. and 35·3° C than that at higher humidities both with this temperature and 35° C. Though the development of the larvae of *X. astia* was the best at 80% R.H. and 35° C, yet its larval life was significantly longer than that at 90% R.H., and this significant retardation was reflected in the combined larval and pupal life.

At 35° C the desiccating influence of 60% R.H. was experienced even by the larvae within the cocoons (tables 26 to 28).* This is shown for cocoons of all species, spun at a favourable temperature of 27 to 30° C and 80 or 90% R.H. Owing to the unfavourable effect of 60% R.H. at this temperature, a few larvae left the recently spun cocoons and died; similarly, the naked pupae failed to grow. Even the stages within some cocoons were also adversely affected by 60% R.H. at 35° C. As the larvae of *X. cheopis* are more tolerant of 35° C than those of *X. brasiliensis* (see p. 622), a desiccating influence of 60 or 80% R.H. was felt even by the stages within cocoons of the former species; this is borne out by the formation of a statistically lower proportion of the advanced pupae than at 90% R.H. In *X. astia* a considerable drying effect of 60% R.H. at 35° C is shown even upon the stages within the cocoons; the proportions of advanced pupae formed and adults emerged at this humidity were statistically much lower than at 90% R.H.

At 36·5° C 80% R.H. also exerted an unfavourable influence on the development of the larvae of *X. astia* (tables 23, 24); there were significantly longer larval life, and combined larval and pupal life of the female, and a lower proportion of cocoons spun than at 90% R.H.

It seems then that the following conditions mark the lower humidity limits for these larvae:

temp. (°C)	R.H. % for *X. cheopi* and *X. brasiliensis*	R.H. % for *X. astia*
13	80	80
17	80	80
22	60	80
27	60	60
32	60	60
35	80	80
36·5	—	80

(*c*) *Optimum humidities*

Humidities that do not affect the development of larvae and pupae adversely, and that provide conditions essential for their most successful development at favourable temperatures, are considered optimum. Under optimum conditions (1) successful development of these stages occurred in the shortest time at the particular temperature, (2) the mortality rate was the least, (3) nearly all the larvae spun cocoons, if not disturbed, and (4) the

---

* In conformity with the usual practice, only cocoons with food particles in their meshes are taken into consideration for assessing the effects of temperature and humidity.

interval between the first and the last cocoon formation or between adult emergences was the shortest.

In order to ascertain the effect of an optimum humidity on the development of the naked resting larvae and pupae, the larvae in the experiments at 90% R.H. and 17° C, marked with an asterisk (*X. cheopis* and *X. brasiliensis*: tables 23, 24), were disturbed twice daily by vigorous shaking, and many naked resting larvae were obtained. Almost all these pupated, and adults emerged from most of the naked pupae. The proportions of adults reared in these experiments were not statistically different from those in which the larvae were not disturbed at the same humidity; this shows that an optimum humidity does not exercise any harmful effect on the successful development of naked resting larvae and pupae. In both these species the active larval life, owing to disturbance at the time of cocoon formation, was significantly prolonged. The combined larval and pupal life of the females was not statistically different from that when most of their larvae spun cocoons; in the males there was a significant shortening of the combined larval and pupal life, if the larvae were disturbed.

The range of optimum humidities for larval development varied according to species and temperature, and will be discussed later (see pp. 621 to 625). At medial temperatures, however, the lower limit is about 75% R.H. in *X. cheopis* (figure 1) and *X. brasiliensis* (figure 3) and 80% R.H. in *X. astia* (figure 4).

(*d*) *High humidities*

The growth of larvae (tables 23, 24) at the high humidities of 97·5 and 100% gave rather discordant results, mainly due to mechanical difficulties caused by the food (see pp. 587 and 593). If the food and sand mixture was disturbed daily, high humidities did not affect the development of larvae and pupae adversely when compared with optimum humidities (figures 1 to 4). In experimenting at a high humidity, one must avoid putting larvae into food at room temperature, and then rapidly raising the temperature, say to 35° C or above. This often causes death, perhaps owing to rapid changes in the water content of food material, and of the spaces in it.

The harmful effect of 100% R.H. on the larvae of three species at 13° C was not so marked as at a higher temperature. This humidity did not exercise any adverse effect on the larvae of *X. brasiliensis* (table 13) when compared with 90% R.H., though it did so in *X. cheopis*, the larvae of which died in a significantly shorter time, and a lower proportion of them reached the resting stage (and after a statistically longer time) than at the latter humidity.

At 17° C the sogginess of the food and sand mixture came on so slowly at 97·5% R.H. that most of the larvae of *X. cheopis* and *X. brasiliensis* (tables 23, 24) spun cocoons in periods not statistically different from those at 90% R.H.; but in these species almost all the larvae became adults in a significantly longer time than that at the optimum humidity.

At 22° C results were not very different. For instance, at 97·5% R.H., if food was occasionally disturbed, most of the larvae of the three species (tables 23, 24) managed to develop successfully when compared with optimum humidities. The active larval life of *X. cheopis* at 97·5% R.H. did not differ statistically from that at 90% R.H.; that of the other two species was prolonged, and the increase is highly significant statistically.

At 27° C and 100% R.H. when the food and sand mixture was not disturbed daily, none of the larvae of *X. cheopis* and *X. brasiliensis* (tables 23, 24) developed into adults; but even then larval life was not statistically prolonged except in *X. brasiliensis*, in which an early crust formation at 100% R.H. resulted in poor cocoon formation and a highly significant retardation in the larval growth when compared with 90 and 94·8% R.H. In one experiment with *X. brasiliensis*, marked with an asterisk, when the mixture was disturbed daily, there was 100% success in rearing adults in a time not significantly longer than at optimum humidities.

At 32° C as the food and sand mixture was often disturbed at 97·5 and 100% R.H., there was a good success in the development of rat-flea larvae. At 97·5% R.H. almost all the larvae of *X. cheopis* and *X. astia* (tables 23, 24) were reared into adults in a period not statistically longer than at 80% R.H. But at 100% R.H., even when the mixture was often disturbed, its choking effect was felt prominently towards the end; consequently, a large number of the males of *X. cheopis* and *X. brasiliensis* died, perhaps of suffocation. This is also borne out by the fact that though the active larval life of these species was shorter, yet the combined larval and pupal life of the males was longer at this humidity than at the other favourable humidities. At 100% R.H. there was an appreciably better success in the development of the larvae of *X. astia* than in the other two species, when kept under identical conditions.

At 35° C the ill effect of 100% R.H. on the larvae of *X. brasiliensis* (table 25) was most pronounced; a significantly higher proportion of them failed to complete their active larval life, and died in a highly significantly shorter time than at 90% R.H. Many of the larvae of *X. cheopis* (tables 23, 24) grew at 97·5 and 100% R.H. with 35·3° C; but about half of them spun cocoons in a highly significantly shorter time than that at 80% R.H. In general, few adults were reared, and the death occurred in different stages of development from second larval instar upwards. At 100% R.H., provided the food and sand mixture was disturbed daily, many of the larvae of *X. astia* developed successfully at 35° C. Almost all the larvae spun cocoons and pupated, and the dead pupae contained fully formed adults. There was a slight but significant retardation in the development of larvae at this humidity when compared with that at 90% R.H. The larval life was also significantly prolonged both at 97·5 and 100% R.H., even when they were not reared into adults. The harmful effect of 97·5 and 100% R.H. at 35° C was so great on the larvae within the cocoons of *X. cheopis* (table 26) and *X. brasiliensis* (table 27), having food in their meshes, that none of them even reached the advanced pupal stage. Even the development of the larvae within such cocoons of *X. astia* (table 28) at these humidities was significantly less than at 90% R.H. I have referred above to technical difficulties which arise at this temperature (p. 606).

At 36·5° C the growth of the larvae of *X. cheopis* (table 29) was adversely affected at 97·5 and 100% R.H.; they died in a highly significantly shorter time than at 90% R.H., ingested very little food and were very sluggish. At 97·5% R.H. a few partially or completely defaecated second-instar larvae were seen, but at 100% R.H. they completely failed to grow, though some of them lived for 7 or 8 days. The ill effect of even 97·5% R.H. was felt by the larvae of *X. brasiliensis* (table 32) at 36° C, as all died in the first instar in a shorter time than at 80% R.H.

At 100 or 97·5% R.H. with 36·5° C the proportion of cocoon formation in *X. astia* (tables 23, 24) was significantly lower than at 90% R.H.; but its larval life at the high humidities was not statistically different from that at the optimum humidity. At 100% R.H. only a few larvae spun cocoons, and no adults emerged; many of the larvae died early owing to crust formation.

At 37° C the larvae of *X. cheopis* (table 32) died in the first instar and in a much shorter time at 100% R.H. than at 80 or 90% R.H. Those of *X. astia* (table 31) behaved similarly, but only one larva survived into the second instar. The larvae of *X. astia* also failed to grow at 97·5 and 100% R.H. with 38° C. Some larvae of this species even grew for a short time at 38° C with 80 and 90% R.H. The fact that its larvae can withstand 97·5% R.H. for a much longer time than 100% R.H. appears to me to indicate the direct adverse effect of a high vapour pressure on the growth of the larvae.

Thus high humidities by themselves exert an adverse effect on the growth of rat-flea larvae, but only with extremely high temperatures. This is possibly due to either of two causes or perhaps both; first, the larvae may be susceptible to 'water poisoning'; secondly, the high vapour pressure may itself exercise a harmful effect on them. Water poisoning, as explained by Buxton (1932, p. 298), is either due to the inability of larvae to lose water by evaporation in the saturated air (which prevents regulation of temperature by evaporation), or the larvae may be actually gaining water of metabolism faster than they can dispose of it. The fact that intrinsic injurious effect of high humidities is only perceptible in combination with high temperatures is in favour of the latter assumption.

XI. Effects of temperature on the growth of flea larvae and pupae

I desired to find out the effect of a temperature on the resting larvae and pupae within cocoons. I made use of cocoons spun at temperatures between 24 and 32° C and an optimum humidity of 80 or 90%, which will be referred to hereafter for the sake of brevity as 'the favourable temperature and humidity'. In order to determine the probable percentages of pupation within the cocoons of different ages, as given in the tables, two methods were adopted. First, some of larvae, after defaecation, from the same batch that spun cocoons were put in a clean, empty tube, and the time required by them to form naked pupae was taken into consideration for calculating the approximate percentage of pupation within cocoons of a given age. Secondly, cocoons of different ages were opened and the percentages of pupation were ascertained.

In order to determine the lethal period, on exposure to certain temperatures, for larvae and pupae, only those that had been maintained at favourable temperature and humidity were used. Then they were immediately replaced in favourable climatic conditions, given food, and observed for mortality after a fairly long time; but in the case of resting larvae and pupae, subsequent growth was taken as a criterion of survival.

When rat-flea larvae, immobilized by cold or heat, revived after $\frac{1}{4}$ to 4 hr. exposure to the favourable temperature and humidity, they could be arranged in two categories: in one they recovered from the shock, and moved about a little; in the other they were incapable of any progressive movement, but could wave the anterior end to and fro. The larvae of the latter category succumbed subsequently in a short time, and are designated 'moribunds'.

A few larvae of the former category, however, completed their larval life; others failed to grow, as they could not ingest food. In some larvae that were exposed to fatal low temperatures, the escaping contents of the alimentary canal glued them to the tubes, which was partly responsible for their death. In nature a higher proportion of such revivals would be able to complete their life cycle, especially in the debris. For these reasons those that revived after 24 hr. exposure to the favourable temperature and humidity were considered as survivals.

In order to assess the effect of the temperature alone on the larvae and the pupae, only the experiments conducted at optimum humidities were taken into consideration, as they give a correct and reliable estimate of this factor. The results have been arranged in four categories: (*a*) fatal low temperatures, (*b*) temperature thresholds for the development of larvae and pupae and for pupal and adult emergence, (*c*) effective temperatures, and (*d*) fatal high temperatures. The range of each of these temperatures differed according to the species (figures 1 to 4).

(*a*) *Fatal low temperatures*

When exposed to 2° C for 1 day, most of the recently hatched larvae of the three species (table 7) revived on subsequent subjection to the favourable temperature and humidity. Two days' exposure was fatal to all such larvae of *X. astia*, but many of the other two species revived subsequently; this indicates that the larvae of *X. astia* possess the least resistance to cold. A subjection of 3 days to 2° C proved fatal to all the larvae of *X. brasiliensis*; a few *X. cheopis* survived, but were mostly moribund, and exposure to 2° C for 5 days was invariably fatal. According to Webster (1930, p. 403), some individual first- and second-instar larvae of *X. cheopis* and *X. astia* withstood an exposure of 24 hr. to 4·4° C (40° F), and grew into adults on subsequent exposure to a favourable temperature; but the mortality rate was high.

It has then been shown in several ways that the recently hatched larvae of *X. cheopis* are more able to withstand 2° C than those of other species (table 7). A few of them survive an exposure of 1 to 3 days. The proportion which revive a few hours after exposure is also higher in *X. cheopis* than in the other species.

Exposure of the recently hatched larvae of the three species (table 8) to 0° C for 1 day was not immediately fatal, as a number of them revived; but all failed to develop except one larva of *X. cheopis*, which reached the cocoon stage after 10 days. The proportion of larvae which revived and the subsequent length of life were statistically greater in *X. cheopis* than in the other two species, when exposed to 90 and 97·5% R.H.; such significant differences existed between *X. astia* and *X. brasiliensis* at 80% R.H., being greater in the latter species. Thus even 1 day's exposure to 0° C demonstrated specific differences in resistance to cold. An exposure of 2 days to 0° C was fatal to all the larvae of *X. astia*, and 3 days to those of the other two species.

Most of the fully fed third-instar larvae of *X. brasiliensis* and *X. cheopis* (table 9), when subjected to 2° C for $\frac{3}{4}$ day, revived, but all subsequently died within 1 or 2 days respectively. When exposed to the same humidity, there was no significant difference between the revival proportions of the two species; but the larvae of *X. cheopis* died after a statistically longer interval than those of *X. brasiliensis*. One day's exposure of such larvae was more harmful, as their revival proportions decreased appreciably. Two days' subjection to 2° C was fatal to all the fed larvae of the three species.

Taking larvae which have ceased to feed, and have emptied the gut, 1 day's exposure (table 10) to 2° C was not totally fatal; half the larvae of *X. cheopis* and *X. brasiliensis* were reared into adults subsequently at the favourable temperature and humidity. A subjection of 4 days proved completely fatal for such larvae of *X. brasiliensis*. A higher susceptibility of the fed larvae than the unfed ones to cold is possibly due to an injury caused by the presence of blood in the gut

TABLE 7. SHOWING THE FATAL EFFECT OF A TEMPERATURE OF $2\pm1°$ C WITH EFFECTIVE HUMIDITIES ON THE RECENTLY HATCHED LARVAE OF THE THREE SPECIES OF *XENOPSYLLA*

				subsequent exposure to 25 to 29° C and 90% R.H.				
				larvae revived after 2–4 hr.		no. of larvae and period in which they died in first instar		
species	R.H. (%)	exposure to 2° C (days)	no. of larvae used	no.	Sig. χ^2 test	larvae	days	remarks
cheopis	90·0	1	150	138	↑	138	0–3	12 larvae grew into third instar in 6 days
brasiliensis	90·0	1	25	18	× ↑	25	0–3	
astia	90·0	1	25	18	× – ↑	25	0–2	1 larva alive after 1 day
brasiliensis	97·5	1	25	20	– – – ↑	25	0–3	
astia	97·5	1	25	21	– – – –	25	0–3	4 larvae alive after 1 day
cheopis	90·0	2	200	122	↑	197	0–3	3 larvae grew into third instar in 6 days
brasiliensis	90·0	2	30	16	– ↑	30	0–2	
astia	90·0	2	50	0	× × ↑	50	0	
cheopis	97·5	2	50	26	– – × ↑	50	0–3	
astia	97·5	2	25	0	× × – ×	25	0	
cheopis	80·0	3	50	6	↑	48	0–3	2 larvae grew into second instar in 5 days
brasiliensis	80·0	3	30	0	– ↑	30	0	
cheopis	90·0	3	350	37	– – ↑	350	0–2	17 larvae alive after 1 day
brasiliensis	97·5	3	60	0	+ – +	60	0	
cheopis	90·0	5	25	0	↑	25	0	
cheopis	97·5	5	25	0	–	25	0	
cheopis	90·0	6	50	0	↑	50	0	
cheopis	97·5	6	50	0	– ↑	50	0	
astia	97·5	6	25	0	– –	25	0	
cheopis	90·0	11	50	0		50	0	

TABLE 8. SHOWING THE FATAL EFFECT OF A TEMPERATURE OF $0\pm1°$ C WITH EFFECTIVE HUMIDITIES ON THE RECENTLY HATCHED LARVAE OF THE THREE SPECIES OF *XENOPSYLLA*

				subsequent exposure to 25 to 29° C and 90% R.H.				
				larvae revived after 2–4 hr.		days in which all the larvae died in first instar		
species	R.H. (%)	exposure to 0° C (days)	no. of larvae used	no.	Sig. χ^2 test	range	mean	Sig. t test
brasiliensis	80·0	1	50	26	↑	0–2	0·54	↑
astia	80·0	1	50	15	+ ↑	0–2	0·32	+ ↑
cheopis	90·0	1	90	60	– × ↑	0–3	*0·76	+ × ↑
brasiliensis	90·0	1	120	37	+ – × ↑	0–2	0·32	+ – × ↑
astia	90·0	1	140	29	× – × – ↑	0–2	0·22	× – × – ↑
cheopis	97·5	1	120	91	× × – × × ↑	0–2	0·83	× × – × × ↑
brasiliensis	97·5	1	50	11	× – × – – × ↑	0–1	0·22	× – × – – × ↑
astia	97·5	1	50	12	× – × – – × –	0–1	0·24	× – × – – × –
brasiliensis	80·0	2	50	1	↑	0–1	0·02	↑
astia	80·0	2	50	0	– ↑	0	0·00	– ↑
cheopis	90·0	2	90	1	– – ↑	0–2	0·02	– – ↑
brasiliensis	90·0	2	195	1	– – – ↑	0–1	0·01	– – – ↑
astia	90·0	2	170	0	– – – – ↑	0	0·00	– – – – ↑
brasiliensis	97·5	2	50	2	– – – – – ↑	0–1	0·04	– – – – × ↑
astia	97·5	2	50	0	– – – – – –	0	0·00	– – – – – –
cheopis	90·0	3	240	0	↑	0	0·00	↑
brasiliensis	90·0	3	90	0	– ↑	0	0·00	– ↑
astia	90·0	3	90	0	– –	0	0·00	– –

* Only 89 larvae died in first instar; one grew and spun cocoon after 10 days.

Table 9. Showing the fatal effect of a temperature of 2±1° C with effective humidities on the fully fed third-instar larvae of the three species of *Xenopsylla*

				subsequent exposure to 25 to 29° C and 90% R.H.				
				larvae revived after 2 to 4 hr.		days in which all the larvae died		
species	R.H. (%)	exposure to 2° C (days)	no. of larvae used	no.	Sig. χ^2 test	range	mean	Sig. *t* test
cheopis	90·0	$\frac{3}{4}$	25	23	↑	0–1·25	0·93	↑
brasiliensis	90·0	$\frac{3}{4}$	23	17	– ↑	0–1	0·65	+ ↑
cheopis	97·5	$\frac{3}{4}$	25	25	– + ↑	1–2	1·12	+ × ↑
brasiliensis	97·5	$\frac{3}{4}$	24	22	– – –	0–1	0·88	– – +
cheopis	80·0	1	38	15	↑	0–2	0·42	↑
astia	80·0	1	30	10	– ↑	0–1	0·33	– ↑
cheopis	90·0	1	25	16	– + ↑	0–1·25	0·66	– + ↑
cheopis	97·5	1	55	34	– + – ↑	0–1	0·62	– + – ↑
brasiliensis	97·5	1	25	6	– – + × ↑	0–1	0·24	– – × × ↑
astia	97·5	1	38	11	– – + × –	0–2	0·32	– – + × –
cheopis	80·0	2	30	0	↑	0	0·00	↑
astia	80·0	2	30	0	– ↑	0	0·00	– ↑
cheopis	90·0	2	90	0	– – ↑	0	0·00	– – ↑
brasiliensis	90·0	2	25	0	– – – ↑	0	0·00	– – – ↑
astia	90·0	2	30	0	– – – – ↑	0	0·00	– – – – ↑
cheopis	97·5	2	30	0	– – – – – ↑	0	0·00	– – – – – ↑
astia	97·5	2	30	0	– – – – – –	0	0·00	– – – – – –
cheopis	90·0	3	30	0	↑	0	0·00	↑
cheopis	97·5	3	60	0	–	0	0·00	–

Table 10. Showing the fatal effect of a temperature of 2±1° C with effective humidities on the full-grown larvae (gut emptied) and one-day-old white naked pupae of the two species of *Xenopsylla*

				subsequent growth on 17 to 32 days' exposure to 24 to 29° C and 90% R.H.			
					stages died		
species	R.H. (%)	exposure to 2° C (days)	no. of larvae or pupae used	adults emerged	R.L	E.P.	A.P.
cheopis	97·5	1	16 D.L.	8♂♂	8	0	0
brasiliensis	90·0	1	14 D.L.	7♂♂	7	0	0
brasiliensis	90·0	4	10 D.L.	0	10	0	0
brasiliensis	97·5	1	15 E.P.	7♀♀	—	0	8
cheopis	80·0	2	20 E.P.	0	—	5	15
cheopis	90·0	2	9 E.P.	1♀	—	1	7
brasiliensis	97·5	4	6 E.P.	0	—	6	0
cheopis	90·0	6	25 E.P.	0	—	25	0
cheopis	97·5	6	30 E.P.	0	—	30	0
brasiliensis	80·0	6	20 E.P.	0	—	20	0
brasiliensis	90·0	6	35 E.P.	0	—	35	0
cheopis	97·5	11	25 E.P.	0	—	25	0
brasiliensis	90·0	10	47 E.P.	0	—	47	0
brasiliensis	80·0	20	20 E.P.	0	—	20	0
cheopis	80·0	20	20 E.P.	0	—	20	0

On the whole, the three humidities tested at 2 and 0° C did not influence statistically the proportions of larvae of the three species (tables 7 to 9), which revived for a period, nor the time taken to die on subsequent exposure to the favourable temperature and humidity.

