Reprinted from the
AUSTRALIAN JOURNAL OF ZOOLOGY

IN VITRO STUDIES ON THE ENVIRONMENTAL BIOLOGY OF *GONIODES COLCHICI* (DENNY) (MALLOPHAGA : ISCHNOCERA)

II.* THE EFFECTS OF TEMPERATURE AND HUMIDITY ON WATER LOSS

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[*Manuscript received August* 24, 1970]

Summary

Weight changes have been used to assess the effect of various conditions of temperature and humidity on the rates of water loss of pheasant body lice, *G. colchici*.

Both living and dead animals, in 0% R.H., lose water at a low but increasing rate from 32 to 40°C; above this temperature, the rate of water loss suddenly increases. Living insects lose water at half the rate of dead ones over this temperature range. At 35°C and 0% R.H., all non-moulting stages lose water at a similar rate. Moulting insects lose water at half the rate of non-moulting ones.

The threshold humidity for survival of these insects is within the range 52·5–62·5% R.H. The weight of mature animals does not vary at different humidities above the threshold.

In general, the patterns of humidity requirements and regulation of water loss of *G. colchici* do not differ from those of other insects. There is no evidence that the dependence of these animals on temperatures in the 30–40°C range for survival and reproduction is connected with more efficient control of water loss under these conditions.

I. Introduction

In a previous paper (Williams 1970) it was shown that *Goniodes colchici*, like all other ischnoceran lice examined, required temperatures in the 30–40°C range in order to survive and reproduce. It is possible that at these temperatures the water balance of the animal is under more severe strain than in insects living at more normal temperatures.

If the water content of an animal is to remain constant, the gain of water must equal the loss. In the case of *G. colchici*, most water is lost by evaporation through the cuticle and tracheal system. Very little water is lost in the faeces since much of it is removed before the faeces are egested.

The water intake of these animals being limited, it is possible that water loss by evaporation is regulated more efficiently at temperatures in the mid-30's than in other conditions, and that this forms the basis for their temperature dependence.

In an attempt to evaluate this possibility, various experiments have been performed to study the way in which temperature affects water loss in these animals.

* Part I, *Aust. J. Zool.*, 1970, **18**, 379–89.

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II. Materials and Methods

All the weights reported in this paper were obtained on a Cahn automatic electrobalance. This ultrasensitive balance is based on the null-balance principle, weight changes being translated into electric currents which are amplified before being fed into a 1-mV potentiometric recorder. The balance is not affected by temperatures up to 100°C, and has a device for changing the weight range during weighing if so desired. The degree of zero shift is negligible.

The balance was set up in conjunction with an incubator (Fig. 1) to allow weighings to be made at known temperatures and humidities. The incubator was held 70 cm off the ground on a metal framework. The balance was placed on a shelf above the incubator, and a suspension wire ran from the weighing side of the balance beam (loop A) through the case of the balance, through a hole drilled in the shelf, and into the incubator via the ventilator hole in the top. To prevent air currents disturbing the wire during weighing, it was surrounded by a perspex tube. A weighing pan was then attached to the wire.

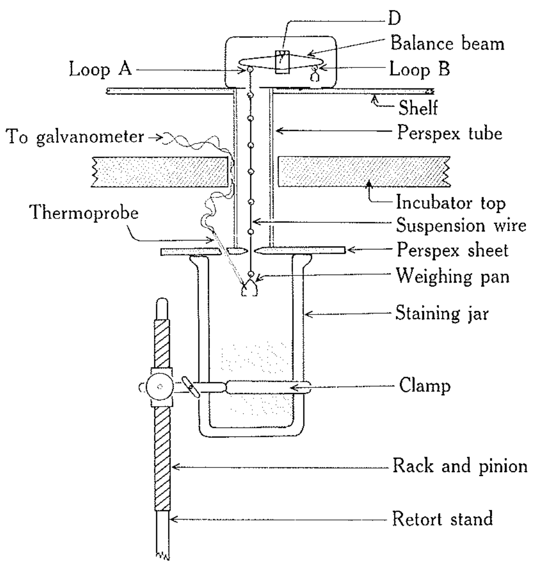

Fig. 1.—Microbalance set up for weighing lice in known temperature and humidity conditions. *D*, Beam balancing mechanism.