After exposure to 2° C for 1 day, subsequent growth occurred in all the early white naked pupae of *X. brasiliensis* (table 10), and adults emerged from about half of them; even 2 days' exposure of similar pupae of *X. cheopis* to this low temperature did not inhibit the subsequent growth in many of them, and a single adult emerged. But a subjection of 4 days or more to 2° C was fatal to all early naked pupae of *X. brasiliensis*, and that of 6 days or more to those of *X. cheopis*.

When subjected to 2° C for 1 day, larvae and pupae within some cocoons of *X. cheopis* and *X. brasiliensis* (table 11), were subsequently reared into adults; many individuals of both species within the unhatched cocoons had made some growth before dying. Two days' exposure to 2° C resulted in a total failure of subsequent adult emergence from the cocoons of *X. brasiliensis*; but a few adults emerged from those of *X. cheopis*, and almost all the remaining cocoons contained advanced pupae. Webster (1930, p. 404) also found that the subjection of cocoons of *X. cheopis* and *X. astia* to 4·4° C (40° F) for 24 hr. had no harmful effect; but that of 48 hr. resulted in a 'marked fall-off in the output of adults'. An exposure of 3 days to 2° C led to a complete failure of emergence of adults from the cocoons of *X. cheopis*; but growth occurred within many of them.

A subjection of 4 days to 2° C was completely fatal to the larvae and the pupae within the cocoons of *X. brasiliensis* (table 11), for it prevented their subsequent growth at the favourable temperature and humidity; but it required 6 days to produce the same effect in those of *X. cheopis*, as was the case with their early naked pupae (table 10). Exposure of stages within the cocoons of both the species to this low temperature for 6 to 20 days was totally fatal. These experiments, along with those on the early naked pupae, suggest that these species cannot hibernate in the larval and the pupal stages.

TABLE 11. SHOWING THE FATAL EFFECT OF A TEMPERATURE OF 2±1° C WITH EFFECTIVE HUMIDITIES ON LARVAE AND PUPAE WITHIN THE COCOONS OF THE TWO SPECIES OF *XENOPSYLLA*

					subsequent growth on 20 to 37 days' exposure to 27 to 29° C and 90% R.H.						
						stages died within cocoons					
species	R.H. (%)	exposure to 2° C (days)	age of cocoons (days)	no. of cocoons used	adults emerged	R.L.	E.P.	A.P.	% of ultimate pupation	probable % of initial pupation	signs of growth
cheopis	90·0	1	4	71	35	2	0	34	97·2	60	+
cheopis	90·0	1	3	30	8	10	0	12	66·7	25	+
brasiliensis	90·0	1	3	65	5	20	1	39	69·2	25	+
**brasiliensis*	97·5	1	3	25	5	8	0	12	68·0	25	+
brasiliensis	97·5	1	4	40	4	6	2	28	85·0	60	+
cheopis	80·0	2	1	40	5	2	0	33	95·0	0	+
cheopis	90·0	2	4	63	6	0	1	56	100	60	+
brasiliensis	80·0	2	1	33	0	22	0	11	33·3	0	+
cheopis	90·0	3	4	60	0	0	15	45	100	60	+
cheopis	90·0	4	3	25	0	9	15	1	64·0	25	+
cheopis	90·0	4	4	40	0	0	17	23	100	60	+
cheopis	97·5	4	3	50	0	22	25	3	56·0	25	+
brasiliensis	80·0	4	4–5	80	0	14	66	0	82·5	80	–
brasiliensis	90·0	4	2–3	40	0	38	2	0	5·0	10	–
cheopis	90·0	5	4	40	0	0	16	24	100	60	+
cheopis	90·0	6	4	60	0	30	30	0	50·0	60	–
cheopis	90·0	7	4–5	60	0	13	47	0	78·3	80	–
brasiliensis	90·0	7	3	25	0	16	9	0	36·0	25	–
cheopis	80·0	20	1	73	0	73	0	0	0·0	0	–
brasiliensis	80·0	20	1	33	0	33	0	0	0·0	0	–

TABLE 12. SHOWING THE FATAL EFFECT OF A TEMPERATURE OF 0±1° C WITH A RELATIVE HUMIDITY OF 90% ON LARVAE AND PUPAE WITHIN THE COCOONS OF *XENOPSYLLA CHEOPIS*

			subsequent growth on 14 to 23 days' exposure to 28 to 30° C and 90% R.H.						
				stages died within cocoons					
exposure to 0° C (hr.)	age of cocoons (days)	no. of cocoons used	adults emerged	R.L.	E.P.	A.P.	% of ultimate pupation	probable % of initial pupation	signs of growth
2·5	4	120	73	1	0	46	99·2	60	+
24·0	4	240	0	20	94	126	91·7	60	+
24·0	3	80	0	38	2	40	52·5	25	+
48·0	3	60	0	28	0	32	53·3	25	+
48·0	4–5	100	0	3	63	34	97·0	80	+
48·0	4–5	250	0	6	35	209	97·6	80	+
72·0	3–4	40	0	23	11	6	42·5	40	+
96·0	4	36	0	16	20	0	55·6	60	–
96·0	5–6	250	0	5	245	0	98·0	100	–

Exposure of the larvae and the pupae within the cocoons of *X. cheopis* (table 12) to 0° C for $2\frac{1}{2}$ hr. did not prevent the subsequent rearing of many adults at the favourable temperature and humidity, and almost all the remaining cocoons contained advanced pupae. One day's exposure to this low temperature, however, stopped the emergence of adults; though some subsequent growth proceeded in about half of them. Some growth, not followed by emergence of adults, occurred in some of the larvae and the pupae within cocoons after their 2 days' exposure to 0° C. A subjection of 3 days to it proved very harmful, as only a few pupae within the cocoons showed slight signs of subsequent growth; but that of 4 days proved completely fatal, and even the pupae within cocoons 5 to 6 days old showed no subsequent signs of growth.

There was a marked variation in the percentages of the larvae which developed within the cocoons of *X. cheopis* and *X. brasiliensis*, when exposed to 2° C for short periods permitting their subsequent development (table 11). This is possibly due to death of the resting larvae, as these appear to be most adversely affected by this low temperature. The fact that the percentage of ultimate pupation within the 3-day-old cocoons of *X. cheopis* was definitely lower than within its 1- or 4-day-old cocoons supports this assumption, because the proportion of the larvae ready for metamorphosis in the former case was higher than in the latter. This is also borne out by the fact that a much higher percentage of mortality occurred in the larval stage in 3-day-old cocoons of *X. cheopis* (table 12) than in its 4-day-old ones, when exposed to 0° C for 1 day. In an experiment with *X. brasiliensis* at 2° C (table 11), marked with an asterisk, seven prepupae contained fully formed teratological adults. They were elongated, following the outline of the prepupa, and were more of the shape of a larva than a pupa, as complete pupation had not occurred in them. Apparently, this unfavourable temperature is more harmful to the emergence of the pupa than to its growth. This also holds good for the emergence of adults, as a fairly high percentage died within the pupal skins.

The resistance of both larvae and pupae of the three species of rat-fleas to cold is not considerable, which is also supported by the experiments of Bacot (1914, p. 643) with *X. cheopis*. They are extremely non-hardy like the early stages of insects infesting stored products, which is in accord with the assumption that in 'insects living under equable conditions', such as fleas, even a 'moderate lowering of the temperature for a long period only results in death' (Uvarov 1931, p. 12). The fact that these stages cannot survive cold storage of 2° C even for a few days can be utilized for the control of these rat-fleas. In these closely allied species there are slight specific differences in the cold resistance of their larvae and pupae.

(*b*) *Thresholds for the development of larvae and pupae and for the pupal and the adult emergence*

The theoretical developmental threshold taken is the average of values obtained by the mathematical expression, $K=\dfrac{dt-DT}{d-D}$ (see Uvarov 1931, p. 23), where t and T are the different temperatures in degrees centigrade, d and D are the average number of days required for the completion of development at the corresponding temperatures; their reciprocals (developmental units) lie on the straight line, and K is the theoretical threshold.

TABLE 13. SHOWING THE EFFECT OF A TEMPERATURE OF 13 ± 0·5° C WITH EFFECTIVE HUMIDITIES ON THE GROWTH OF RECENTLY HATCHED LARVAE OF THE THREE SPECIES OF *XENOPSYLLA*

species	R.H. (%)	no. of larvae used	no. of larvae and period in which they died: larvae	days: range	days: mean	Sig. t test	larvae reached the resting stage: no.	Sig. χ^2 test	days in which larvae reached the resting stage: range	mean	Sig. t test	no. of larvae and period in which they pupated: larvae	days: range	days: mean	Sig. t test	remarks
cheopis	80	148	148	10–142	61·05	↑	41	↑	72–118	92·73	↑	0	—	—		
brasiliensis	80	146	146	16–123	37·91	× ↑	10	× ↑	78– 92	86·10	– ↑	0	—	—		
cheopis	90	148	144	10–158	66·86	– × ↑	50	– × ↑	72–135	92·94	– – ↑	4	101–120	115·25	↑	1} advanced pupae died
brasiliensis	90	192	187	8–140	41·66	× – × ↑	29	× + × ↑	77–116	85·41	× – × ↑	5	94–140	105·60	– ↑	1}
cheopis	100	107	104	15–158	55·32	– × + × ↑	13	× – × – ↑	84–135	106·54	× × × × ↑	3	112–125	120·67	– – ↑	1 cocoon formed in 113 days
brasiliensis	100	149	142	8–118	36·91	× – × – ×	14	× – × – –	77– 92	84·14	+ – + – ×	7	94–111	102·00	+ – ×	6 cocoons formed in 92·33 ± 2·11 days
astia	80	150	150	5– 76	14·78	↑ } All larvae died in first instar										
astia	90	111	111	5– 98	20·49	× ↑ }										
astia	100	96	96	2– 79	14·60	– × }										

TABLE 14. SHOWING THE EFFECT OF A TEMPERATURE OF 13 ± 0·5° C WITH EFFECTIVE HUMIDITIES ON THE GROWTH OF LARVAE AND PUPAE WITHIN THE COCOONS OF THE THREE SPECIES OF *XENOPSYLLA*

species	R.H. (%)	exposure to 13° C (days)	age of cocoons (days)	no. of cocoons used	no. of adults and period in which they emerged on exposure to 13° C: adults	days	stages found dead on subsequent exposure of 15 to 28 days to 28 to 32° C and 90% R.H.: R.L.	Pr.	E.P.	A.P.	% of ultimate pupation	probable % of initial pupation	% of pupae showed signs of growth	remarks
cheopis	60	208	2–3	52	0	—	15	29	2	6	15·4	10	75·0	
**cheopis*	60	55	5	50	2	33	0	0	5	43	100	100	90·0	
brasiliensis	60	193	1–2	80	0	—	40	40	0	0	0·0	0	—	
brasiliensis	60	171	3	88	0	—	35	33	10	10	22·7	25	50·0	14 larvae left cocoons and died
astia	60	201	4	45	0	—	6	14	4	21	55·6	60	84·0	
cheopis	80	218	1	72	6	65	4	0	5	57	94·4	0	92·6	6 naked advanced pupae died
cheopis	80	165	2–3	47	4	57	4	23	3	13	42·6	10	85·0	3 naked pupae and 4 larvae died
brasiliensis	80	171	2–3	145	13	†40–63	18	74	13	27	36·6	10	75·5	4 larvae left cocoons and died
astia	80	201	3	30	4	48	3	17	2	4	33·3	25	80·0	
cheopis	90	165	2–3	45	1	57	9	16	1	18	44·4	10	95·0	4} larvae left cocoons and died
cheopis	90	208	3	60	4	33–43	5	3	0	48	86·7	25	100	5}
brasiliensis	90	192	2–3	45	0	—	6	34	0	5	11·1	10	100	
astia	90	199	1	25	0	—	25	0	0	0	0·0	0	—	
cheopis	100	172	3	100	9	43–55	16	0	8	67	84·0	25	90·5	
cheopis	100	129	2–3	40	0	—	35	0	5	0	12·5	10	0·0	} no development due to early fungus growth
brasiliensis	100	156	2	45	0	—	34	11	0	0	0·0	0	—	}
brasiliensis	100	135	2–3	80	0	—	61	0	9	10	23·8	10	52·6	4 larvae left cocoons and died
astia	100	200	2–3	25	0	—	22	0	2	1	12·0	10	33·3	

† The complete combined resting larval and pupal life within the cocoon was 74·37 days (calculated average).

In calculating the average theoretical threshold, I rejected certain figures which were judged to be aberrant.

The recently hatched larvae of *X. astia* (table 13) failed to grow at 13° C even with an optimum humidity of 90 %, though they ingested a little food. The capacity of some of them to live for a very long time indicates that their developmental threshold is about 13° C; this is borne out by the fact that their theoretical threshold of development is calculated to be 13·1° C (table 21). In contrast, similar larvae of *X. cheopis* and *X. brasiliensis* (table 13) grew at 13° C; in both the species a very few of the larvae that completed their larval life pupated at 90% R.H., and two larvae of *X. cheopis* even spun flimsy cocoons after 58 and 65 days. The growth, however, did not produce adults; the main obstacle was the failure of pupal emergence. The fact that the larvae of *X. cheopis* lived for a longer time at each humidity than those of *X. brasiliensis* and that a higher proportion of them completed their active larval life at 80 or 90% R.H. (both differences being highly significant) denotes that the former species is less adversely affected by this temperature. On the other hand, at 90 or 100% R.H. the larvae of *X. brasiliensis* completed their active larval life in a highly significantly shorter period than those of *X. cheopis*; consequently, the developmental threshold of the larva of the former species can only be a little lower than that of the latter; this is corroborated by the theoretical threshold of development, which is lower in *X. brasiliensis* (table 19) than in *X. cheopis* (table 15).

TABLE 15. SHOWING THE DURATIONS OF THE ACTIVE LARVAL LIFE OF *XENOPSYLLA CHEOPIS* AT DIFFERENT TEMPERATURES, AND THEIR RECIPROCALS (DEVELOPMENTAL UNITS); THE THERMAL CONSTANTS (DAY-DEGREES) AND THEORETICAL THRESHOLD OF DEVELOPMENT FOR ITS LARVA

serial order	R.H. (%)	temp. (° C)	mean duration (days)	develop-mental units	thermal constants when threshold taken as 12·21° C	theoretical threshold calculated from the following pairs: pair	threshold (° C)
A	90·0	13·0	92·94	0·011	73·4226	AB	11·24
B	90·0	17·0	28·38	0·035	135·9402	AC	11·62
C	90·0	22·0	12·33	0·081	120·7107	AD	11·59
D	90·0	27·0	8·51	0·118	125·8629	AE	11·63
E	90·0	29·0	7·32	0·137	122·9028	BC	13·16
F	90·0	32·0	7·42	0·135	146·8418	BD	12·72
G	90·0	35·0	8·34	0·120	190·0686	BE	12·83
H	80·0	35·3	9·32	0·107	215·1988	CD	10·86
I	80·0	36·5	14·00	0·071	340·0600	CE	11·77
						DE	14·70

TABLE 16. SHOWING THE DURATIONS OF LIFE OF THE STAGES WITHIN THE COCOON OF *XENOPSYLLA CHEOPIS* AT DIFFERENT TEMPERATURES, AND THEIR RECIPROCALS (DEVELOPMENTAL UNITS); THE COMBINED THERMAL CONSTANTS (DAY-DEGREES) AND THEORETICAL THRESHOLD OF DEVELOPMENT FOR ITS RESTING LARVA AND PUPA WITHIN THE COCOON

serial order	R.H. (%)	temp. (° C)	mean duration (days)	develop-mental units	thermal constants when threshold taken as 11·83° C	theoretical threshold calculated from the following pairs: pair	threshold (° C)
A	90·0	13·0	93·36*	0·011	109·2312	BC	12·14
B	90·0	17·0	34·80	0·029	179·9160	BD	11·61
C	90·0	22·0	17·16	0·058	174·5172	BE	11·98
D	90·0	27·0	12·19	0·082	184·9223	CE	11·57
E	90·0	29·0	10·27	0·097	176·3359		
F	90·0	32·0	9·76	0·102	196·8592		
G	90·0	35·0	10·96	0·091	253·9432		

* Calculated combined resting larval and pupal life within the cocoon.

TABLE 17. SHOWING THE DURATIONS OF THE COMBINED LARVAL AND PUPAL LIFE OF THE FEMALE OF *XENOPSYLLA CHEOPIS* AT DIFFERENT TEMPERATURES, AND THEIR RECIPROCALS (DEVELOPMENTAL UNITS); THE COMBINED THERMAL CONSTANTS (DAY-DEGREES) AND THEORETICAL THRESHOLD OF DEVELOPMENT FOR ITS LARVA AND PUPA

serial order	R.H. (%)	temp. (° C)	mean duration (days)	develop-mental units	thermal constants when threshold taken as 12·11° C	theoretical threshold calculated from the following pairs	
						pair	threshold (° C)
A	90·0	17·0	57·14	0·018	279·4146	AB	12·46
B	90·0	22·0	27·20	0·037	269·0080	AC	12·16
C	90·0	27·0	18·64	0·054	277·5496	AD	12·44
D	90·0	29·0	15·73	0·064	265·6797	BC	11·11
E	90·0	32·0	16·05	0·062	319·2345	BD	12·40
F	90·0	35·0	17·51	0·057	400·8039		
G	80·0	35·3	19·00	0·053	440·6100		

TABLE 18. SHOWING THE DURATIONS OF THE COMBINED LARVAL AND PUPAL LIFE OF THE MALE OF *XENOPSYLLA CHEOPIS* AT DIFFERENT TEMPERATURES, AND THEIR RECIPROCALS (DEVELOPMENTAL UNITS); THE COMBINED THERMAL CONSTANTS (DAY-DEGREES) AND THEORETICAL THRESHOLD OF DEVELOPMENT FOR ITS LARVA AND PUPA

serial order	R.H. (%)	temp. (° C)	mean duration (days)	develop-mental units	thermal constants when threshold taken as 12·28° C	theoretical threshold calculated from the following pairs	
						pair	threshold (° C)
A	90·0	17·0	71·29	0·014	336·4888	AB	12·32
B	90·0	22·0	34·48	0·029	335·1456	AC	12·18
C	90·0	27·0	23·19	0·043	341·3568	AD	12·24
D	90·0	29·0	20·26	0·049	338·7472	BC	11·73
E	90·0	32·0	18·65	0·054	367·7780	BD	12·03
F	90·0	35·0	21·88	0·046	497·1136	CD	13·17
G	80·0	35·3	22·43	0·045	516·3386		

When twenty recently hatched larvae of *X. cheopis* were subjected to 10·5 ± 1·5° C at 80% R.H., they did not grow; they did not even eat any food. Three of them survived 75 days, and were reared into adults after spinning cocoons at 23 to 25° C. This shows that the threshold of development of the larva of *X. cheopis* is either 10·5° C or a little higher.

In order to determine the thresholds for the development of pupae and for the pupal and the adult emergence of the three species, their cocoons, containing larvae and pupae, and spun at the favourable temperature and humidity, were exposed to 13° C for very long periods. As none of them emerged subsequently, under favourable conditions, it is taken for granted that they had died owing to an exposure to 13° C.

Larvae within the cocoons of the three species (table 14) failed to grow at 60% R.H. with 13° C, as there was no appreciable difference between the probable initial and the ultimate percentages of pupation; but many of their pupae showed signs of growth at this humidity, for they contained fully formed adults. Indeed, a few sluggish adults emerged from the cocoons of *X. cheopis* in one experiment, marked with an asterisk. Apparently then the resting larvae of the three species (like the recently hatched ones see p. 597) cannot grow at 60% R.H. with 13° C. There was, however, no indication of the desiccating influence of 80% R.H. on the growth of these stages within cocoons; on the other hand, emergence of adults was slightly better than at higher humidities.