Conditions of humidity around the weighing pan could be regulated, as the pan was enclosed in a 100-ml staining jar, which could be racked up and down. A perspex sheet formed the roof of the humidity chamber. Sulphuric acid solutions of the required strength in the jar provided the desired humidity (Solomon 1951).

A thermocouple, previously calibrated to read temperatures from 44 to 32°C, was let into the perspex roof of the humidity chamber to lie 1 cm from the weighing pan. This continuously monitored the temperature in the chamber.

The balance was calibrated using weights of the highest precision (U.S. National Bureau of Standards, Class M) to weigh quantities in the 0–1 mg range. At this sensitivity, it was possible to read weights to 0·0001 mg.

Most insects used in the following experiments were maintained at 35°C, in an incubator contained in a Gooch crucible unit (Williams 1970) having a relative humidity of 75%. Other experimental animals were obtained direct from one of three pheasants housed in an enclosure.

III. Results

(*a*) *Weight Ranges of Incubator-maintained and Natural Lice*

Since the following experiments were based on weight changes of lice undergoing various treatments, it was desirable to compare the weight of a natural population of lice with similar animals maintained artificially.

Figure 2 shows the weight distributions of various forms of lice on removal from the pheasants, compared to similar animals from the incubator. Females obtained from the incubator had a weight range of 0·550–0·730 mg, whether or not they were fecund. Females taken from birds showed a different pattern of weight distribution. Non-fecund females had the lowest weight range, of 0·430–0·670 mg; females having a developing ovum were slightly heavier, in the 0·430–0·690 mg range; those with a fully developed ovum or ova were much heavier, ranging from 0·490 to 0·710 mg.

Incubator males tended to be heavier than males obtained from a bird (0·340–0·460 mg compared with 0·320–0·440 mg).

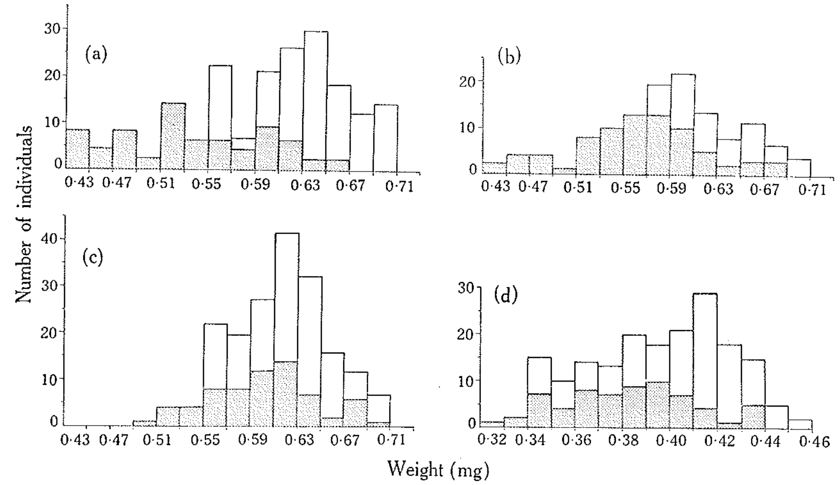

Fig. 2.—Distribution by weight of *G. colchici* removed from incubator populations and from a bird: (*a*) non-fecund female; (*b*) female with developing ovum; (*c*) female with fully developed ovum; (*d*) male. Shaded areas represent animals removed from a bird. Unshaded areas represent animals removed from the incubator.

(*b*) *Rates of Water Loss of* G. colchici

(i) *Effect of Temperature*

For this experiment, five fecund female lice of average weight 0·630 mg, taken from the incubator population at various times, were placed in the weighing chamber at 44°C and 0% R.H. The temperature was allowed to fall to 32°C, and the weight recorded at every 1·2 deg C drop in temperature. The time taken for each temperature drop was recorded. A similar sample of lice, of average weight 0·505 mg, was killed by freezing in a dry-ice air-stream, and then subjected to the same experimental treatment. From the data, the mean rate of water loss, expressed as a percentage of the initial body weight (W_o), per 1 hr, for each 1·2 deg C drop was calculated.

Both living and dead lice lost water at a low but increasing rate from 32 to 40°C (Fig. 3). (Living lice lost 0·2–2·0%/hr of W_o; dead lice lost 1·7–4·0%/hr.) From 40 to 44°C, the rate of water loss suddenly increased, to 2·0–5·0%/hr of W_o for living and 4·0–10·0%/hr for dead lice. It may be noted that living lice lost water at about half the rate of dead ones.

(ii) *Effect of Time*

For this experiment, living animals only were used. Two samples, of six or seven each, of adult males, adult non-fecund females, fecund females, third-instar nymphs, third-instar nymphs undergoing a moult, and newly moulted adults were placed in a Gooch crucible unit, and the humidity maintained at 0% R.H. by means of silica gel. The animals were confined at 35°C. The first batch was weighed every 2 hr for 12 hr, and the second every 2 hr from 12 to 24 hr.

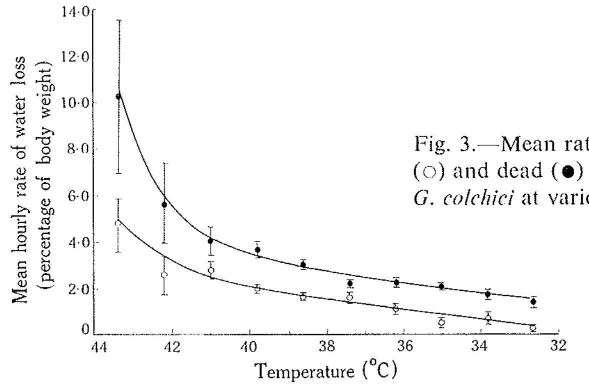

Fig. 3.—Mean rate of water loss of live (○) and dead (●) fecund female *G. colchici* at various temperatures.

The results are shown in Figure 4. Since these animals were able to eat normally during this treatment, the weight changes noted can be inferred as being almost entirely due to water loss. The adults and third-instar nymphs [Fig. 4(*a*)] lost water at a similar rate over this 24-hr period, at about 1·6%/hr of W_o. Animals in the

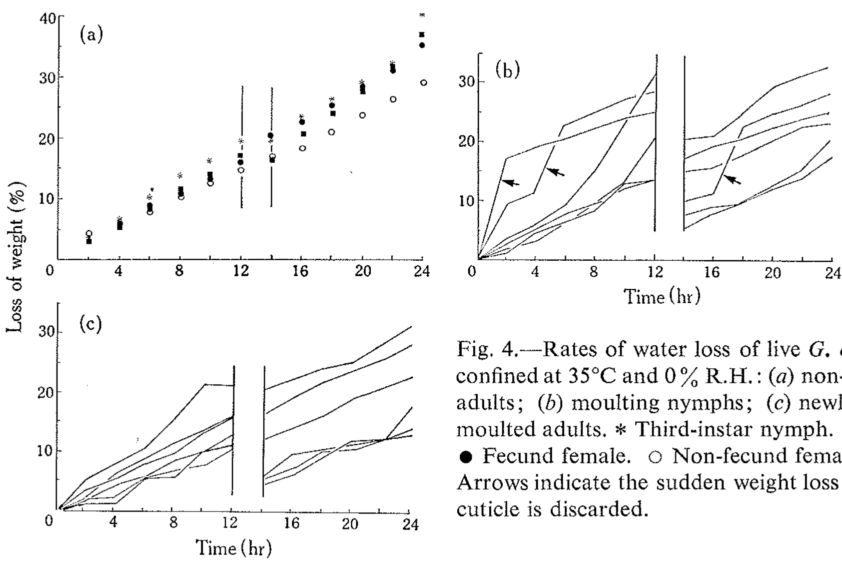

Fig. 4.—Rates of water loss of live *G. colchici* confined at 35°C and 0% R.H.: (*a*) non-moulting adults; (*b*) moulting nymphs; (*c*) newly moulted adults. * Third-instar nymph. ■ Male. ● Fecund female. ○ Non-fecund female. Arrows indicate the sudden weight loss when the cuticle is discarded.