In *X. astia* the resting larvae within cocoons (table 14) failed to grow at 13° C even with 80, 90 and 100 % R.H.; in this they resemble the recently hatched larvae (table 13). Many pupae, however, showed signs of growth at this temperature, and adults emerged from a very few at 80 % R.H. The growth of pupae but not that of larvae within the cocoons of *X. astia* at 13° C, especially with 60 % R.H., can only be explained on the assumption that the high water requirements of the larva cannot be met under these conditions; but the pupa, perhaps because it is a non-feeding stage and has to depend mostly on water stored during the larval stage, was not so adversely affected, especially when within a cocoon spun at the favourable temperature and humidity. Furthermore, the theoretical threshold of development for the stages within the cocoons (probably pupae) of *X. astia* was 12·34° C, appreciably lower than that of its larva (table 21).

TABLE 19. SHOWING THE DURATIONS OF THE ACTIVE LARVAL LIFE OF *XENOPSYLLA BRASILIENSIS* AT DIFFERENT TEMPERATURES, AND THEIR RECIPROCALS (DEVELOPMENTAL UNITS); THE THERMAL CONSTANTS (DAY-DEGREES) AND THEORETICAL THRESHOLD OF DEVELOPMENT FOR ITS LARVA

						theoretical threshold calculated from the following pairs	
serial order	R.H. (%)	temp. (° C)	mean duration (days)	developmental units	thermal constants when threshold taken as 11·65° C	pair	threshold (° C)
A	90·0	13·0	85·41	0·012	115·3035	AB	10·85
B	90·0	17·0	29·88	0·033	159·8580	AC	11·29
C	90·0	22·0	13·62	0·073	140·9670	AD	11·30
D	90·0	27·0	9·27	0·108	142·2945	AE	11·28
E	90·0	29·0	8·30	0·120	144·0050	BC	12·81
F	97·5	32·0	8·30	0·120	168·9050	BD	12·50
G	90·0	35·0	10·40	0·096	242·8400	BE	12·38
						CD	11·34
						CE	11·08

TABLE 20. SHOWING THE DURATIONS OF THE COMBINED LARVAL AND PUPAL LIFE OF THE FEMALE OF *XENOPSYLLA BRASILIENSIS* AT DIFFERENT TEMPERATURES, AND THEIR RECIPROCALS (DEVELOPMENTAL UNITS); THE COMBINED THERMAL CONSTANTS (DAY-DEGREES) AND THEORETICAL THRESHOLD OF DEVELOPMENT FOR ITS LARVA AND PUPA

						theoretical threshold calculated from the following pairs	
serial order	R.H. (%)	temp. (° C)	mean duration (days)	developmental units	thermal constants when threshold taken as 11·89° C	pair	threshold (° C)
A	80·0	17·0	56·24	0·018	287·3864	AB	11·97
B	90·0	22·0	28·21	0·035	285·2031	AC	11·75
C	90·0	27·0	19·36	0·052	292·5296	AD	11·84
D	90·0	29·0	16·91	0·059	289·3301	BC	11·06
E	97·5	32·0	17·08	0·059	343·4788	BD	11·52
						CD	13·20

At 80 and 100 % R.H. only a few larvae within the cocoons of *X. brasiliensis* (table 14) pupated; but many pupae showed signs of growth, and adults emerged from a very few at the former humidity. In *X. cheopis* there was a comparatively better growth of larvae and pupae within the cocoons, as is indicated by higher percentages of ultimate pupation and advanced pupae formed at 80, 90 and 100 % R.H. A fairly high mortality occurred within the cocoons that contained the larvae about to undergo metamorphosis; this is borne out by the fact that there was a very high pupation within the 1-day-old cocoons of

X. cheopis, that only contained the resting larvae; whilst a very large number of larvae died in the prepupal stage within cocoons 2 or 3 days old, which contained many larvae ready for the metamorphic change. A low percentage of pupation within the cocoons of *X. brasiliensis* was due to this cause. The fact that in both these species growth occurred in a much higher percentage of the pupae than of the larvae shows that the developmental thresholds of pupae are lower than those of larvae; this is corroborated by slightly lower theoretical developmental thresholds for stages within the cocoons of these species, being 11·83° C in *X. cheopis* and 11·63° C in *X. brasiliensis*. A few of the naked pupae of *X. cheopis* showed signs of growth; but the naked resting larvae of both species died.

The larva and pupa of each of the three species have different thresholds of development, which are lower in the latter. This is perhaps due to the lower water requirements of the pupa than those of the larva. The difference between the developmental thresholds of the larva and the pupa is much less in *X. cheopis* or *X. brasiliensis* than in *X. astia*. The developmental threshold of the larva of *X. astia* is either 13° C or a little higher; that of its pupa is definitely lower. The thresholds of development for the larvae of *X. cheopis* and *X. brasiliensis* are much lower than 13° C, and the developmental threshold of the larva of the latter species is a little lower. I cannot agree with Mellanby (1933, p. 200) that the development zero of the pupation of *X. cheopis* 'appears to be about 15° C', as its pupae were formed even at 13° C.

TABLE 21. SHOWING THE DURATIONS OF THE ACTIVE LARVAL LIFE OF *XENOPSYLLA ASTIA* AT DIFFERENT TEMPERATURES, AND THEIR RECIPROCALS (DEVELOPMENTAL UNITS); THE THERMAL CONSTANTS (DAY-DEGREES) AND THEORETICAL THRESHOLD OF DEVELOPMENT FOR ITS LARVA

						theoretical threshold calculated from the following pairs	
serial order	R.H. (%)	temp. (° C)	mean duration (days)	developmental units	thermal constants when threshold taken as 13·13° C	pair	threshold (° C)
A	80·0	17·0	38·89	0·026	150·5043	AB	13·76
B	80·0	22·0	15·30	0·065	135·7110	AC	13·59
C	90·0	27·0	9·89	0·101	137·1743	AD	13·51
D	90·0	29·0	8·77	0·114	139·1799	AE	13·38
E	80·0	32·0	7·56	0·132	142·6572	BC	12·86
F	90·0	35·0	7·17	0·139	156·8079	BD	12·60
G	90·0	36·5	9·74	0·103	227·6238	BE	12·23
H	90·0	37·0	12·50	0·080	298·3750		
I	90·0	38·0	18·00	0·056	447·6600		

On the whole, the theoretical thresholds for the combined development of active larvae and stages within cocoons of the three species of rat-fleas (tables 17, 18, 20, 22)* were slightly higher than the values for their separate developments (tables 15, 16, 19, 21). This is due to the fact that the thresholds of their pupal and adult emergence were higher than those of the development of their previous stages, which is borne out by the failure of a high percentage of prepupae and adults to extricate themselves from larval and pupal skins at 13° C (table 14).

---

* The theoretical thresholds for the development of stages within cocoons and for the combined development of larva and pupa of the male of *X. brasiliensis* were 11·63 and 11·68° C respectively, and for those of *X. astia* were 12·34 and 13·34° C.

TABLE 22. SHOWING THE DURATIONS OF THE COMBINED LARVAL AND PUPAL LIFE OF THE FEMALE OF *XENOPSYLLA ASTIA* AT DIFFERENT TEMPERATURES, AND THEIR RECIPROCALS (DEVELOPMENTAL UNITS); THE COMBINED THERMAL CONSTANTS (DAY-DEGREES) AND THEORETICAL THRESHOLD OF DEVELOPMENT FOR ITS LARVA AND PUPA

serial order	R.H. (%)	temp. (° C)	mean duration (days)	develop-mental units	thermal constants when threshold taken as 13·16° C	theoretical threshold calculated from the following pairs: pair	threshold (° C)
A	90·0	17·0	61·77	0·016	237·1968	AB	12·62
B	80·0	22·0	28·83	0·035	254·8572	AC	12·56
C	90·0	27·0	18·99	0·053	262·8216	AD	12·84
D	90·0	29·0	15·89	0·063	251·6976	AE	12·59
E	97·5	32·0	14·04	0·071	264·5136	BC	12·35
F	90·0	35·0	13·66	0·073	298·3344	BD	13·40
G	90·0	36·5	16·92	0·059	394·9128	BE	12·51
						CD	16·75
						CE	12·82

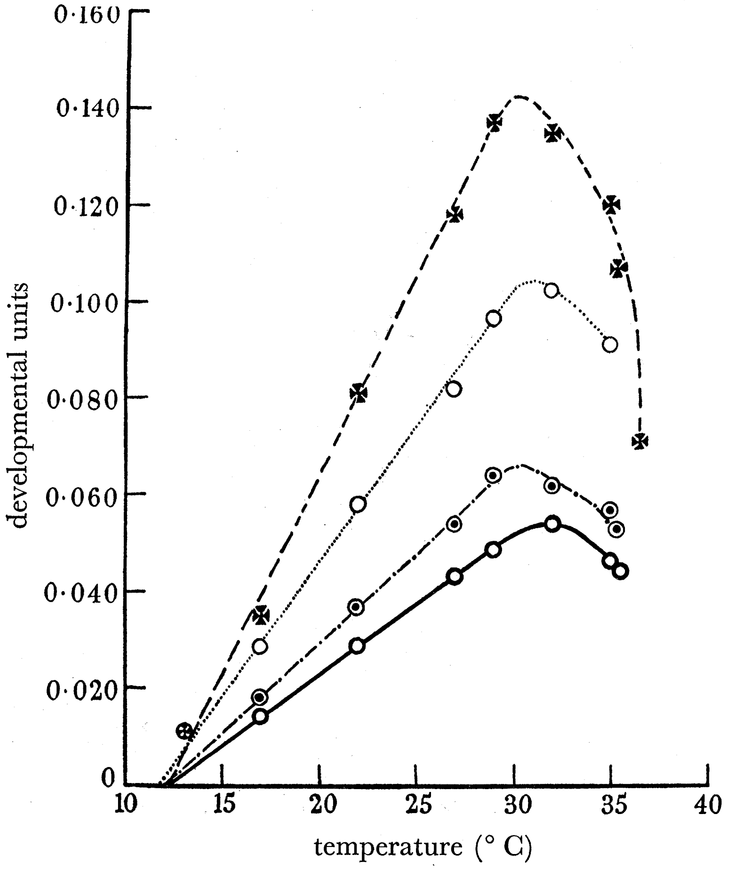

FIGURE 5. Temperature-velocity curves for the development of the pre-adult stages of *X. cheopis* at constant temperatures with optimum humidities. ✠---✠ active larval stage; ○······○ resting larval and pupal stages within the cocoon; ⊙-·-·-⊙ combined larval and pupal stages of the female; **O——O** combined larval and pupal stages of the male. Data in tables 15 to 18.

The observed threshold of 10·5 ± 1·5° C for the larva of *X. cheopis* is very close to its theoretical value; this also appears to be the case with the larvae and the pupae of the three species of rat-fleas. This is contrary to the observations of previous workers on other insects (see, for instance, Shelford 1930, pp. 182, 189; Imms 1937, p. 209).

(*c*) *Effective temperatures*

In order to ascertain the exact relationship between the speed of development and temperature, the developmental durations at different temperatures with an optimum humidity of 80 or 90% (except in the case of *X. brasiliensis* and *X. astia* at 32° C with 97·5% R.H.) have been taken into consideration, and the temperature-velocity curves for the completion of active larval life, larval and pupal life within cocoons, and the combined larval and pupal life of both the sexes have been plotted. The optimum temperature at which these temperature-velocity curves deviate from the straight line varied according to the species, being 30° C in *X. cheopis* (figure 5), 29° C in *X. brasiliensis* (figure 6) and 33·5° C in *X. astia* (figure 7). The range of medial temperatures, which is represented by the straight portion of a temperature-velocity curve is 17 to 30° C for *X. cheopis* and 17 to 29° C for *X. brasiliensis*. For the active larval stage of *X. astia* (figure 7), in order to obtain the temperature-velocity curve that is in agreement with a theoretical threshold higher than 13° C, at which its larvae have failed to grow (table 13), longer developmental periods at 80% R.H. than those at an optimum humidity of 90% for 17 and 22° C have been selected. The reciprocal of developmental duration for the larva of *X. astia* at the latter humidity and 22° C lies much higher than the point for this temperature on the straight portion of the curve. The temperature-velocity curve, however, evinces a tendency between 23 and 33·5° C to conform a straight line.

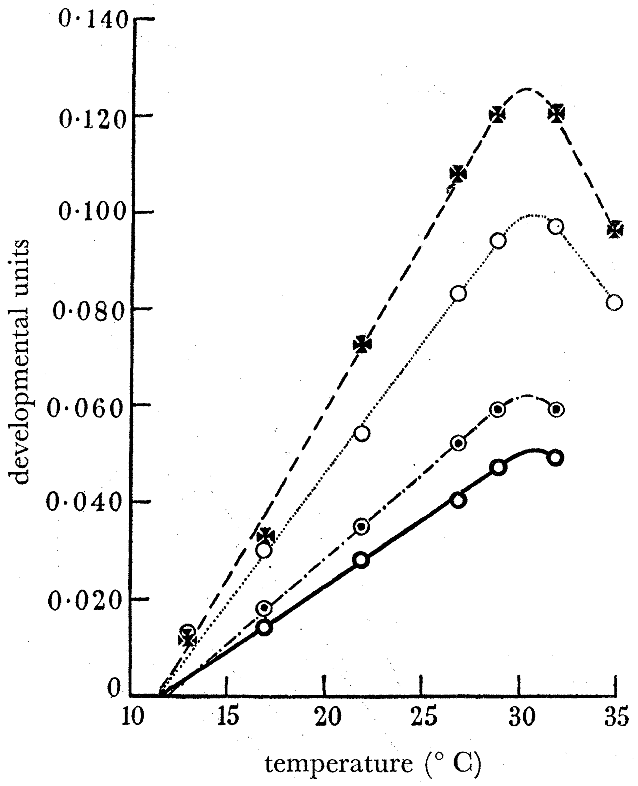

FIGURE 6. Temperature-velocity curves for the development of the pre-adult stages of *X. brasiliensis* at constant temperatures with favourable humidities. Explanation as in figure 5. Data in tables 19, 20, and reciprocals of mean durations of the stages, marked †, in tables 14, 24, 27.

The thermal constants (day-degrees), were calculated by the formula $(T-K)\,D=C$ (see Uvarov 1931, p. 22), where D is the time required for development in days at the temperature T in degrees centigrade, K is the theoretical threshold of development, and C is the thermal constant. The constants were approximately of equal value for the medial temperatures (tables 15 to 22); but temperatures below or above these gave lower and higher thermal constants respectively.

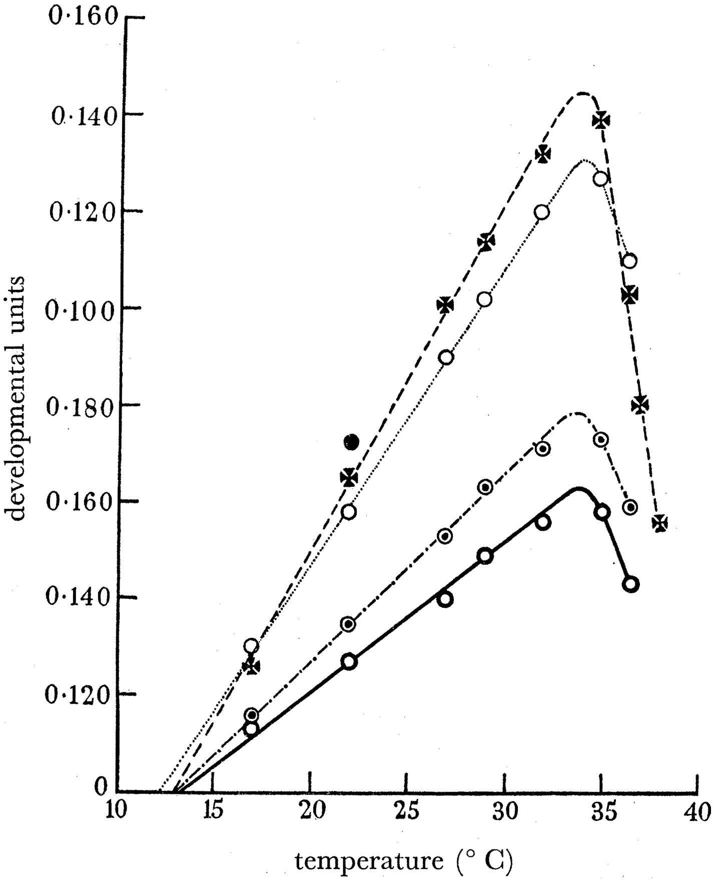

FIGURE 7. Temperature-velocity curves for the development of the pre-adult stages of *X. astia* at constant temperatures with favourable humidities. ● represents the developmental unit of the active larval stage at 90 % R.H. and 17 or 22° C. Other explanations as in figure 5. Data in tables 21, 22, and reciprocals of mean durations of the stages, marked †, in table 24.

At 17° C the optimum humidity for rearing the larvae of the three species of rat-fleas (tables 23, 24) was higher than 80 %, approximating to 90 % for those of *X. astia*. Owing to a great retarding effect of this temperature on the larval development of *X. astia*, its combined larval and pupal life was much longer than in the other two species at the optimum humidity, which was not the case at other effective temperatures; in other words, 17° C is not within the range of medial temperatures for this species. There was, however, no significant difference between the combined larval and pupal life of *X. cheopis* and *X. brasiliensis* at 17° C.

At 22° C 80 and 90 % R.H. were optimum for the development of the larvae of *X. cheopis* and *X. brasiliensis* (tables 23, 24). The proportions of the naked resting larvae of *X. cheopis* that were reared into adults at both these humidities were statistically higher than those at lower humidities. Though the active larval life of *X. cheopis* was significantly prolonged at 80% R.H. when compared with that at 90% R.H., yet its combined larval and pupal life was statistically shorter at the former humidity. In *X. brasiliensis* a (highly significant statistically) difference was found, but only in the combined larval and pupal life of the male, the duration being shorter at 80% R.H. According to Edney (1945, p. 406), the larval duration of this species increased significantly from 12·4 days at 90% R.H. to 16·7 days at 80% R.H. with 24° C; this shows that the former humidity is only optimum for the larval development of *X. brasiliensis*. The difference between his observation and mine, I believe, is due to his use of bran and ox blood, now known to be an imperfect food, and to incomplete control of temperature (Edny 1945, p. 400). Only 90% R.H. at 22° C was the optimum humidity for the larvae of *X. astia*; at this humidity average durations of larval life and of combined larval and pupal life were significantly the shortest. In *X. brasiliensis* the combined larval and pupal life of both sexes at 22° C with 90% R.H. was longer than in the other two species (the difference having high statistical significance).

At 27° C the range of optimum humidity for larval development of the three species (tables 23, 24) appears to be 80 to 94·8%. In many cases the combined larval and pupal life differed statistically within these humidities; the differences were slight, and possibly due to the light of a pilot lamp located inside the incubator. The intermittent light might have influenced the developmental rate to a certain extent (see Edney 1945, p. 403). There was no statistical difference in the active larval life of *X. cheopis* at 80, 90 and 94·8% R.H. A significant prolongation of the combined larval and pupal life of its male at 94·8% was believed to be due to a mechanical difficulty. At 27° C and 90% R.H. the combined larval and pupal life of *X. brasiliensis* was significantly longer than that of both the sexes of *X. cheopis* and that of the female of *X. astia*. The combined larval and pupal life of the male of *X. astia*, however, did not differ statistically from that of *X. brasiliensis*; though it was significantly longer than that of *X. cheopis*. The fact that at 29° C and 90% R.H. the combined larval and pupal life of *X. brasiliensis* was statistically longer than that of the other two species, indicates that the developmental rate in this species was intrinsically the slowest, as this temperature is optimum for *X. brasiliensis*; no such difference, however, existed between the other two species.

At 32° C 80% R.H. satisfied practically all the conditions of an optimum humidity for the three species (tables 23, 24). A significant retardation in the development of the larvae of *X. cheopis* at 90% R.H. when compared with that at 80% R.H. was due to slight crust formation. The combined larval and pupal life of *X. brasiliensis* was statistically much longer than that of the other two species at 80% R.H., perhaps because this temperature is above the optimum for the larva of this species (figure 6). The same is true of the larvae of *X. cheopis*, as its combined larval and pupal life was highly significantly longer than that of *X. astia*.

The detrimental effect of 35° C on recently hatched larvae was greatest in *X. brasiliensis* (table 25); none of them was reared into adult even with an optimum humidity of 90%. Moreover, the larval growth was not simultaneous, as all the stages from the first larva to cocoon were found after 10 to 16 days at 80 and 90% R.H. At 90% R.H. a few larvae pupated and many of the pupae contained fully formed adults suggests that this humidity is nearest to the insects' optimum requirements. Edney (1945, pp. 404, 405) has recorded

TABLE 23. SHOWING THE PROPORTIONS OF CERTAIN STAGES IN THE LIFE CYCLE, INTO V
OF *XENOPSYLLA* GREW, WHEN MAINTAINED AT CERTAIN T

	temp. (° C)	R.H. (%)	no. of larvae used	larvae spun cocoons		larvae reached the adult stage		no. of naked resting larvae	naked resting larvae formed pupae	
es				no.	Sig. χ^2 test	no.	Sig. χ^2 test		no.	Sig. χ^2 test
	17·0	60·0	251	135	↑ ↑	113	↑ ↑	†134	110	↑
	17·0	80·0	164	43	× ↑	89	— ↑	121	115	× ↑
	17·0	90·0	130	9	× × ↑	121	× × ↑	121	118	× — ↑
	17·0	90·0	156	132	× × × ↑	147	× × — ↑	24	20	— — + ↑
	17·0	97·5	151	145	× × × × ↑	145	× × — — ↑	6	6	— — — — ↑
	17·0	100	117	57	— × × × × ↑	12	× × × × × ↑	60	44	— × × — — ↑
	17·0	100	114	100	× × × — + ×	7	× × × × × —	14	12	— — — — — —
nsis	17·0	60·0	192	88	↑ —	65	↑ +	†60	51	↑
nsis	17·0	80·0	157	75	— ↑	99	× ↑	82	82	× ↑
nsis	17·0	90·0	154	36	× × ↑	150	× × ↑	118	118	× — ↑
nsis	17·0	90·0	110	83	× × × ↑	105	× × — ↑	†28	27	— — — ↑
nsis	17·0	97·5	152	134	× × × + ↑	152	× × — + ↑	18	18	— — — — ↑
nsis	17·0	100	113	101	× × × + — ↑	26	— × × × × ↑	8	8	— — — — — ↑
nsis	17·0	100	146	98	× × × — × ×	0	× × × × × ×	27	27	— — — — — —
	17·0	80·0	235	150	↑	146	↑	†81	62	↑
	17·0	90·0	137	133	× ↑	135	× ↑	†5	4	— ↑
	17·0	97·5	105	94	× + ↑	97	× + ↑	8	8	— — ↑
	17·0	100	110	55	+ × × ↑	19	× × × ↑	8	0	× + ×
	17·0	100	36	25	— × × —	21	— × × ×	0	0	
	22·0	50·0	70	24	↑	12	↑	†21	12	↑
	22·0	60·0	173	167	× ↑	166	× ↑	†7	6	— ↑
	22·0	80·0	208	202	× — ↑	206	× — ↑	6	6	— — ↑
	22·0	90·0	172	154	× + × ↑	166	× — — ↑	12	12	+ — — ↑
	22·0	97·5	175	172	× — — ×	172	× — — —	1	1	— — — —
nsis	22·0	60·0	148	140	↑	139	↑	9	7	↑
nsis	22·0	80·0	145	142	— ↑	137	— ↑	7	5	— ↑
nsis	22·0	90·0	90	90	— — ↑	89	— — ↑	†1	0	— —
nsis	22·0	97·5	95	90	— — —	80	+ + ×	0	0	
	22·0	60·0	120	104	↑	87	↑	†28	12	↑
	22·0	80·0	96	96	× ↑	94	× ↑	†2	2	— ↑
	22·0	90·0	130	129	× — ↑	129	× — ↑	1	1	— — ↑
	22·0	97·5	110	108	× — — ↑	107	× — — ↑	†3	1	— — — ↑
	22·0	97·5	30	30	— — — —	28	+ — — —	†2	2	— — — —
	27·0	50·0	60	31	↑	24	↑	†10	7	↑
	27·0	50·0	140	140	× ↑	140	× ↑	0	0	
	27·0	60·0	158	158	× — ↑	158	× — ↑	0	0	
	27·0	80·0	102	101	× — — ↑	102	× — — ↑	1	1	—
	27·0	90·0	117	117	× — — — ↑	117	× — — — ↑	0	0	
	27·0	94·8	150	150	× — — — — ↑	150	× — — — — ↑	0	0	
	27·0	100	160	160	× — — — — —	0	× × × × × ×	0	0	
ensis	27·0	50·0	60	14	↑	14	↑	8	7	↑
ensis	27·0	50·0	132	88	× ↑	74	× ↑	†34	29	— ↑
ensis	27·0	60·0	138	130	× × ↑	128	× × ↑	†4	3	— — ↑
ensis	27·0	80·0	131	131	× × + ↑	129	× × + ↑	†2	2	— — —
ensis	27·0	90·0	117	115	× × — — ↑	115	× × — — ↑	0	0	
ensis	27·0	94·8	25	25	× × — — — ↑	25	× × — — — ↑	0	0	
ensis	27·0	100	30	30	× × — — — — ↑	30	× × — — — — ↑	0	0	
ensis	27·0	100	25	7	— × × × × × ×	0	+ × × × × × ×	—	—	
	27·0	60·0	127	119	↑	119	↑	4	0	
	27·0	80·0	136	136	× ↑	136	× ↑	0	0	
	27·0	90·0	148	148	× — ↑	147	+ — ↑	0	0	
	27·0	94·8	20	19	— — —	19	— — —	0	0	
	29·0	60·0	143	143	↑	140	↑	†3	0	
	29·0	90·0	213	213	—	213	—	0	0	
ensis	29·0	60·0	150	150	↑	147	↑	†3	0	
ensis	29·0	90·0	130	130	—	130	—	0	0	
	29·0	60·0	85	67	↑	64	↑	†4	0	↑
	29·0	90·0	90	90	×	88	×	†2	2	—
	32·0	60·0	159	157	↑	156	↑	1	1	↑
	32·0	80·0	150	148	— ↑	148	— ↑	0	0	
	32·0	90·0	132	132	— — ↑ ↑	132	— — ↑ ↑	0	0	
	32·0	97·5	163	161	— — — ↑	160	— — — ↑	3	1	—
	32·0	100	161	161	— — — —	99	× × × ×	0	0	
ensis	32·0	60·0	121	106	↑	98	↑	†16	9	↑
ensis	32·0	80·0	173	170	× ↑	170	× ↑	1	0	— ↑
ensis	32·0	97·5	124	124	× — ↑	122	× — ↑	0	0	
ensis	32·0	100	183	178	× — —	101	× × ×	†5	5	— —
	32·0	60·0	136	75	↑	63	↑	†12	2	↑
	32·0	60·0	86	12	× ↑	12	× ↑	1	0	— ↑
	32·0	80·0	143	141	× × ↑	142	× × ↑	2	1	— — ↑
	32·0	97·5	169	164	× × — ↑	164	× × — ↑	0	0	
	32·0	100	160	156	× × — —	156	× × — —	4	4	+ — —
	35·0	80·0	178	166	↑	94	↑	†33	19	↑
	35·0	90·0	154	148	— ↑ —	104	× ↑ ×	6	6	— ↑
	35·3	80·0	105	92	— + ↑	46	— × ↑	13	1	× × ↑

INTO WHICH THE RECENTLY HATCHED LARVAE OF THE THREE SPECIES

RTAIN TEMPERATURES AND HUMIDITIES

g larvae upae	naked resting larvae reached the adult stage		stages died			
χ^2 test	no.	Sig. χ^2 test	R.L.	E.P.	A.P.	remarks
	0	↑	24	90	20	4 larvae died early
	46	× ↑	6	12	57 }	larvae disturbed to prevent cocoon formation
	112	× × ↑	3	2	4 }	
↑	15	× + × ↑	4	0	5	
— ↑	0	— — × + ↑	0	6	0	
— — ↑	0	— × × × — ↑	16	—	—‡	
— — —	0	— + × × — —	2	—	—‡	
	0	↑	9	42	9	67 larvae died in different active instars
	24	× ↑	0	22	36 }	larvae disturbed to prevent cocoon formation
	114	× × ↑	0	0	4 }	
↑	23	× × + ↑	1	0	4	1 larva left cocoon and died
— ↑	18	× × — — ↑	0	0	0	
— — ↑	0	— — × × × ↑	—	—	—‡	4 } larvae died early
— — —	0	— × × × × —	—	—	—‡	21 } not disturbed after cocoon formation
	0	↑	19	37	25	8 larvae died in third instar
	3	× ↑	1	0	1	1 larva left cocoon and died
	3	× — ↑	0	4	1	3 larvae died early
	0	— — —	8	—	—‡	47 } larvae died early
	0		—	—	—‡	11 }
	0	↑	9	12		12 } 37 larvae died in 4 days
	0	— ↑	1	6		1 } larvae left cocoons and pupated
	4	× + ↑	0	2		
↑	12	× × — ↑	0	0	0	6 } larvae died early
—	1	+ — — —	0	0	0	2 }
	0	↑	2	7	0	
	0	— ↑	2	5	0	1 active third-instar larva died
	0	— —	1	0	0	
	0		0	10		5 active larvae and 10 pupae within cocoons died
	0	↑	16	12		5 larvae died early
	0	— ↑	0	0	2	
	0	— — ↑	0	0	1	
↑	0	— — — ↑	2	0	1	
—	0	— — — —	0	0	2	
	0	↑	3	7		26 larvae died early
	0	|	0	0	0	
	0	|	0	0	0	
	1	—	0	0	0	
	0		0	0	0	
	0		0	0	0	
	0		—	—	—‡	
	0	↑	1	7		38 } active larvae died in different instars
	0	— ↑	5	29		24 }
	0	— — ↑	1	3		6 larvae died early
—	0	— — —	0	2		
	0		0	0	0	2 larvae died early
	0		0	0	0	
	0		0	0	0	1 } days' initial exposure of food to the humidity
	—		—	—	—‡	7 }
	0		4	0	0	4 larvae died in first instar
	0		0	0	0	
	0		1	0	0	1 larva died within cocoon
	0		0	0	0	1 larva died early
	0		3	0	0	
	0		0	0	0	
	0		3	0	0	
	0		0	0	0	
	0	↑	4	0	0	17 larvae died early
	0	—	0	0	2	
	0	↑	0	1	0	2 } second instar larvae died
	0	|	0	0	0	2 }
	0	|	0	0	0	fungus growth
	0	—	2	1	0	
	0		0	62		
	0	↑	7	9		7 } larvae died early
	0	— ↑	1	0		2 }
	0	|	0	2		2 pupae died within cocoons
	0	— —	0	77		
	0	↑	10	0	2	61 } larvae died early
	0	— ↑	1	0	0	73 }
	1	— — ↑	1	0	0	
	0	|	0	0	0	5 larvae died early
—	0	— — —	0	4		food disturbed daily
	0	↑	31	13	37	1 second- and 2 third-instar larvae died
	0	— ↑	6	16	28	
↑	0	— — ↑	27	6	26	

32·0	100	160	156	× × — —	156	× × — —	4	4	+ — —
35·0	80·0	178	166	↑	94	↑	†33	19	↑
35·0	90·0	154	148	— ↑ —	104	× ↑ ×	6	6	— ↑
35·3	80·0	105	92	— + ↑	46	— × ↑	13	1	× × ↑
35·3	97·5	160	76	× × × ↑	3	× × × ↑	84	2	× × —
35·3	100	151	79	× × × —	1	× × × —	—	—	
35·0	80·0	154	153	↑	154	↑	1	1	↑
35·0	90·0	148	145	— ↑	148	— ↑	3	3	— ↑
35·0	100	94	93	— — ↑	67	× × ↑	1	1	— — ↑
35·0	100	80	75	+ — — ↑	0	× × × ↑	5	5	— — — ↑
35·0	97·5	80	78	— — — — ↑	0	× × × — ↑	2	2	— — — —
35·0	97·5	80	0	× × × × × ↑	0	× × × — — ↑	0	0	
35·0	100	120	0	× × × × × —	0	× × × — — —	0	0	
36·5	80·0	40	12	↑	8	↑	†2	2	↑
36·5	90·0	40	34	× ↑	17	— ↑	6	2	—
36·5	97·5	40	20	— × ↑	12	— — ↑	—	—	
36·5	100	40	4	— × ×	0	× × ×	6	—	

† Wherever the combined total of the cocoons and the naked resting and active larvae exceeds the number

‡ Different stages could not be determined due to clod formation and sogginess of the food and sand mixtur

-	0	— — —	0	4		food disturbed daily
	0	↑	31	13	37	1 second- and 2 third-instar larvae died
	0	— ↑	6	16	28	
↾	0	— — ↑	27	6	26	
-	0	— — —	—	—	—‡	increase in pressure killed different stages from second larval instar to adult
	—		—	—	—‡	
	1	↑	0	0	0	
	3	— ↑	0	0	0	
↾	0	— — ↑	0	0	27	
- ↑	0	— + — ↑	—	—	—‡	no adult emergence due to rise in temperature and pressure
- —	0	— — — —	—	—	—‡	
	0		0	0	0	increase in pressure killed all the larvae in third instar
	0		0	0	0	
	0	↑	0	2	2	2 pupae within cocoons and 28 larvae died
	0	—	8	8	7	
	0		—	—	—‡	all larvae grew
	0		—	—	—‡	crust killed 25 larvae in 3 days

: number of the larvae used, it is due to the defaecated larvae having left the cocoons.
nd mixture.

24. SHOWING THE DURATIONS OF CERTAIN STAGES IN THE LIFE CYCLE, INTO WHICH THE RECE
GREW, WHEN MAINTAINED AT CERTAIN TEMPERATURES AND HUMIDITIES (CONT

			no. of larvae spun cocoons and duration of the active larval life				no. of cocoons from which adults emerged and duration of the resting larval and pupal life				no. o of thei ar
				days				days			
es	temp. (° C)	R.H. (%)	larvae	range	mean	Sig. t test	cocoons	range	mean	Sig. t test	♀♀
	17·0	60·0	135	31–47	37·27	↑	113	29–39	34·15	↑	46
	17·0	80·0	43	23–36	30·02	× ↑	—	—	—		68
	17·0	90·0	9	33–41	36·33	− × ↑	—	—	—		61
	17·0	90·0	132	23–39	28·38	× + × ↑ ↑	132	28–43	34·80	− ↑ ↑	72
	17·0	97·5	145	23–36	28·56	× × × − ↑	145	32–46	39·48	× ×	67
	17·0	100	57	27–39	32·49	× × × × × ↑	—	—	—		9
	17·0	100	100	23–37	27·28	× × × + × ×	—	—	—		6
nsis	17·0	60·0	88	30–44	37·70	↑	65	26–39	32·45	↑	36
nsis	17·0	80·0	75	25–38	31·16	× ↑	75	25–39	29·87	× ↑	59
nsis	17·0	90·0	36	29–36	33·03	× × ↑	—	—	—		71
nsis	17·0	90·0	83	25–34	29·88	× × × ↑ × ↑	82	26–42	†33·40	− × ↑ − ↑	58
nsis	17·0	97·5	134	25–35	30·57	× − × − ↑	134	29–42	35·19	× × ×	84
nsis	17·0	100	101	25–37	30·68	× − × + − ↑	—	—	—		26
nsis	17·0	100	98	27–39	33·05	× × − × × ×	—	—	—		0
	17·0	80·0	150	30–50	38·89	↑	146	26–45	31·78	↑	75
	17·0	90·0	133	29–45	†35·32	× ↑ × ×	132	26–43	†33·34	× ↑ + −	75
	17·0	97·5	94	29–45	38·57	− × ↑	94	26–41	33·26	× − ↑	52
	17·0	100	55	30–46	39·05	− × − ↑	—	—	—		15
	17·0	100	25	31–45	36·40	+ − + +	21	26–37	30·48	− + ×	17
	22·0	50·0	24	26–42	33·29	↑	12	15–21	19·00	↑	6
	22·0	60·0	167	13–20	16·04	× ↑	166	14–22	17·70	− ↑	78
	22·0	80·0	202	10–15	12·62	× × ↑	202	14–22	17·19	+ − ↑	109
	22·0	90·0	154	10–15	12·33	× × + ↑ ↑	154	13–22	17·16	+ − − ↑ ↑	105
	22·0	97·5	172	10–17	12·08	× × × −	172	14–22	17·84	− − + +	96
nsis	22·0	60·0	140	14–20	16·99	↑	139	13–21	17·24	↑	80
nsis	22·0	80·0	142	11–16	13·28	× ↑	137	14–24	†18·50	× ↑	72
nsis	22·0	90·0	90	11–17	13·62	× − ↑ × ↑	89	14–24	19·24	× − ↑ × ↑	43
nsis	22·0	97·5	90	11–19	15·18	× × ×	80	15–27	19·51	× + −	40
	22·0	60·0	104	18–28	22·20	↑	87	13–22	16·83	↑	56
	22·0	80·0	96	13–19	15·30	× ↑	94	13–23	17·49	− ↑	48
	22·0	90·0	129	11–17	†13·66	× × ↑ × −	129	13–21	†17·30	− − ↑ − ×	68
	22·0	97·5	108	11–17	14·48	× × × ↑	107	13–24	17·18	− − − ↑	58
	22·0	97·5	30	15–23	17·90	× × × ×	28	14–20	17·00	− − − −	15
	27·0	50·0	31	15–27	21·81	↑	24	12–18	14·50	↑	15
	27·0	50·0	140	12–20	15·33	× ↑	140	9–16	12·31	× ↑	76
	27·0	60·0	158	8–14	10·22	× × ↑	158	9–15	11·91	× + ↑	86
	27·0	80·0	101	7–11	8·71	× × × ↑	101	8–15	11·10	× × × ↑	62
	27·0	90·0	117	7–11	8·51	× × × − ↑ ↑	117	9–15	12·19	× − − × ↑ ↑	64
	27·0	94·8	150	7–11	8·63	× × × − − ↑	150	9–15	12·47	× − × × −	81
	27·0	100	160	7–10	8·38	× × × + − +	0	—	—		0
nsis	27·0	50·0	14	20–23	21·29	↑	14	11–16	13·43	↑	11
nsis	27·0	50·0	88	16–24	20·74	− ↑	74	11–15	12·35	× ↑	50
nsis	27·0	60·0	130	11–17	12·85	× × ↑	128	9–15	11·52	× × ↑	78
nsis	27·0	80·0	131	8–14	9·95	× × × ↑	129	10–16	12·82	− − × ↑	70
nsis	27·0	90·0	115	8–11	9·27	× × × × ↑ × ↑	115	9–16	†11·99	× − + × ↑ − ↑	76
nsis	27·0	94·8	25	8–10	9·08	× × × × − ↑	25	11–16	12·52	− − × − − ↑	18
nsis	27·0	100	30	8–11	10·13	× × × − × × ↑	30	10–14	11·87	× − − + − −	18
nsis	27·0	100	7	10–12	10·86	× × × − × × −	0	—	—		0
	27·0	60·0	119	11–17	13·62	↑	119	9–14	10·81	↑	70
	27·0	80·0	136	7–12	10·16	× ↑	136	9–15	11·77	× ↑	73
	27·0	90·0	148	7–12	9·89	× − ↑ × ×	147	9–15	†11·17	− + ↑ × ×	98
	27·0	94·8	19	8–11	9·26	× × +	19	9–15	12·00	× − −	11
	29·0	60·0	143	8–12	10·03	↑	140	8–14	10·09	↑	70
	29·0	90·0	213	6– 9	7·32	× ↑	213	8–13	10·27	− ↑	126
nsis	29·0	60·0	150	9–14	11·35	↑	147	9–13	10·08	↑	78
nsis	29·0	90·0	130	7–10	8·30	× × ↑	130	9–13	†10·66	× + ↑	68
	29·0	60·0	67	11–15	12·45	↑	64	7–13	9·75	↑	22
	29·0	90·0	90	7–11	8·77	× × ×	88	7–12	†9·78	− + ×	37
	32·0	60·0	157	8–13	9·85	↑	156	8–12	9·03	↑	83
	32·0	80·0	148	5– 8	6·78	× ↑ ↑	148	8–13	9·60	× ↑ ↑	71
	32·0	90·0	132	6– 9	7·42	× × ↑ ↑	132	8–11	9·76	× − ↑ ↑	78
	32·0	97·5	161	5– 8	6·56	× + × ↑	160	8–12	9·56	× − − ↑	81
	32·0	100	161	5– 8	6·53	× × × −	99	8–13	9·93	× − − +	80
nsis	32·0	60·0	106	9–14	11·58	↑	98	8–12	10·14	↑	36
nsis	32·0	80·0	170	7–10	8·44	× ↑ × ↑	170	8–14	9·92	− ↑ + ↑	84
nsis	32·0	97·5	124	7–11	8·30	× − ↑	122	8–13	†10·31	− × ↑	64
nsis	32·0	100	178	6–11	7·82	× × ×	101	8–14	9·69	+ − ×	76
	32·0	60·0	75	10–16	12·09	↑	63	7–10	8·10	↑	33
	32·0	60·0	12	10–13	11·50	− ↑	12	7–11	8·75	− ↑	5
	32·0	80·0	141	6– 9	7·56	× × ↑ × ×	141	6–10	7·82	− + ↑ × ×	86
	32·0	97·5	164	6–11	7·77	× × − ↑	164	6–11	†8·32	− − × ↑	77
	32·0	100	156	6– 9	7·48	× × − +	156	6–11	8·08	− − − −	81
	35·0	80·0	166	7–11	8·70	↑	94	8–13	10·17	↑	72
	35·0	90·0	148	7–11	8·34	× ↑ ↑ ×	104	9–14	10·96	× ↑ ↑ ×	61
	35·3	80·0	92	7–12	9·32	× × ↑	46	9–13	10·57	− −	39
	35·3	97·5	76	7–13	8·24	× − × ↑	—	—	—		3
	35·3	100	79	6–12	8·24	× − × −	—	—	—		1

E RECENTLY HATCHED LARVAE OF THE THREE SPECIES OF *XENOPSYLLA*
(CONTINUATION OF EXPERIMENTS IN TABLE 23)

no. of ♀♀ and duration of their combined larval and pupal life				no. of ♂♂ and duration of their combined larval and pupal life			
	days				days		
♀♀	range	mean	Sig. *t* test	♂♂	range	mean	Sig. *t* test
46	62–69	64·89	↑	67	71–81	75·63	↑
68	51–65	56·04	× ↑	21	67–75	69·95	× ↑
61	53–63	56·92	× − ↑	60	65–73	68·35	× × ↑
72	52–64	57·14	× + − ↑ ↑	75	64–78	71·29	× − × ↑ ↑
67	55–70	60·57	× × × × ↑	78	70–80	74·37	+ × × × ↑
9	59–74	64·44	− × × × × ↑	3	77–85	81·33	× × × × × ↑
6	56–59	58·00	× − − − − ×	1	73	73·00	− − + − − −
36	57–69	64·75	↑	29	71–83	75·00	↑
59	52–62	56·24	× ↑	40	67–75	69·98	× ↑
71	51–63	57·32	× + ↑	79	65–74	68·14	× × ↑
58	51–65	58·33	× × − ↑ − ↑	47	66–76	†71·26	× + × ↑ − ↑
84	55–67	61·08	× × × × ↑	68	70–76	73·38	× × × ×
26	57–75	62·35	× × × × −	0	—	—	
0	—	—		0	—	—	
75	58–72	64·37	↑	71	69–86	77·13	↑
75	56–70	61·77	× ↑ × ×	60	70–84	†77·57	− ↑ × ×
52	59–72	66·10	+ × ↑	45	72–86	78·87	+ − ↑
15	60–77	68·27	× × − ↑	4	74–79	76·00	− − − ↑
17	59–72	64·18	− + − +	4	74–78	75·75	− − − −
6	46–49	47·00	↑	6	51–55	52·67	↑
78	28–33	30·08	× ↑	88	34–41	36·99	× ↑
109	24–31	26·55	× × ↑	97	32–36	34·01	× × ↑
105	24–30	27·20	× × × ↑ ↑	61	31–37	34·48	× × × ↑ ↑
96	24–31	26·91	× × − −	76	31–36	33·74	× × − ×
80	29–36	31·51	↑	59	36–41	37·88	↑
72	25–32	27·72	× ↑	65	34–39	†36·15	× ↑
43	26–31	28·21	× − ↑ × ↑	46	34–40	37·20	+ × ↑ × ↑
40	26–35	30·15	× × ×	40	32–46	38·90	+ × ×
56	33–40	36·23	↑	31	40–48	42·77	↑
48	26–31	28·83	× ↑	46	34–40	†36·85	× ↑
68	25–30	26·94	× × ↑ − ×	61	33–37	35·44	× × ↑ × ×
58	25–33	27·69	× × × ↑	49	33–41	36·33	× − × ↑
15	29–35	32·20	× × × ×	13	37–40	37·85	× + × ×
15	31–36	33·80	↑	9	38–43	38·67	↑
76	23–27	25·29	× ↑	64	28–35	30·44	× ↑
86	18–22	20·31	× × ↑	72	23–26	24·28	× × ↑
62	15–20	18·00	× × × ↑	40	21–26	22·65	× × × ↑
64	17–20	18·64	× × × × ↑ ↑	53	21–25	23·19	× × × − ↑ ↑
81	17–20	18·64	× × × × −	69	21–24	23·65	× × × × +
0	—	—		0	—	—	
11	33–34	33·82	↑	3	38	38·00	↑
50	27–34	31·34	× ↑	24	35–38	35·83	× ↑
78	20–25	22·95	× × ↑	50	26–29	26·76	× × ↑
70	18–22	20·24	× × × ↑	59	24–27	25·69	× × × ↑
76	17–20	19·36	× × × × ↑ × ↑	39	24–26	†24·90	× × × × ↑ × ↑
18	19–22	20·11	× × × − × ↑	7	24–26	25·43	× × × − − ↑
18	19–22	20·17	× × × − × −	12	24–25	24·75	× × × × − −
0	—	—		0	—	—	
70	20–25	22·74	↑	49	25–29	26·82	↑
73	16–22	19·40	× ↑	63	23–27	24·87	× ↑
98	16–22	18·99	× + ↑ − +	49	24–27	†25·14	× − ↑ × −
11	18–19	18·91	× − −	8	24–26	24·50	× − −
70	17–20	18·31	↑	70	21–25	21·87	↑
126	14–18	15·73	× ↑	87	19–22	20·26	× ↑
78	18–21	19·59	↑	69	21–26	23·12	↑
68	16–20	16·91	× × ↑	62	20–22	†21·19	× × ↑
22	18–21	19·77	↑	42	22–27	23·31	↑
37	14–17	15·89	× − ×	51	19–22	†20·39	× − ×
83	16–19	17·54	↑	73	19–24	20·38	↑
71	13–16	14·42	× ↑ ↑	77	17–21	18·18	× ↑ ↑
78	14–17	16·05	× × ↑ ↑	54	17–19	18·65	× × ↑ ↑
81	13–16	14·42	× − × ↑	79	17–19	17·84	× × × ↑
80	13–17	15·43	× × × ×	19	18–21	19·21	× × × ×
36	18–22	19·86	↑	62	20–25	22·65	↑
84	16–18	16·75	× ↑ × ↑	86	19–23	19·93	× ↑ × ↑
64	16–19	17·08	× + ↑	58	19–23	†20·24	× + ↑
76	15–19	16·25	× × ×	25	19–22	20·36	× + −
33	17–21	18·55	↑	30	20–23	21·43	↑
5	17–19	18·20	− ↑	7	18–24	21·71	− ↑
86	13–16	13·94	× × ↑ × ×	56	17–19	17·66	× × ↑ × ×
77	12–17	14·04	× × − ↑	87	16–20	†17·91	× × − ↑
81	13–17	13·75	× × − −	75	17–19	17·52	× × − ×
72	16–20	17·74	↑	22	19–24	21·45	↑
61	16–19	17·51	− ↑ ↑ ×	43	19–24	21·88	− ↑ ↑ ×
39	17–20	19·00	× × ↑	7	21–25	22·43	− −
3	18–20	18·67	− − − ↑	0	—	—	
1	16	16·00	− − + −	0	—	—	

[illegible]	[illegible]	[illegible]	7–11	[illegible]	│		│	[illegible]	[illegible]	[illegible]	│		│	[illegible]
35·0	90·0	148	7–11	8·34	× ↑	↑	×	104	9–14	10·96	× ↑	↑	×	61
35·3	80·0	92	7–12	9·32	× × ↑	│		46	9–13	10·57	— —	│		39
35·3	97·5	76	7–13	8·24	× — × ↑	│		—	—	—		│		3
35·3	100	79	6–12	8·24	× — × —	│		—	—	—		│		1
35·0	80·0	153	6–10	7·92	↑	│		153	6–10	7·92	↑	│		81
35·0	90·0	145	6– 9	7·17	× ↑	×		145	6–10	†7·87	— ↑	×		87
35·0	100	93	6– 9	8·09	— × ↑			67	6–10	8·15	— —			33
35·0	100	75	6– 9	8·15	— × — ↑			0	—	—				0
35·0	97·5	78	6–10	8·04	— × — —			0	—	—				0
35·0	97·5	0	—	—				0	—	—				0
35·0	100	0	—	—				0	—	—				0
36·5	80·0	12	9–14	11·00	↑			8	8– 9	8·63	↑			7
36·5	90·0	34	7–12	9·74	+ ↑			17	8–12	†9·12	— ↑			13
36·5	97·5	20	7–10	9·40	× — ↑			12	8–11	8·42	— —			11
36·5	100	4	10–12	10·50	— — +			0	—	—				0

BIOLOGICAL SCIENCES
THE ROYAL SOCIETY
PHILOSOPHICAL TRANSACTIONS OF

61	16–19	17·51	–	↑			↑	×	43	19–24	21·88	–	↑	↑	×
39	17–20	19·00	×	×	↑				7	21–25	22·43	–	–		
3	18–20	18·67	–	–	–	↑			0	—	—				
1	16	16·00	–	–	+	–			0	—	—				
81	13–16	14·02	↑						73	17–20	17·88	↑			
87	13–15	13·66	×	↑			×		61	16–18	†17·15	×	↑	×	
33	13–15	14·42	×	×					34	16–19	17·56	–	+		
0	—	—							0	—	—				
0	—	—							0	—	—				
0	—	—							0	—	—				
0	—	—							0	—	—				
7	17–19	18·43	↑						1	21	21·00	↑			
13	16–19	16·92	×	↑					4	23–24	†23·50	+	↑		
11	16–19	17·00	×	–					1	21	21·00	–	+		
0	—	—							0	—	—				

BLE 25. SHOWING THE EFFECTS OF THE TEMPERATURES OF 35·0 ± 0·2 AND 35·3 ± 0·3° C (MARK
ON THE GROWTH OF RECENTLY HATCHED LARVAE OF THE TWO SPE

s	R.H. (%)	no. of larvae used	larvae died in feeding state		no. of larvae and period in which they died in different instars without cocoon formation			Sig. *t* test	larvae spun cocoons		duration of active larval life to cocoon formation (days)		Sig te
						days							
			no.	Sig. χ^2 test	larvae	range	mean		no.	Sig. χ^2 test	range	mean	
	60	203	†154	↑	†186	4–24	12·38	↑	17	↑	12–20	15·29	↑
	60	160	124	–	151	2–25	10·79	×	9	–	14–19	15·89	–
sis	60	240	235	× ↑	240	1–25	6·69	× ↑	0	× ↑	—	—	
sis	80	155	40	× ↑	123	4–23	12·84	× ↑	32	× ↑	10–14	11·28	↑
sis	90	151	35	× – ↑	73	3–20	13·14	× – ↑	78	× × ↑	9–16	10·40	×
sis	100	186	129	× × ×	156	3–13	8·55	× × ×	30	× – ×	9–12	10·40	×

† In these columns, the first indicates number of larvae which died in any instar but while feeding continued: the secon

TABLE 26. SHOWING THE EFFECT OF A TEMPERATURE OF 35 ± 0·2° C WITH EFFECTIVE
AND PUPAE WITHIN ONE- OR TWO-DAY-OLD COCOONS OF *XEN*

R.H. (%)	presence or absence of food in the cocoon wall	no. of cocoons used	advanced pupae formed		adults emerged		days in which adults emerged	stages found dead 21 to 45 days' expo	
			no.	Sig. χ^2 test	no.	Sig. χ^2 test		R.L.	E.P.
60·0	+	95	30	↑	8	↑	9	61	4
80·0	+	25	14	+ ↑	9	× ↑	7–13	5	6
90·0	+	40	33	× + ↑	13	× – ↑	9–13	6	1
90·0	–	40	39	× × – ↑	14	× – – ↑	9–18	0	1
97·5	–	39	39	× × + – ↑	16	× – – – ↑	9–16	0	0
100	–	40	40	× × + – – ↑	6	– – – – + ↑	9–11	0	0
97·5	+	25	0	× × × × × × ↑	0	– × × × × – ↑	—	18	7
100	+	80	0	× × × × × × – ↑	0	+ × × × × × – ↑	—	30	50
97·5	+	80	0	× × × × × × – –	0	+ × × × × × – –	—	—	—

TABLE 27. SHOWING THE EFFECT OF A TEMPERATURE OF 35 ± 0·2° C WITH EFFECTIVE HU
PUPAE WITHIN ONE- OR TWO-DAY-OLD COCOONS OF *XENOPSYL*

R.H. (%)	presence or absence of food in the cocoon wall	no. of cocoons used	advanced pupae formed		adults emerged		days in which adults emerged	stages foun 21 to 46 da	
			no.	Sig. χ^2 test	no.	Sig. χ^2 test		R.L.	E
60·0	+	40	13	↑	0	↑	—	22	
60·0	–	40	0	× ↑	0	– ↑	—	37	
80·0	+	40	22	– × ↑	5	– – ↑	7– 9	11	
90·0	+	89	29	– × + ↑	7	– – – ↑	†9–11	52	
90·0	–	80	33	– × – – ↑	1	– – + – ↑	11	40	
97·5	+	40	0	× – × × × ↑	0	– – – – – ↑	—	40	
97·5	–	79	47	× × – × + × ↑	0	– – × + – – ↑	—	29	
100	+	42	0	× – × × × – × ↑	0	– – – – – – – ↑	—	42	
100	–	80	74	× × × × × × × ×	0	– – × + – – – –	—	6	

† The complete combined resting larval and pupal life within the cocoon was 12·36 day

TABLE 28. SHOWING THE EFFECT OF A TEMPERATURE OF 35 ± 0·2° C WITH EFFECTIVE
AND PUPAE WITHIN ONE- OR TWO-DAY-OLD COCOONS OF *XEN*

R.H. (%)	presence or absence of food in the cocoon wall	no. of cocoons used	advanced pupae formed		adults emerged		days in which adults emerged	stages found d 36 to 45 days'	
			no.	Sig. χ^2 test	no.	Sig. χ^2 test		R.L.	E.P.
60·0	–	40	15	↑	10	↑	7– 9	24	1
60·0	+	28	18	– ↑	9	– ↑	9–11	8	2
90·0	+	46	46	× × ↑	46	× × ↑	9–11	0	0
90·0	–	42	42	× × – ↑	42	× × – ↑	7–14	0	0
97·5	+	40	31	× – × × ↑	14	– – × × ↑	9	0	9
97·5	–	80	70	× + + + – ↑	70	× × + + × ↑	7–15	7	3
100	–	80	80	× × – – × × ↑	80	× × – – × × ↑	7–12	0	0
100	+	40	15	– – × × × × ×	0	× × × × × × ×	—	15	10

(MARKED WITH AN ASTERISK) WITH EFFECTIVE HUMIDITIES WO SPECIES OF *XENOPSYLLA*

active cocoon days)		no. of larvae and period in which they reached the resting stage				stages died		
			days					
mean	Sig. *t* test	larvae	range	mean	Sig. *t* test	R.L.	E.P.	A.P.
15·29	↑	32	13–20	16·56	↑	44	3	2
15·89	—	27	12–19	16·81	—	36	0	0
—		5	20–25	21·80	× ↑	5	0	0
11·28	↑	83	10–18	13·11	× ↑	115	0	0
10·40	× ↑	38	10–15	12·92	× — ↑	104	3	9
10·40	× —	27	9–13	11·22	× × ×	57	0	0

: the second includes also those which died after final defaecation.

ECTIVE HUMIDITIES ON THE GROWTH OF LARVAE OF *XENOPSYLLA CHEOPIS*

und dead after days' exposure		
E.P.	A.P.	remarks
4	22	13 larvae left cocoons and 2 died as A.P.
6	5	
1	20	2 larvae left cocoons and died
1	25	
0	23	1 naked A.P. died
0	34	
7	0	Larvae and E.P. disintegrated within cocoons
50	0	
—	—	

TIVE HUMIDITIES ON THE GROWTH OF LARVAE AND *ENOPSYLLA BRASILIENSIS*

tages found dead after 21 to 46 days' exposure			
R.L.	E.P.	A.P.	remarks
22	5	13	3 } larvae left cocoons and died
37	3	0	3
11	7	17	
52	8	22	13 larvae left cocoons and died
40	7	32	
40	0	0	2 } larvae left cocoons and died
29	3	47	2
42	0	0	3
6	0	74	

12·36 days (calculated average).

ECTIVE HUMIDITIES ON THE GROWTH OF LARVAE OF *XENOPSYLLA ASTIA*

es found dead after o 45 days' exposure			
.	E.P.	A.P.	remarks
	1	5	8 larvae left cocoons and died
	2	9	2 naked E.P. died
	0	0	
	0	0	
	9	17	
	3	0	10 larvae left cocoons and 3 pupated
	0	0	1 naked pupa bred successfully
	10	15	

success in rearing adults from the larvae of *X. brasiliensis* at 35° C with 80% R.H., which, in view of my observations, would only be possible at a slightly lower temperature.

At an optimum humidity of 90%, the adverse effect of 35° C on the development of the recently hatched larvae of *X. cheopis* (tables 23, 24) was shown by a statistically longer combined larval and pupal life and a lower emergence of adults than at 32° C; also by a significant retardation in growth at 35° C when compared with *X. astia*. At 35° C much greater success in the breeding of the larvae of *X. cheopis* was attained at 90% R.H. than at 80% R.H.; most of them spun cocoons at the former humidity, and the remainder formed naked pupae from which no adults emerged. Within some cocoons death occurred in different stages of development; but comparatively few larvae died in the resting stage. In contrast 35° C was not at all harmful for *X. astia*, for all its larvae produced adults at 80 and 90% R.H.; but its optimum range of humidity was narrowed down to about 90%.

Similarly, the harmful effect of 35° C was experienced by the resting larvae within the cocoons of *X. cheopis* (table 26) and *X. brasiliensis* (table 27), even at an optimum humidity. Its very marked detrimental effect on *X. brasiliensis* was evinced by the absence of any significant difference between the proportions of advanced pupae formed and adults emerged at 60% R.H. and at 80 or 90% R.H. The harmful effect of 35° C was less on *X. cheopis* than on *X. brasiliensis*, which is indicated by a better development of the resting larvae within the cocoons of the former species at the optimum humidity of 90%.

The larvae and the pupae of the three species also demonstrated specific differences at 36 and 36·5° C. In *X. brasiliensis* (tables 30, 32) both of them failed to grow completely: the larvae of *X. cheopis* (tables 29, 30), however, grew, a few completing their larval growth and dying as resting larvae within 17 days at 80% R.H.; they produced no adults. On the

TABLE 29. SHOWING THE EFFECT OF A TEMPERATURE OF 36·5±0·3° C WITH EFFECTIVE HUMIDITIES ON THE GROWTH OF RECENTLY HATCHED LARVAE OF *XENOPSYLLA CHEOPIS*

R.H. (%)	no. of larvae used	days in which all the larvae died in different instars: range	mean	Sig. *t* test	larvae died in different instars: I: no.	Sig. χ^2 test	II: no.	Sig. χ^2 test	III: no.	Sig. χ^2 test
80·0	160	3–18	7·98	↑	106	↑	49	↑	5	↑
90·0	80	5–14	8·83	– ↑	65	+ ↑	15	– ↑	0	– ↑
97·5	350	2–16	7·77	– × ↑	287	× – ↑	63	× – ↑	0	× – ↑
100	80	4– 9	5·49	× × ×	80	× × ×	0	× × ×	0	– – –

TABLE 30. SHOWING THE EFFECT OF A TEMPERATURE OF 36·5±0·3° C WITH EFFECTIVE HUMIDITIES ON THE GROWTH OF LARVAE AND PUPAE WITHIN THE COCOONS OF THE TWO SPECIES OF *XENOPSYLLA*

species	R.H. (%)	exposure to 36·5° C (days)	subsequent exposure to 23 to 32° C and 80% R.H. (days)	age of cocoons (days)	no. of cocoons used	stages found dead: R.L.	Pr.	E.P.	A.P.	% of ultimate pupation	probable % of initial pupation	signs of growth
cheopis	80·0	37	12	3	40	25	5	5	5	25·0	25	+
**cheopis*	80·0	6	26	3–4	56	9	6	30	11	73·2	40	+
cheopis	80·0	8	20	1–2	55	52	3	0	0	0.0	0	–
cheopis	90·0	16	0	1	45	45	0	0	0	0·0	0	–
cheopis	97·5	33	0	1	25	25	0	0	0	0·0	0	–
cheopis	97·5	33	0	5	25	2	0	7	16	92·0	100	+
brasiliensis	80·0	21	29	1–2	40	40	0	0	0	0·0	0	–
brasiliensis	80·0	6	19	4	25	1	8	16	0	64·0	60	–
brasiliensis	90·0	25	13	1–2	40	40	0	0	0	0·0	0	–

Table 31. Showing the effects of different high temperatures with effective humidities on the growth of recently hatched larvae of *Xenopsylla astia*

			no. of larvae and period in which they died in different instars without cocoon formation				no. of larvae spun cocoons and duration of active larval life				no. of larvae and period in which they reached the resting stage				
temp. (° C)	R.H. (%)	no. of larvae used	larvae	days range	days mean	Sig. *t* test	larvae	days range	days mean	Sig. *t* test	larvae	days range	days mean	Sig. *t* test	no. of larvae pupated
37	80·0	40	27	7–22	13·30	↑	13	9–14	11·62	↑	6	14–18	15·33	↑	0
37	90·0	25	17	4–21	12·47	– ↑	8	11–13	12·50	–	3	13–17	15·67	–	0
37	100	15	15	2– 8	3·87	× ×	0	—	—		0	—	—		0
38	80·0	100	100	2–19	4·70	↑	0	—	—		1	16	16·00	↑	0
38	90·0	115	115	2–21	6·37	× ↑	0	—	—		2	16–20	18·00	–	0
38	97·5	125	125	1–11	4·51	– × ↑	0	—	—		0	—	—		0
38	100	115	115	1– 5	2·50	× × ×	0	—	—		0	—	—		0

other hand, the recently hatched larvae of *X. astia* (tables 23, 24) were reared into adults at 36·5° C; but the success was only partial even with an optimum humidity of 90%.

Resting larvae within the cocoons of *X. cheopis* (table 30) failed to pupate at 36·5° C except in an experiment, marked with an asterisk, at 80% R.H., in which both the resting larvae and the pupae showed subsequent growth after a relatively short exposure of 6 days to 36·5° C. Some pupae, in other experiments, showed signs of development and contained fully formed dead adults.

At 37° C with 80 and 90% R.H. only the recently hatched larvae of *X. astia* (table 31) grew; some of them even spun cocoons, but none pupated. There was no significant difference in their development at these two humidities. Only 9·1 and 13% larvae of this species showed signs of growth at 38° C with 80 and 90% R.H. respectively; a very few of them actually completed active larval life, but died in the resting stage without spinning cocoons.

Between 17 and 32° C at an optimum humidity the active larval life of *X. cheopis* (table 24) was materially shorter than that of either of the other species (the difference being highly significant); that of *X. brasiliensis* was similarly shorter than that of *X. astia* at 17, 27 and 29° C; though these differences were not always represented in the combined larval and pupal life of these species, which suggests that speed of cocoon formation depends on the water requirements of the larva (see p. 586).

The temperature range for the development of larvae and pupae differed in the three species (figures 1 to 4). In view of the above findings it is reasonable to suggest that the

Table 32. Showing the fatal effect of different high temperatures with effective humidities on the recently hatched larvae of the two species of *Xenopsylla*

				days in which all larvae died in first instar		
species	temp. (° C)	R.H. (%)	no. of larvae used	range	mean	Sig. *t* test
brasiliensis	36	80·0	105	1–14	6·87	↑
brasiliensis	36	97·5	100	1– 9	5·36	×
brasiliensis	37	80·0	63	2– 4	2·71	× ↑
cheopis	37	80·0	112	4–13	7·91	× ↑
brasiliensis	37	90·0	40	2– 4	2·40	– × ↑
cheopis	37	90·0	80	4– 8	6·56	× × × ↑
cheopis	37	100·0	92	2– 5	3·29	× × × ×
brasiliensis	38	80·0	50	1– 2	1·04	↑
cheopis	38	80·0	45	1– 3	1·89	× ↑
brasiliensis	38	90·0	78	1– 2	1·01	– × ↑
cheopis	38	90·0	190	1– 3	1·94	× – ×

growth of these stages will be possible between 13·5 and 38° C in *X. astia*, between 12 and 36·5° C in *X. cheopis* and between 12 and 35·5° C in *X. brasiliensis*. The temperature range permitting the development of adults from the larvae of these species will vary in the same order. This supports the assumption of Hirst (1926, p. 250) 'that *X. astia* is adapted to a slightly higher temperature' than *X. cheopis*. The lower limit of effective temperature for the larvae and the pupae of *X. brasiliensis* and *X. cheopis* is almost the same; but its upper limit in the latter species is higher. Consequently, I cannot agree with Cragg (1921, p. 388) and Hirst (1927, p. 283) that *X. brasiliensis* 'is better adapted to warmth and dampness than *cheopis*'.

(*d*) *Fatal high temperatures*

In considering the true thermal death-points of larvae and pupae of the three species of rat-fleas, a distinction, as pointed out by Mellanby (1932*a*, pp. 229, 230), must be made between the cessation of growth owing to desiccation and to heat. As these factors operate independently of each other, only experiments at high temperatures with favourable humidities are considered. Even starvation, owing to exhaustion of the food reserves, may cause early death, as increase in temperature enhances the rate of metabolism (see Wigglesworth 1939, p. 362). This perhaps was responsible for the individual variation found in the heat resistance of a stage of the same species.

At 36° C the recently hatched larvae of *X. brasiliensis* (table 32) failed to grow; at 37° C they died in a much shorter period than at 36° C; 38° C was more rapidly fatal, as almost all of them were killed within a day. The resting larvae and the pupae within the cocoons (table 30) failed to grow at 36·5° C, and 6 days' exposure proved completely fatal. No growth occurred at 37° C in the recently hatched larvae of *X. cheopis* (table 32), and at 38° C they died in a much shorter time than at 37° C. At both these temperatures such larvae of *X. cheopis* survived significantly longer than those of *X. brasiliensis*; thus the former species can withstand the harmful effect of a high temperature better. Whenever the larvae were examined at a favourable room temperature after subjection to these dangerous temperatures, the living ones were at first inactive, and under the influence of heat stupor.

The degree of humidity did not influence the period before death of recently hatched larvae at the rapidly fatal temperatures, such as 37 and 38° C for *X. brasiliensis* and 38° C for *X. cheopis* (table 32); but at less rapidly fatal temperatures that allowed the larvae to live long, humidity had a highly significant effect on the time elapsing before death; for instance, the period was longer at 80% R.H. than at higher humidities in the case of *X. brasiliensis* at 36° C and in *X. cheopis* at 37° C. This is perhaps owing to their ability to bring down the body temperature slightly at the lower humidity. This is unexpected, for it is known that flea larvae lose water rapidly, so that at these high temperatures a reverse effect of humidity would be expected. It seems unlikely that so small an insect, with its high ratio of surface to volume, can lower its body temperature to any useful extent by evaporation (Mellanby 1932*a*, pp. 229, 231). Buxton, however (1933, p. 339), was of the opinion 'that even a small insect may be able to regulate its temperature a little if the external temperature is not so high...particularly if the insect is continually feeding'. The larvae of *X. cheopis* and *X. brasiliensis* ingested a little food at temperatures which were fatal after an interval, as they were slightly red; thus my experiments support the latter's assumption. Mellanby (1932*a*, p. 226, figure 4) found that the large fed larvae of *X. cheopis* died within a day at 37° C with 0 to 90% R.H., when not given any food.

All the three fed larval instars of *X. cheopis*, when subjected to 40 ± 1° C at 90% R.H. for 90 min., died within a day though subsequently maintained under favourable conditions; but shorter exposures to this temperature were not completely fatal. Mellanby (1932*a*, p. 225, figure 3), however, found that the large larvae of *X. cheopis* could survive 1 hr. subjection to 39·7° C with 0 to 90% R.H. An exposure of fully fed larvae of *X. astia* to 40 ± 1° C with 90% R.H. for 6 hr. was fatal to all instars.

Table 33. Showing the fatal effect of a temperature of 45·5 ± 0·3° C with effective humidities on the recently hatched larvae of the three species of *Xenopsylla*

				subsequent exposure to 28 to 31° C and 90% R.H.				
				larvae revived after one hour		days in which all the larvae died in first instar		
species	R.H. (%)	exposure to 45·5° C (min.)	no. of larvae used	no.	Sig. χ^2 test	range	mean	Sig. *t* test
cheopis	80	1	60	54	↑	0–3	1·03	↑
cheopis	80	2	30	23	− ↑	0–3	0·97	− ↑
brasiliensis	90	2	60	11	× × ↑	0–3	0·23	× × ↑
astia	90	2	60	60	+ × × ↑	1–3	1·25	+ + × ↑
cheopis	80	3	60	20	× × − ×	0–1	0·33	× × − ×
cheopis	80	4	60	3	↑	0–1	0·05	↑
brasiliensis	90	4	30	0	− ↑	0	0·00	− ↑
astia	90	4	60	32	× ×	0–3	0·63	× ×
cheopis	80	5	60	0	↑	0	0·00	↑
cheopis	80	6	60	0	− ↑	0	0·00	− ↑
brasiliensis	90	6	30	0	− − ↑	0	0·00	− − ↑
astia	90	6	60	0	− − −	0	0·00	− − −

One minute's exposure of the recently hatched larvae of *X. cheopis* (table 33) to 45·5° C completely prevented further growth; although most of them revived after an hour's exposure to favourable conditions. After 3 min. exposure the larvae were moribund. Two minutes' subjection of similar larvae of the three species to 45·5° C demonstrated specific differences in the proportion which revived and the period before death occurred; the period was shortest in *X. brasiliensis*, longest in *X. astia*. Specific differences in resistance to 45·5° C were also indicated in the period of exposure which is immediately fatal, which was 4, 5 and 6 min. in *X. brasiliensis*, *X. cheopis* and *X. astia* respectively.

Table 34. Showing the fatal effect of a temperature of 45·5 ± 0·3° C with different humidities on the fed third-instar larvae of *Xenopsylla cheopis*

			larvae revived on subsequent exposure to 27 to 30° C and 90% R.H.		
			after 1 hr.		after 24 hr.
R.H. (%)	exposure to 45·5° C (min.)	no. of larvae used	no.	Sig. χ^2 test	no.
60	2	20	15	↑	0
*80	2	60	29	− ↑	5 (D.L.)
90	2	20	10	− − ↑	6 (D.L.)
60	3	40	10	× + − ↑	6 (D.L.)
90	3	70	16	× × + −	2 (D.L.)
80	5	30	0	↑	0
90	5	30	0	− ↑	0
90	10	30	0	− − ↑	0
80	15	40	0	− − −	0

All the third-instar larvae of *X. cheopis* (table 34), containing blood in their gut, subjected to 45·5° C for 2 or 3 min., revived after 15 min. exposure to favourable conditions. The fully fed larvae died within a day; but a few which had not emptied the gut completely lived more than a day, dying in the resting stage, except in one experiment, marked with an asterisk, in which three spun cocoons and became adults after 15 and 18 days. The prolonged cocoon life of these few adults is attributed to a retarding effect of short exposure to high temperature, as happens in other insects (see Uvarov 1931, p. 19).

TABLE 35. SHOWING THE FATAL EFFECT OF A TEMPERATURE OF 45·5 ± 0·3° C WITH A RELATIVE HUMIDITY OF 80 % ON THE ACTIVE THIRD-INSTAR LARVAE (GUT EVACUATED) OF *XENOPSYLLA CHEOPIS*

		larvae revived on subsequent exposure to 26 to 29° C and 90 % R.H.				
		after ½ hr.		after 24 hr.		
exposure to 45·5° C (min.)	no. of larvae used	no.	Sig. χ^2 test	no.	Sig. χ^2 test	no. of larvae died in the resting stage
2	10	10	↑	6	↑	4
3	20	20	– ↑	8	– ↑	20
4	60	17	× × ↑	17	– – ↑	60
5	20	0	× × +	0	× × +	20

All the fully fed larvae of *X. cheopis* which had emptied the gut (table 35), when exposed to 45·5° C for 2 min., revived quickly, and 60 % of them grew into adults after 12 days. But 3 min. at this high temperature stopped the further development, though some survived more than a day.

Exposure to 45·5° C for 5 min. was immediately fatal to all the larval instars of *X. cheopis*. In similar experiments it was found that fully fed larvae after the gut is empty are rather more resistant to these high temperatures than either recently hatched larvae or those in the third instar which are still feeding.

When exposed to 45·5° C for 1 min., almost all the early naked pupae of the three species (table 36) showed signs of subsequent growth, and many emerged successfully; even 2 min. exposure was not completely fatal, as a very few were subsequently reared.

TABLE 36. SHOWING THE FATAL EFFECT OF A TEMPERATURE OF 45·5 ± 0·3° C WITH EFFECTIVE HUMIDITIES ON THE EARLY NAKED PUPAE OF THE THREE SPECIES OF *XENOPSYLLA*

					subsequent growth on 18 to 27 days' exposure to 26 to 29° C and 90 % R.H.			advanced pupae formed	
						pupae died			
species	R.H. (%)	exposure to 45·5° C (min.)	age of pupae (days)	no. of pupae used	adults emerged	E.P.	A.P.	no.	Sig. χ^2 test
cheopis	80	1	1–2	40	29	4	7	36	↑
brasiliensis	80	1	1–2	15	6	3	6	12	– ↑
brasiliensis	90	1	1–2	20	14	0	6	20	– – ↑
astia	80	1	2–3	20	15	2	3	18	– – –
cheopis	90	2	1–2	20	1	15	4	5	↑
brasiliensis	80	2	2	18	1	12	5	6	– ↑
astia	80	2	2	20	1	4	15	16	× ×
cheopis	80	3	1–2	20	0	20	0	0	↑
brasiliensis	90	3	2	20	0	20	0	0	– ↑
astia	90	3	2	25	0	25	0	0	– –
cheopis	90	4	4	20	0	20	0	0	↑
brasiliensis	90	4	2	36	0	36	0	0	–
cheopis	80	5	1–2	20	0	20	0	0	↑
brasiliensis	90	5	2	20	0	20	0	0	– ↑
astia	90	5	2	22	0	22	0	0	– –

In the latter case the pupae of *X. astia* showed subsequent growth more frequently than those of the other two species (differences highly significant). Subjection to 45·5° C for 3 or more min. prevented the subsequent growth of the pupae of the three species.

The stages within the cocoons (whether larvae or pupae), when exposed to 45·5° C for 1 or 2 min., were subsequently reared into adults in all species (table 37); exposure for 3 min. was generally fatal, but not always so. The absence of any material difference between the ultimate and the initial percentages of pupation within the cocoons of *X. cheopis* and *X. brasiliensis*, when subjected to 45·5° C for 3 min., indicates that this exposure had affected the resting larvae more adversely than the pupae, as the larvae failed to pupate; but such was not the case with *X. astia*.*

TABLE 37. SHOWING THE FATAL EFFECT OF A TEMPERATURE OF 45·5 ± 0·3° C WITH DIFFERENT HUMIDITIES ON THE LARVAE AND THE PUPAE WITHIN THE COCOONS OF THE THREE SPECIES OF *XENOPSYLLA*

					subsequent growth on 10 to 50 days' exposure to 26 to 29° C and R.H. 90%						
						stages died within cocoons					
species	R.H. (%)	exposure to 45·5° C (min.)	age of cocoons (days)	no. of cocoons used	adults emerged	R.L.	E.P.	A.P.	% of ultimate pupation	probable % of initial pupation	signs of growth
cheopis	80·0	1	2	30	30	0	0	0	100	0	+
brasiliensis	60·0	1	4	45	45	0	0	0	100	60	+
astia	80·0	1	5	60	59	1	0	0	98·3	100	+
cheopis	80·0	2	3–4	72	72	0	0	0	100	40	+
brasiliensis	90·0	2	3–4	72	70	0	1	1	100	40	+
astia	90·0	2	5	40	39	0	0	1	100	100	+
cheopis	80·0	3	3–4	40	14	25	0	1	37·5	40	+
cheopis	90·0	3	4	43	20	18	3	2	58·1	60	+
brasiliensis	80·0	3	4	40	10	19	3	8	52·5	60	+
brasiliensis	90·0	3	3–4	40	2	22	5	11	45·0	40	+
**astia*	90·0	3	3–4	42	24	1	3	14	97·6	40	+
cheopis	80·0	4	3–4	20	2	13	2	3	35·0	40	+
cheopis	90·0	4	3	40	0	29	6	5	27·5	25	+
brasiliensis	90·0	4	4–5	80	0	16	64	0	80·0	80	–
astia	90·0	4	4	69	20	0	8	41	100	60	+
cheopis	80·0	5	2–3	40	0	36	4	0	10·0	10	–
cheopis	90·0	5	5	60	0	3	57	0	95·0	100	–
brasiliensis	90·0	5	4–5	80	0	20	60	0	75·0	80	–
astia	80·0	5	4	30	0	11	19	0	63·3	60	–
astia	90·0	5	5	50	0	0	50	0	100	100	–

Subjection of stages within the cocoons (table 37) for 4 min. to 45·5° C demonstrated specific differences in heat resistance. It put an end to the subsequent growth of both the larvae and the pupae within the cocoons of *X. brasiliensis*; the evidence for this statement is the absence of any material difference between the ultimate and the initial percentages of pupation, and also the lack of any sign of growth in the pupae. In *X. cheopis* signs of subsequent growth were found in a few of the pupae within cocoons; they contained fully formed adults, though most of them failed to extricate themselves from the pupal skins. An even better subsequent development of stages within the cocoons of *X. astia* occurred than in *X. cheopis*, as there was no mortality even in the larval stage. But an exposure of 5 min. to 45·5° C was fatal to both the larvae and the pupae within the cocoons of the three species, and inhibited all subsequent growth; as it required only 3 min. to produce the same effect in larvae ready to spin cocoons, or on naked pupae (tables 35, 36), one concludes that it takes 2 min. to attain this temperature within cocoons.

---

* In an experiment, marked with an asterisk, two elongated and deformed adults were formed within the larval skins.

XII. Dormancy

Hibernation, aestivation or diapause has never been recorded in any stage of the three species of rat-flea (*Xenopsylla*). They are holodynamic, and reproduction can continue in them indefinitely generation after generation.

The fact that the larvae and the pupae of these rat-fleas died quickly at fatal low temperatures (see p. 612) and fatal humidities (see p. 597) also suggests that hibernation and aestivation cannot occur in them. In *Nosopsyllus fasciatus*, however, Bacot (1914, pp. 474, 539, 610) has stated that both of these occur in larvae within 'hard cocoons'. In my view the evidence is inconclusive, in the light of modern work. As hard and soft cocoons were formed under more or less identical conditions in his experiments, it is suggested that the larval food had at first a poor nutritive value, which gradually improved on longer exposure to the moist air. Consequently, two types of cocoons—hard and soft—were formed, depending on the quality of the food eaten by the larvae; it will be remembered (see p. 591) that robust flea larvae reared at favourable humidities spin hard cocoons and weak larvae reared at low humidities spin soft ones. The food factor and Bacot's defective control of humidity and temperature (which often varied about the critical borderline) resulted in different developmental rates, and showed many peaks in the curves of adult emergence (pp. 538–543). In view of these defects in method, natural enough in the work of a pioneer, I cannot accept Bacot's evidence for aestivation and hibernation.

In my extensive experience, diapause does not occur in the three species of rat-fleas. For about 10 years I have kept flourishing stocks at room temperature and humidity of Bombay; over fifty flea-breeding jars were maintained, and in most of them fleas have continued breeding. The population of *X. cheopis* remained abundant throughout; but that of *X. brasiliensis* decreased considerably in the hot months of April to June, and that of *X. astia* in the cold months of December to February. It is suggested that, at least in the Bombay area, these fleas can breed throughout the year under the equable climatic conditions in deep burrows well protected against the sun. In superficial burrows the fleas can perhaps only continue to breed as long as the conditions remain favourable; at low humidity and unfavourable low or high temperature the arrest of development is likely to be only temporary.

XIII. Summary

In India there are three closely similar species of rat-fleas (*Xenopsylla cheopis*, *X. brasiliensis* and *X. astia*) feeding on the same host. They differ in abundance and distribution, in space and season. This may in part be due to different reaction to temperature and humidity. Flea counts (i.e. the number of fleas per rat, under certain standard conditions) are generally thought valuable for assessing rat-flea populations. This method is open to objection, as the number of fleas per rat has little relation to their actual population. In this paper I have subjected the larvae and the pupae of three Indian species of rat-fleas to controlled conditions in order to ascertain their effects on these insects. As these stages are exposed in nature to equable climatic conditions, I only determined the effects of constant temperatures and humidities. For the normal breeding of flea larvae the presence of soil or debris is necessary.

Larval food, comprising blood and yeast, underwent diverse changes at different humidities, which affected flea breeding materially. It appeared that at low effective humidities much food was consumed by larvae in order to gain more metabolic water so as to compensate for an excessive water loss through evaporation. The food increased in volume at optimum and high humidities. The ill effect of high humidities on early stages of fleas was solely due to the food; larvae and pupae died owing to the formation of a crust and a compact mass, and when the food and sand mixture became soggy; this could be avoided by disturbing the mixture so as to allow free access of air. I attribute the paucity of fleas in the burrows of *Millardia meltada* and *Gunomys kok* to the presence of excessive moisture and debris rich in organic material, abundance of fleas in those of *Tatera indica* to the absence of excessive moisture; I believe that the moderate flea infestation generally found in the burrows of domestic rats is due to the absence of excessive moisture and much organic material. Fungus by itself never interfered with rearing fleas; indeed it appeared to serve as a food for their larvae.

Cocoon formation seemed to depend on the capacity of the full-grown larva, after emptying the gut, to part with water for silk secretion: this was inhibited at critical temperatures and very low effective humidities; under these conditions, it seems that evaporation was so intense as to reduce the amount of water available in the larva. The size and texture of cocoons were controlled by the air moisture; cocoons spun at low humidities were small, soft and loose, whilst those formed at optimum and high humidities were large, hard and with close meshes. At high humidities the presence of food in the cocoon wall caused death of the insect inside, apparently from suffocation. The protection afforded by the cocoon against fatal desiccation and extreme effective temperatures was limited but definite.

For the larvae of *Xenopsylla cheopis* and *X. brasiliensis*, 45% R.H. was just below the critical limit at 22 and 27° C, 50% R.H. at 17 and 32° C, and 60% R.H. at 13 and 35° C; for those of *X. astia* 50% R.H. at 22 to 32° C, and 60% R.H. at 17, 35 and 38° C. The effective humidity range for larvae varied according to the species, being much narrower in *X. astia* and slightly wider in *X. cheopis* than in *X. brasiliensis*; it also varied with the temperature, being narrower at extreme effective temperatures than at the medial ones. My figures show that the limits of favourable humidity cannot be defined by some simple physical factor, such as a particular value of saturation deficiency.

The size of adults was influenced by the humidity at which larvae were bred, being greatest at optimum humidities. The most important factor for the development of rat-flea larvae is the moisture content of the diet and solid surroundings rather than that of the atmosphere of the burrow.

At low humidities the larval development was greatly retarded; only those larvae and pupae that remained within cocoons developed successfully, and there was a variable mortality due to variation in cocoon formation. At the lowest effective humidity, even the development of stages within cocoons was adversely affected. The development of larvae was quickest and their mortality least at optimum humidities; these have no adverse effect on the development of resting larvae and pupae without cocoons. The optimum humidity range for development of the larvae of *X. cheopis* and *X. brasiliensis* approximated to 90% R.H. at 13, 17 and 35° C and 80 to 90% R.H. at 22 to 32° C, and of those of *X. astia* 90% R.H. at 17, 22, 35 and 36·5° C, and 80 to 90% R.H. at 27 to 32° C.

In rearing flea larvae at the high humidities of 97·5 and 100% discordant results were obtained, attributed to unequal disturbance of food and sand mixture; when often disturbed, there was no retardation in development. A high humidity, however, was itself unfavourable to larval growth at very high, effective temperatures.

Recently hatched larvae on subjection to 2° C for 1 to 3 days or to 0° C for 1 day showed specific differences in their resistance to cold: the resistance was least in *X. astia* and greatest in *X. cheopis*. All such larvae of *X. astia*, *X. brasiliensis* and *X. cheopis* were killed at 2° C in 2, 3 and 5 days respectively; at 0° C those of *X. astia* were killed in 2 days and of the other species in 3 days. The larvae and the pupae within cocoons and the naked pupae of *X. cheopis* were more resistant to cold than those of *X. brasiliensis*, as it required 6 days to kill all in *X. cheopis* and 4 days in *X. brasiliensis* at 2° C.

The developmental threshold of the larva was higher than that of the pupa; the difference was greatest in *X. astia*. The theoretical threshold for development of the larva was 12·2° C in *X. cheopis*, 11·7° C in *X. brasiliensis* and 13·1° C in *X. astia*, and that of the stages within cocoons was 11·8° C in the first species, 11·6° C in the second and 12·3° C in the third. The threshold was higher for the combined development of the larva and the pupa than for these stages separately, in each species.

The effective temperature range for the larva varied according to the species, apparently being 12 to 35·5° C in *X. brasiliensis*, 12 to 36·5° C in *X. cheopis* and 13·5 to 38° C in *X. astia*. The medial temperatures for their development varied from 17 to 29° C in the first species, 17 to 30° C in the second and 23 to 33·5° C in the third.

For the larvae and pupae of all species 45·5° C was very rapidly fatal. At this temperature all the recently hatched larvae of *X. brasiliensis*, *X. cheopis* and *X. astia* were killed in 4, 5 and 6 min. respectively; an exposure for 2 min. showed that resistance to this temperature was least in the first species and greatest in the third. The further development of the naked pupae of the three species (or of mature larvae of *X. cheopis* after evacuation of the gut) was stopped on 3 min. exposure to 45·5° C; but to produce the same effect in the stages within cocoons required 4 or 5 min., according to species.

Hibernation, aestivation or diapause does not occur in the larval or pupal stage of the three species.

It is, indeed, a pleasant duty to express my gratitude to Maj.-Gen. Sir Sahib Singh Sokhey, Director, Haffkine Institute, Bombay, for affording me many facilities in carrying out this work. Professor P. A. Buxton, F.R.S., has kindly read through and revised my manuscript and made many valuable suggestions, for which I am obliged. Messrs K. D. Gumaste, T. N. Raste and P. M. Salvekar have facilitated my work by their help in the compilation and statistical calculation of the data, for which I am thankful.

REFERENCES

Advisory Committee 1908 *J. Hyg., Camb.* **8**, 236–259.
Advisory Committee 1910 *J. Hyg., Camb.* **10**, 566–568.
Advisory Committee 1912 *J. Hyg., Camb.*, Plague Supplement, **2**, 300–325.
Ahmad, T. 1937 *Indian J. Agric. Sci.* **7**, 156–167.
Bacot, A. 1914 *J. Hyg., Camb.*, Plague Supplement, **3**, 447–654.

Bishopp, F. C. 1931 *Fmrs' Bull. U.S. Dep. Agric.* no. 897, pp. 1–16.

Buxton, P. A. 1932 *Biol. Rev.* **7**, 275–320.

Buxton, P. A. 1933 *Trans. R. Soc. Trop. Med. Hyg.* **26**, 325–356.

Buxton, P. A. 1938 *Indian J. Med. Res.* **26**, 505–530.

Buxton, P. A. & Mellanby, K. 1934 *Bull. Ent. Res.* **25**, 171–175.

Cragg, F. W. 1921 *Indian J. Med. Res.* **9**, 374–397.

Cragg, F. W. 1923 *Indian J. Med. Res.* **10**, 953–961.

Cragg, F. W. & Swaminath, C. S. 1923 *Indian J. Med. Res.* **10**, 979–989.

Edney, E. B. 1945 *Bull. Ent. Res.* **35**, 399–416.

Edney, E. B. 1947 *Bull. Ent. Res.* **38**, 389–404.

Evans, A. C. 1935 *Parasitology*, **27**, 291–298.

Ewing, H. E. 1931 *Amer. Nat.* **65**, 360–369.

Fisher, R. A. 1941 *Statistical methods for research workers*, 8th ed. Edinburgh and London: Oliver and Boyd.

Fisher, R. A. & Yates, F. 1943 *Statistical tables for biological, agricultural and medical research*, 2nd ed. London and Edinburgh: Oliver and Boyd.

Fraenkel, G. & Blewett, M. 1943 *Trans. R. Ent. Soc. Lond.* **93**, 457–490.

Fraenkel, G. & Blewett, M. 1944 *Bull. Ent. Res.* **35**, 127–139.

Hirst, L. F. 1926 *Ceylon J. Sci.* (D), **1**, 155–271.

Hirst, L. F. 1927 *Ceylon J. Sci.* (D), **1**, 277–455.

Hopkins, G. H. E. 1935 *Parasitology*, **27**, 480–488.

Imms, A. D. 1937 *Recent advances in entomology*, 2nd ed. London: J. & A. Churchill.

Ingram, A. 1927 *Publ. S. Afr. Inst. Med. Res.* **20**, 222–256.

Leeson, H. S. 1932 *Bull. Ent. Res.* **23**, 25–31.

Leeson, H. S. 1936 *Parasitology*, **28**, 403–409.

Mellanby, K. 1932*a* *J. Exp. Biol.* **9**, 222–231.

Mellanby, K. 1932*b* *Parasitology*, **24**, 419–428.

Mellanby, K. 1933 *Bull. Ent. Res.* **24**, 197–202.

Mellanby, K. 1935 *Biol. Rev.* **10**, 317–333.

Sharif, M. 1937 *Parasitology*, **29**, 225–238.

Shelford, V. E. 1930 *Laboratory and field ecology*, 2nd ed. Baltimore: Williams & Wilkins Co.

Sikes, E. K. 1930 *Parasitology*, **22**, 242–259.

Sikes, E. K. 1931 *Parasitology*, **23**, 243–249.

Ullyett, G. C. 1936 *Bull. Ent. Res.* **27**, 195–217.

Uvarov, B. P. 1931 *Trans. R. Ent. Soc. Lond.* **79**, 1–247.

Webster, W. J. 1930 *Indian J. Med. Res.* **18**, 391–405.

Webster, W. J. & Chitre, G. D. 1930*a* *Indian J. Med. Res.* **18**, 337–345.

Webster, W. J. & Chitre, G. D. 1930*b* *Indian J. Med. Res.* **18**, 407–425.

Wigglesworth, V. B. 1932 *Quart. J. Micr. Sci.* **75**, 131–150.

Wigglesworth, V. B. 1939 *The principles of insect physiology*. London: Methuen & Co.

Key to abbreviations used in the tables

A.P., advanced pupae, which contained fully formed adults of brown colour.

D.L., defaecated larvae, which are white, third-instar, active larvae after the expulsion of food from their gut.

E.P., early pupae, which are of white colour without any trace of adult formation.

Pr., prepupae, which represent an ill-defined transitional stage between the resting larva and the pupa, having all the underdeveloped pupal parts but the larval shape.

R.H. (%), relative humidity percentage.

R.L., resting larvae, which are immobilized, defaecated, third larval instars ready for pupation.

Sig. t test, t test of significance.

Sig. χ^2 test, χ^2 test of significance.

−, not significant in significant test columns, and absent in others.

+, significant at 5 % level in significant test columns, and present in others.

×, significant at 1 % level.

TABLE 23. SHOWING THE PROPORTIONS OF CERTAIN STAGES IN THE LIFE CYCLE, INTO WHICH THE RECENTLY HATCHED LARVAE OF THE THREE SPECIES OF *XENOPSYLLA* GREW, WHEN MAINTAINED AT CERTAIN TEMPERATURES AND HUMIDITIES

species	temp. (° C)	R.H. (%)	no. of larvae used	larvae spun cocoons		larvae reached the adult stage		no. of naked resting larvae	naked resting larvae formed pupae		naked resting larvae reached the adult stage		stages died			remarks
				no.	Sig. χ^2 test	no.	Sig. χ^2 test		no.	Sig. χ^2 test	no.	Sig. χ^2 test	R.L.	E.P.	A.P.	
cheopis	17·0	60·0	251	135	↑ ↑	113	↑ ↑	†134	110	↑	0	↑	24	90	20	4 larvae died early
[illegible]	17·0	80·0	164	43	× ↑	89	− ↑	121	115	× ↑	46	× ↑	6	12	57	larvae disturbed to prevent cocoon formation
	17·0	90·0	130	9	× × ↑	121	× × ↑	121	118	× − ↑	112	× × ↑	3	2	4	
	17·0	90·0	156	132	× × × ↑	[illegible]	× × − ↑	24	20	− − + ↑	15	× + × ↑	4	0	5	
	17·0	97·5	151	145	× × × × ↑	145	× × − − ↑	6	6	− − − − ↑	0	− − × + ↑	0	6	0	
	17·0	100	117	57	− × × × × ↑	12	× × × × × ↑	60	44	− × × − − ↑	0	− × × × − ↑	16	—	—‡	
	17·0	100	114	100	× × × − + ×	7	× × × × × −	14	12	− − − − − −	0	− + × × − −	2	—	—‡	
nsis	17·0	60·0	192	88	↑ −	65	↑ +	†60	51	↑	0	↑	9	42	9	67 larvae died in different active instars
nsis	17·0	80·0	157	75	− ↑	99	× ↑	82	82	× ↑	24	× ↑	0	22	36	larvae disturbed to prevent cocoon formation
nsis	17·0	90·0	154	36	× × ↑	150	× × ↑	118	118	× − ↑	114	× × ↑	0	0	4	
nsis	17·0	90·0	110	83	× × × ↑	105	× × − ↑	†28	27	− − − ↑	23	× × + ↑	1	0	4	1 larva left cocoon and died
nsis	17·0	97·5	152	134	× × × + ↑	152	× × − + ↑	18	18	− − − − ↑	18	× × − − ↑	0	0	0	
nsis	17·0	100	113	101	× × × + − ↑	26	− × × × × ↑	8	8	− − − − − ↑	0	− − × × × ↑	—	—	—‡	4 larvae died early
nsis	17·0	100	146	98	× × × − × ×	0	× × × × × ×	27	27	− − − − − −	0	− × × × × −	—	—	—‡	21 not disturbed after cocoon formation
	17·0	80·0	235	150	↑	146	↑	†81	62	↑	0	↑	19	37	25	8 larvae died in third instar
	17·0	90·0	137	133	× ↑	135	× ↑	†5	4	− ↑	3	× ↑	1	0	1	1 larva left cocoon and died
	17·0	97·5	105	94	× + ↑	97	× + ↑	8	8	− − ↑	3	× − ↑	0	4	1	3 larvae died early
	17·0	100	110	55	+ × × ↑	19	× × × ↑	8	0	× + ×	0	− − −	8	—	—‡	47 larvae died early
	17·0	100	36	25	− × × −	21	− × × ×	0	0		0		—	—	—‡	11
	22·0	50·0	70	24	↑	12	↑	†21	12	↑	0	↑	9	12		12 37 larvae died in 4 days
	22·0	60·0	173	167	× ↑	166	× ↑	†7	6	− ↑	0	− ↑	1	6		1 larvae left cocoons and pupated
	22·0	80·0	208	202	× − ↑	206	× − ↑	6	6	− − ↑	4	× + ↑	0	2		
	22·0	90·0	172	154	× + × ↑	166	× − − ↑	12	12	+ − − ↑	12	× × − ↑	0	0	0	6 larvae died early
	22·0	97·5	175	172	× − − ×	172	× − − −	1	1	− − − −	1	+ − − −	0	0	0	2
nsis	22·0	60·0	148	140	↑	139	↑	9	7	↑	0	↑	2	7	0	
nsis	22·0	80·0	145	142	− ↑	137	− ↑	7	5	− ↑	0	− ↑	2	5	0	1 active third-instar larva died
nsis	22·0	90·0	90	90	− − ↑	89	− − ↑	†1	0	− −	0	− −	1	0	0	
ensis	22·0	97·5	95	90	− − −	80	+ + ×	0	0		0		0	10		5 active larvae and 10 pupae within cocoons died
	22·0	60·0	120	104	↑	87	↑	†28	12	↑	0	↑	16	12		5 larvae died early
	22·0	80·0	96	96	× ↑	94	× ↑	†2	2	− ↑	0	− ↑	0	0	2	
	22·0	90·0	130	129	× − ↑	129	× − ↑	1	1	− − ↑	0	− − ↑	0	0	1	
	22·0	97·5	110	108	× − − ↑	107	× − − ↑	†3	1	− − − ↑	0	− − − ↑	2	0	1	
	22·0	97·5	30	30	− − − −	28	+ − − −	†2	2	− − − −	0	− − − −	0	0	2	
	27·0	50·0	60	31	↑	24	↑	†10	7	↑	0	↑	3	7		26 larvae died early
	27·0	50·0	140	140	× ↑	140	× ↑	0	0		0		0	0	0	
	27·0	60·0	158	158	× − ↑	158	× − ↑	0	0		0		0	0	0	
	27·0	80·0	102	101	× − − ↑	102	× − − ↑	1	1	−	1	−	0	0	0	
	27·0	90·0	117	117	× − − − ↑	117	× − − − ↑	0	0		0		0	0	0	
	27·0	94·8	150	150	× − − − − ↑	150	× − − − − ↑	0	0		0		0	0	0	
	27·0	100	160	160	× − − − − −	0	× × × × × ×	0	0		0		—	—	—‡	
ensis	27·0	50·0	60	14	↑	14	↑	8	7	↑	0	↑	1	7		38 active larvae died in different instars
ensis	27·0	50·0	132	88	× ↑	74	× ↑	†34	29	− ↑	0	− ↑	5	29		24
ensis	27·0	60·0	138	130	× × ↑	128	× × ↑	†4	3	− − ↑	0	− − ↑	1	3		6 larvae died early
ensis	27·0	80·0	131	131	× × + ↑	129	× × + ↑	†2	2	− − −	0	− − −	0	2		
ensis	27·0	90·0	117	115	× × − − ↑	115	× × − − ↑	0	0		0		0	0	0	2 larvae died early
ensis	27·0	94·8	25	25	× × − − − ↑	25	× × − − − ↑	0	0		0		0	0	0	
ensis	27·0	100	30	30	× × − − − − ↑	30	× × − − − − ↑	0	0		0		0	0	0	1 days' initial exposure of food to the humidity
ensis	27·0	100	25	7	− × × × × × ×	0	+ × × × × × ×	—	—		—		—	—	—‡	7
	27·0	60·0	127	119	↑	119	↑	4	0		0		4	0	0	4 larvae died in first instar
	27·0	80·0	136	136	× ↑	136	× ↑	0	0		0		0	0	0	
	27·0	90·0	148	148	× − ↑	147	+ − ↑	0	0		0		1	0	0	1 larva died within cocoon
	27·0	94·8	20	19	− − −	19	− − −	0	0		0		0	0	0	1 larva died early
s	29·0	60·0	143	143	↑	140	↑	†3	0		0		3	0	0	
s	29·0	90·0	213	213	−	213	−	0	0		0		0	0	0	
ensis	29·0	60·0	150	150	↑	147	↑	†3	0		0		3	0	0	
ensis	29·0	90·0	130	130	−	130	−	0	0		0		0	0	0	
	29·0	60·0	85	67	↑	64	↑	†4	0	↑	0	↑	4	0	0	17 larvae died early
	29·0	90·0	90	90	×	88	×	†2	2	−	0	−	0	0	2	
s	32·0	60·0	159	157	↑	156	↑	1	1	↑	0	↑	0	1	0	2 second instar larvae died
s	32·0	80·0	150	148	− ↑	148	− ↑	0	0		0		0	0	0	2
s	32·0	90·0	132	132	− − ↑ ↑	132	− − ↑ ↑	0	0		0		0	0	0	fungus growth
s	32·0	97·5	163	161	− − − ↑	160	− − − ↑	3	1	−	0	−	2	1	0	
s	32·0	100	161	161	− − − −	99	× × × ×	0	0		0		0	62		
ensis	32·0	60·0	121	106	↑	98	↑	†16	9	↑	0	↑	7	9		7 larvae died early
ensis	32·0	80·0	173	170	× ↑	170	× ↑	1	0	− ↑	0	− ↑	1	0		2
ensis	32·0	97·5	124	124	× − ↑	122	× − ↑	0	0		0		0	2		2 pupae died within cocoons
ensis	32·0	100	183	178	× − −	101	× × ×	†5	5	− −	0	− −	0	77		
	32·0	60·0	136	75	↑	63	↑	†12	2	↑	0	↑	10	0	2	61 larvae died early
	32·0	60·0	86	12	× ↑	12	× ↑	1	0	− ↑	0	− ↑	1	0	0	73
	32·0	80·0	143	141	× × ↑	142	× × ↑	2	1	− − ↑	1	− − ↑	1	0	0	
	32·0	97·5	169	164	× × − ↑	164	× × − ↑	0	0		0		0	0	0	5 larvae died early
	32·0	100	160	156	× × − −	156	× × − −	4	4	+ − −	0	− − −	0	4		food disturbed daily
s	35·0	80·0	178	166	↑	94	↑	†33	19	↑	0	↑	21	13	37	1 second- and 2 third-instar larvae died
s	35·0	90·0	154	148	− ↑ −	104	× ↑ ×	6	6	− ↑	0	− ↑	6	16	28	
s	35·3	80·0	105	92	− + ↑	46	− × ↑	13	1	× × ↑	0	− − ↑	27	6	26	
s	35·3	97·5	160	76	× × × ↑	3	× × × ↑	84	2	× × −	0	− − −	—	—	—‡	increase in pressure killed different stages from second larval instar to adult
s	35·3	100	151	79	× × × −	1	× × × −	—	—		—		—	—	—‡	
	35·0	80·0	154	153	↑	154	↑	1	1	↑	1	↑	0	0	0	
	35·0	90·0	148	145	− ↑	148	− ↑	3	3	− ↑	3	− ↑	0	0	0	
	35·0	100	94	93	− − ↑	67	× × ↑	1	1	− − ↑	0	− − ↑	0	0	27	
	35·0	100	80	75	+ − − ↑	0	× × × ↑	5	5	− − − ↑	0	− + − ↑	—	—	—‡	no adult emergence due to rise in temperature and pressure
	35·0	97·5	80	78	− − − − ↑	0	× × × − ↑	2	2	− − − −	0	− − − −	—	—	—‡	
	35·0	97·5	80	0	× × × × × ↑	0	× × × − − ↑	0	0		0		0	0	0	increase in pressure killed all the larvae in third instar
	35·0	100	120	0	× × × × × −	0	× × × − − −	0	0		0		0	0	0	
	36·5	80·0	40	12	↑	8	↑	†2	2	↑	0	↑	0	2	2	2 pupae within cocoons and 28 larvae died
	36·5	90·0	40	34	× ↑	17	− ↑	6	2	−	0	−	8	8	7	
	36·5	97·5	40	20	− × ↑	12	− − ↑	—	—		0		—	—	—‡	all larvae grew
	36·5	100	40	4	− × ×	0	× × ×	6	—		0		—	—	—‡	crust killed 25 larvae in 3 days

† Wherever the combined total of the cocoons and the naked resting and active larvae exceeds the number of the larvae used, it is due to the defaecated larvae having left the cocoons.
‡ Different stages could not be determined due to clod formation and sogginess of the food and sand mixture.

TABLE 24. SHOWING THE DURATIONS OF CERTAIN STAGES IN THE LIFE CYCLE, INTO WHICH THE RECENTLY HATCHED LARVAE OF THE THREE SPECIES OF *XENOPSYLLA* GREW, WHEN MAINTAINED AT CERTAIN TEMPERATURES AND HUMIDITIES (CONTINUATION OF EXPERIMENTS IN TABLE 23)

ies	temp. (° C)	R.H. (%)	no. of larvae spun cocoons and duration of the active larval life				no. of cocoons from which adults emerged and duration of the resting larval and pupal life				no. of ♀♀ and duration of their combined larval and pupal life				no. of ♂♂ and duration of their combined larval and pupal life			
				days				days				days				days		
			larvae	range	mean	Sig. *t* test	cocoons	range	mean	Sig. *t* test	♀♀	range	mean	Sig. *t* test	♂♂	range	mean	Sig. *t* test
	17·0	60·0	135	31–47	37·27	↑	113	29–39	34·15	↑	46	62–69	64·89	↑	67	71–81	75·63	↑
	17·0	80·0	43	23–36	30·02	× ↑	—	—	—		68	51–65	56·04	× ↑	21	67–75	69·95	× ↑
	17·0	90·0	9	33–41	36·33	− × ↑	—	—	—		61	53–63	56·92	× − ↑	60	65–73	68·35	× × ↑
	17·0	90·0	132	23–39	28·38	× + × ↑ ↑	132	28–43	34·80	− ↑ ↑	72	52–64	57·14	× + − ↑ ↑	75	64–78	71·29	× − × ↑ ↑
	17·0	97·5	145	23–36	28·56	× × × − ↑	145	32–46	39·48	× ×	67	55–70	60·57	× × × × ↑	78	70–80	74·37	+ × × × ↑
	17·0	100	57	27–39	32·49	× × × × × ↑	—	—	—		9	59–74	64·44	− × × × × ↑	3	77–85	81·33	× × × × × ↑
	17·0	100	100	23–37	27·28	× × × + × ×	—	—	—		6	56–59	58·00	× − − − − ×	1	73	73·00	− − + − − −
nsis	17·0	60·0	88	30–44	37·70	↑	65	26–39	32·45	↑	36	57–69	64·75	↑	29	71–83	75·00	↑
nsis	17·0	80·0	75	25–38	31·16	× ↑	75	25–39	29·87	× ↑	59	52–62	56·24	× ↑	40	67–75	69·98	× ↑
nsis	17·0	90·0	36	29–36	33·03	× × ↑	—	—	—		71	51–63	57·32	× + ↑	79	65–74	68·14	× × ↑
nsis	17·0	90·0	83	25–34	29·88	× × × ↑ × ↑	82	26–42	†33·40	− × ↑ − ↑	58	51–65	58·33	× × − ↑ − ↑	47	66–76	†71·26	× + × ↑ − ↑
nsis	17·0	97·5	134	25–35	30·57	× − × − ↑	134	29–42	35·19	× × ×	84	55–67	61·08	× × × × ↑	68	70–76	73·38	× × × ×
nsis	17·0	100	101	25–37	30·68	× − × + − ↑	—	—	—		26	57–75	62·35	× × × × −	0	—	—	
nsis	17·0	100	98	27–39	33·05	× × − × × ×	—	—	—		0	—	—		0	—	—	
	17·0	80·0	150	30–50	38·89	↑	146	26–45	31·78	↑	75	58–72	64·37	↑	71	69–86	77·13	↑
	17·0	90·0	133	29–45	†35·32	× ↑ × ×	132	26–43	†33·34	× ↑ + −	75	56–70	61·77	× ↑ × ×	60	70–84	†77·57	− ↑ × ×
	17·0	97·5	94	29–45	38·57	− × ↑	94	26–41	33·26	× − ↑	52	59–72	66·10	+ × ↑	45	72–86	78·87	+ − ↑
	17·0	100	55	30–46	39·05	− × − ↑	—	—	—		15	60–77	68·27	× × − ↑	4	74–79	76·00	− − − ↑
	17·0	100	25	31–45	36·40	+ − + +	21	26–37	30·48	− + ×	17	59–72	64·18	− + − +	4	74–78	75·75	− − − −
	22·0	50·0	24	26–42	33·29	↑	12	15–21	19·00	↑	6	46–49	47·00	↑	6	51–55	52·67	↑
	22·0	60·0	167	13–20	16·04	× ↑	166	14–22	17·70	− ↑	78	28–33	30·08	× ↑	88	34–41	36·99	× ↑
	22·0	80·0	202	10–15	12·62	× × ↑	202	14–22	17·19	+ − ↑	109	24–31	26·55	× × ↑	97	32–36	34·01	× × ↑
	22·0	90·0	154	10–15	12·33	× × + ↑ ↑	154	13–22	17·16	+ − − ↑ ↑	105	24–30	27·20	× × × ↑ ↑	61	31–37	34·48	× × × ↑ ↑
	22·0	97·5	172	10–17	12·08	× × × −	172	14–22	17·84	− − + +	96	24–31	26·91	× × − −	76	31–36	33·74	× × − ×
nsis	22·0	60·0	140	14–20	16·99	↑	139	13–21	17·24	↑	80	29–36	31·51	↑	59	36–41	37·88	↑
nsis	22·0	80·0	142	11–16	13·28	× ↑	137	14–24	†18·50	× ↑	72	25–32	27·72	× ↑	65	34–39	†36·15	× ↑
nsis	22·0	90·0	90	11–17	13·62	× − ↑ × ↑	89	14–24	19·24	× − ↑ × ↑	43	26–31	28·21	× − ↑ × ↑	46	34–40	37·20	+ × ↑ × ↑
nsis	22·0	97·5	90	11–19	15·18	× × ×	80	15–27	19·51	× + −	40	26–35	30·15	× × ×	40	32–46	38·90	+ × ×
	22·0	60·0	104	18–28	22·20	↑	87	13–22	16·83	↑	56	33–40	36·23	↑	31	40–48	42·77	↑
	22·0	80·0	96	13–19	15·30	× ↑	94	13–23	17·49	− ↑	48	26–31	28·83	× ↑	46	34–40	†36·85	× ↑
	22·0	90·0	129	11–17	†13·66	× × ↑ × −	129	13–21	†17·30	− − ↑ − ×	68	25–30	26·94	× × ↑ − ×	61	33–37	35·44	× × ↑ × ×
	22·0	97·5	108	11–17	14·48	× × × ↑	107	13–24	17·18	− − − ↑	58	25–33	27·69	× × × ↑	49	33–41	36·33	× − × ↑
	22·0	97·5	30	15–23	17·90	× × × ×	28	14–20	17·00	− − − −	15	29–35	32·20	× × × ×	13	37–40	37·85	× + × ×
	27·0	50·0	31	15–27	21·81	↑	24	12–18	14·50	↑	15	31–36	33·80	↑	9	38–43	38·67	↑
	27·0	50·0	140	12–20	15·33	× ↑	140	9–16	12·31	× ↑	76	23–27	25·29	× ↑	64	28–35	30·44	× ↑
	27·0	60·0	158	8–14	10·22	× × ↑	158	9–15	11·91	× + ↑	86	18–22	20·31	× × ↑	72	23–26	24·28	× × ↑
	27·0	80·0	101	7–11	8·71	× × × ↑	101	8–15	11·10	× × × ↑	62	15–20	18·00	× × × ↑	40	21–26	22·65	× × × ↑
	27·0	90·0	117	7–11	8·51	× × × − ↑ ↑	117	9–15	12·19	× − − × ↑ ↑	64	17–20	18·64	× × × × ↑ ↑	53	21–25	23·19	× × × − ↑ ↑
	27·0	94·8	150	7–11	8·63	× × × − − ↑	150	9–15	12·47	× − × × −	81	17–20	18·64	× × × × −	69	21–24	23·65	× × × × +
	27·0	100	160	7–10	8·38	× × × + − +	0	—	—		0	—	—		0	—	—	
nsis	27·0	50·0	14	20–23	21·29	↑	14	11–16	13·43	↑	11	33–34	33·82	↑	3	38	38·00	↑
nsis	27·0	50·0	88	16–24	20·74	− ↑	74	11–15	12·35	× ↑	50	27–34	31·34	× ↑	24	35–38	35·83	× ↑
nsis	27·0	60·0	130	11–17	12·85	× × ↑	128	9–15	11·52	× × ↑	78	20–25	22·95	× × ↑	50	26–29	26·76	× × ↑
nsis	27·0	80·0	131	8–14	9·95	× × × ↑	129	10–16	12·82	− − × ↑	70	18–22	20·24	× × × ↑	59	24–27	25·69	× × × ↑
nsis	27·0	90·0	115	8–11	9·27	× × × × ↑ × ↑	115	9–16	†11·99	× − + × ↑ − ↑	76	17–20	19·36	× × × × ↑ × ↑	39	24–26	†24·90	× × × × ↑ × ↑
nsis	27·0	94·8	25	8–10	9·08	× × × × − ↑	25	11–16	12·52	− − × − − ↑	18	19–22	20·11	× × × − × ↑	7	24–26	25·43	× × × − − ↑
nsis	27·0	100	30	8–11	10·13	× × × − × × ↑	30	10–14	11·87	× − − + − −	18	19–22	20·17	× × × − × −	12	24–25	24·75	× × × × − −
nsis	27·0	100	7	10–12	10·86	× × × − × × −	0	—	—		0	—	—		0	—	—	
	27·0	60·0	119	11–17	13·62	↑	119	9–14	10·81	↑	70	20–25	22·74	↑	49	25–29	26·82	↑
	27·0	80·0	136	7–12	10·16	× ↑	136	9–15	11·77	× ↑	73	16–22	19·40	× ↑	63	23–27	24·87	× ↑
	27·0	90·0	148	7–12	9·89	× − ↑ × ×	147	9–15	†11·17	− + ↑ × ×	98	16–22	18·99	× + ↑ − +	49	24–27	†25·14	× − ↑ × −
	27·0	94·8	19	8–11	9·26	× × +	19	9–15	12·00	× − −	11	18–19	18·91	× − −	8	24–26	24·50	× − −
	29·0	60·0	143	8–12	10·03	↑	140	8–14	10·09	↑	70	17–20	18·31	↑	70	21–25	21·87	↑
	29·0	90·0	213	6– 9	7·32	× ↑	213	8–13	10·27	− ↑	126	14–18	15·73	× ↑	87	19–22	20·26	× ↑
nsis	29·0	60·0	150	9–14	11·35	↑	147	9–13	10·08	↑	78	18–21	19·59	↑	69	21–26	23·12	↑
nsis	29·0	90·0	130	7–10	8·30	× × ↑	130	9–13	†10·66	× + ↑	68	16–20	16·91	× × ↑	62	20–22	†21·19	× × ↑
	29·0	60·0	67	11–15	12·45	↑	64	7–13	9·75	↑	22	18–21	19·77	↑	42	22–27	23·31	↑
	29·0	90·0	90	7–11	8·77	× × ×	88	7–12	†9·78	− + ×	37	14–17	15·89	× − ×	51	19–22	†20·39	× − ×
	32·0	60·0	157	8–13	9·85	↑	156	8–12	9·03	↑	83	16–19	17·54	↑	73	19–24	20·38	↑
	32·0	80·0	148	5– 8	6·78	× ↑ ↑	148	8–13	9·60	× ↑ ↑	71	13–16	14·42	× ↑ ↑	77	17–21	18·18	× ↑ ↑
	32·0	90·0	132	6– 9	7·42	× × ↑ ↑	132	8–11	9·76	× − ↑ ↑	78	14–17	16·05	× × ↑ ↑	54	17–19	18·65	× × ↑ ↑
	32·0	97·5	161	5– 8	6·56	× + × ↑	160	8–12	9·56	× − − ↑	81	13–16	14·42	× − × ↑	79	17–19	17·84	× × × ↑
	32·0	100	161	5– 8	6·53	× × × −	99	8–13	9·93	× − − +	80	13–17	15·43	× × × ×	19	18–21	19·21	× × × ×
nsis	32·0	60·0	106	9–14	11·58	↑	98	8–12	10·14	↑	36	18–22	19·86	↑	62	20–25	22·65	↑
nsis	32·0	80·0	170	7–10	8·44	× ↑ × ↑	170	8–14	9·92	− ↑ + ↑	84	16–18	16·75	× ↑ × ↑	86	19–23	19·93	× ↑ × ↑
nsis	32·0	97·5	124	7–11	8·30	× − ↑	122	8–13	†10·31	− × ↑	64	16–19	17·08	× + ↑	58	19–23	†20·24	× + ↑
nsis	32·0	100	178	6–11	7·82	× × ×	101	8–14	9·69	+ − ×	76	15–19	16·25	× × ×	25	19–22	20·36	× + −
	32·0	60·0	75	10–16	12·09	↑	63	7–10	8·10	↑	33	17–21	18·55	↑	30	20–23	21·43	↑
	32·0	60·0	12	10–13	11·50	− ↑	12	7–11	8·75	− ↑	5	17–19	18·20	− ↑	7	18–24	21·71	− ↑
	32·0	80·0	141	6– 9	7·56	× × ↑ × ×	141	6–10	7·82	− + ↑ × ×	86	13–16	13·94	× × ↑ × ×	56	17–19	17·06	× × ↑ × ×
	32·0	97·5	164	6–11	7·77	× × − ↑	164	6–11	†8·32	− − × ↑	77	12–17	14·04	× × − ↑	87	16–20	†17·91	× × − ↑
	32·0	100	156	6– 9	7·48	× × − +	156	6–11	8·08	− − − −	81	13–17	13·75	× × − −	75	17–19	17·52	× × − ×
	35·0	80·0	166	7–11	8·70	↑	94	8–13	10·17	↑	72	16–20	17·74	↑	22	19–24	21·45	↑
	35·0	90·0	148	7–11	8·34	× ↑ ↑ ×	104	9–14	10·96	× ↑ ↑ ×	61	16–19	17·51	− ↑ ↑ ×	43	19–24	21·88	− ↑ ↑ ×
	35·3	80·0	92	7–12	9·32	× × ↑	46	9–13	10·57	− −	39	17–20	19·00	× × ↑	7	21–25	22·43	− −
	35·3	97·5	76	7–13	8·24	× − × ↑	—	—	—		3	18–20	18·67	− − − ↑	0	—	—	
	35·3	100	79	6–12	8·24	× − × −	—	—	—		1	16	16·00	− − + −	0	—	—	
	35·0	80·0	153	6–10	7·92	↑	153	6–10	7·92	↑	81	13–16	14·02	↑	73	17–20	17·88	↑
	35·0	90·0	145	6– 9	7·17	× ↑ ×	145	6–10	†7·87	− ↑ ×	87	13–15	13·66	× ↑ ×	61	16–18	†17·15	× ↑ ×
	35·0	100	93	6– 9	8·09	− × ↑	67	6–10	8·15	− −	33	13–15	14·42	× ×	34	16–19	17·56	− +
	35·0	100	75	6– 9	8·15	− × − ↑	0	—	—		0	—	—		0	—	—	
	35·0	97·5	78	6–10	8·04	− × − −	0	—	—		0	—	—		0	—	—	
	35·0	97·5	0	—	—		0	—	—		0	—	—		0	—	—	
	35·0	100	0	—	—		0	—	—		0	—	—		0	—	—	
	36·5	80·0	12	9–14	11·00	↑	8	8– 9	8·63	↑	7	17–19	18·43	↑	1	21	21·00	↑
	36·5	90·0	34	7–12	9·74	+ ↑	17	8–12	†9·12	− ↑	13	16–19	16·92	× ↑	4	23–24	†23·50	+ ↑
	36·5	97·5	20	7–10	9·40	× − ↑	12	8–11	8·42	− −	11	16–19	17·00	× −	1	21	21·00	− +
	36·5	100	4	10–12	10·50	− − +	0	—	—		0	—	—		0	—	—	

BLE 25. Showing the effects of the temperatures of 35·0 ± 0·2 and 35·3 ± 0·3° C (marked with an asterisk) with effective humidities on the growth of recently hatched larvae of the two species of *Xenopsylla*

s	R.H. (%)	no. of larvae used	larvae died in feeding state		no. of larvae and period in which they died in different instars without cocoon formation			Sig. t test	larvae spun cocoons		duration of active larval life to cocoon formation (days)		Sig. t test	no. of larvae and period in which they reached the resting stage			Sig. t test	stages died		
			no.	Sig. χ² test	larvae	days range	days mean		no.	Sig. χ² test	range	mean		larvae	days range	days mean		R.L.	E.P.	A.P.
	60	203	†154	†	†186	4–24	12·38	†	17	†	12–20	15·29	†	32	13–20	16·56	†	44	3	2
	60	160	124	–	151	2–25	10·79	×	9	–	14–19	15·89	–	27	12–19	16·81	–	36	0	0
nsis	60	240	235	× †	240	1–25	6·69	× †	0	× †	—	—		5	20–25	21·80	× †	5	0	0
nsis	80	155	40	× †	123	4–23	12·84	× †	32	× †	10–14	11·28	†	83	10–18	13·11	× †	115	0	0
nsis	90	151	35	× – †	73	3–20	13·14	× – †	78	× × †	9–16	10·40	× †	38	10–15	12·92	× – †	104	3	9
nsis	100	186	129	× × ×	156	3–13	8·55	× × ×	30	× – ×	9–12	10·40	× –	27	9–13	11·22	× × ×	57	0	0

† In these columns, the first indicates number of larvae which died in any instar but while feeding continued: the second includes also those which died after final defaecation.

Table 26. Showing the effect of a temperature of 35 ± 0·2° C with effective humidities on the growth of larvae and pupae within one- or two-day-old cocoons of *Xenopsylla cheopis*

R.H. (%)	presence or absence of food in the cocoon wall	no. of cocoons used	advanced pupae formed		adults emerged		days in which adults emerged	stages found dead after 21 to 45 days' exposure			remarks
			no.	Sig. χ² test	no.	Sig. χ² test		R.L.	E.P.	A.P.	
60·0	+	95	30	†	8	†	9	61	4	22	13 larvae left cocoons and 2 died as A.P.
80·0	+	25	14	+ †	9	× †	7–13	5	6	5	
90·0	+	40	33	× + †	13	× – †	9–13	6	1	20	2 larvae left cocoons and died
90·0	–	40	39	× × – †	14	× – – †	9–18	0	1	25	
97·5	–	39	39	× × + – †	16	× – – – †	9–16	0	0	23	1 naked A.P. died
100	–	40	40	× × + – – †	6	– – – – + †	9–11	0	0	34	
97·5	+	25	0	× × × × × × †	0	– × × × × – †	—	18	7	0	Larvae and E.P. disintegrated within cocoons
100	+	80	0	× × × × × × – †	0	+ × × × × × – †	—	30	50	0	
97·5	+	80	0	× × × × × × – –	0	+ × × × × × – –	—	—	—	—	

Table 27. Showing the effect of a temperature of 35 ± 0·2° C with effective humidities on the growth of larvae and pupae within one- or two-day-old cocoons of *Xenopsylla brasiliensis*

R.H. (%)	presence or absence of food in the cocoon wall	no. of cocoons used	advanced pupae formed		adults emerged		days in which adults emerged	stages found dead after 21 to 46 days' exposure			remarks
			no.	Sig. χ² test	no.	Sig. χ² test		R.L.	E.P.	A.P.	
60·0	+	40	13	†	0	†	—	22	5	13	3} larvae left cocoons and died
60·0	–	40	0	× †	0	– †	—	37	3	0	3}
80·0	+	40	22	– × †	5	– – †	7– 9	11	7	17	
90·0	+	89	29	– × + †	7	– – – †	†9–11	52	8	22	13 larvae left cocoons and died
90·0	–	80	33	– × – – †	1	– – + – †	11	40	7	32	
97·5	+	40	0	× – × × × †	0	– – – – – – †	—	40	0	0	2}
97·5	–	79	47	× × – × + × †	0	– – × + – – †	—	29	3	47	2} larvae left cocoons and died
100	+	42	0	× – × × × – × †	0	– – – – – – – – †	—	42	0	0	3}
100	–	80	74	× × × × × × × ×	0	– – × + – – – –	—	6	0	74	

† The complete combined resting larval and pupal life within the cocoon was 12·36 days (calculated average).

Table 28. Showing the effect of a temperature of 35 ± 0·2° C with effective humidities on the growth of larvae and pupae within one- or two-day-old cocoons of *Xenopsylla astia*

R.H. (%)	presence or absence of food in the cocoon wall	no. of cocoons used	advanced pupae formed		adults emerged		days in which adults emerged	stages found dead after 36 to 45 days' exposure			remarks
			no.	Sig. χ² test	no.	Sig. χ² test		R.L.	E.P.	A.P.	
60·0	–	40	15	†	10	†	7– 9	24	1	5	8 larvae left cocoons and died
60·0	+	28	18	– †	9	– †	9–11	8	2	9	2 naked E.P. died
90·0	+	46	46	× × †	46	× × †	9–11	0	0	0	
90·0	–	42	42	× × – †	42	× × – †	7–14	0	0	0	
97·5	+	40	31	× – × × †	14	– – × × †	9	0	9	17	
97·5	–	80	70	× + + + – †	70	× × + + × †	7–15	7	3	0	10 larvae left cocoons and 3 pupated
100	–	80	80	× × – – × × †	80	× × – – × × †	7–12	0	0	0	1 naked pupa bred successfully
100	+	40	15	– – × × × × ×	0	× × × × × × ×	—	15	10	15	