moulting condition, as shown by third-instar nymphs [Fig. 4(*b*)] lost water at a much lower rate, under 1%/hr of W_o. At the moment of moulting, weight loss was much greater. This was due mainly to water loss, but also to the weight of the discarded

cuticle. Newly moulted adults displayed the entire range of rates of water loss [Fig. 4(*c*)], from the low rates typical of the moulting condition to those shown by mature adults.

(*c*) *Effect of Humidity Changes on Weight*

Fifteen adult females and five adult male lice were removed from a pheasant, weighed, placed in a Gooch crucible unit at nearly 100% R.H. over distilled water, and put in the incubator at 35°C. After 2 days, the insects were removed and re-weighed; counts were made of the number of eggs laid and within each female. The lice were then returned to the crucible unit over 90% R.H., and replaced in the incubator. This process was repeated every 2 days, the humidity being dropped to 80, 70, 60, 55, and 52·5% successively. The population died in 50% R.H.

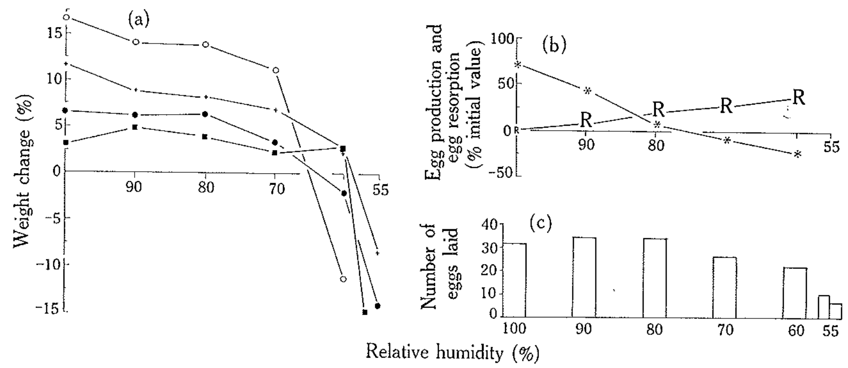

Fig. 5.—Changes in weight (*a*), egg production and resorption (*b*), and egg-laying (*c*), in *G. colchici* subjected to 10% drops in humidity from 100 to 55% R.H. ○ Non-fecund females. + Female with developing ovum. ● Female with fully developed ovum. ■ Male. * Mean number of eggs within each female expressed as a percentage of the initial value. R, number of ova undergoing resorption within each female as a percentage of total females.

Figure 5(*a*) shows the weight changes of representative animals exposed to this treatment. Animals immature at the start of the experiment registered the largest initial increases in weight, gaining over 15% of W_o in the first 2 days, at 100% R.H. Mature females with fully developed ova showed the least increase in weight amongst the females, usually about 7·5% of W_o. Males showed the least increase in weight of all, in the region of 3·0% of W_o.

As the relative humidity dropped from 100% to 70%, the animals showed little change in weight. Between 70 and 60% R.H., one female and one male louse rapidly lost weight and died. Between 60 and 55% R.H., six female and four male lice died after rapidly losing weight. A further three females died in 52·5% R.H. The last five females died in 50% R.H.

The number of eggs laid [Fig. 5(*c*)] remained steady, oscillating about 30 in each 2-day period, at relative humidities down to 60%. The number of eggs laid fell sharply in 55% and in 52·5% R.H., due largely to the deaths of many of the females.

The number of ova within each female [Fig. 5(*b*)] rose sharply in 100% R.H. compared to the number present when the lice were removed from the bird. As the humidity decreased, the number of developing ova also fell, until at 75% R.H. fewer ova were developing than originally. Coincidental with the fall in ova production, the incidence of egg resorption increased [Fig. 5(*b*)].

In order that the transition humidity could be more exactly defined, a second, similar, experiment was performed. In this case, 18 female and 10 male adults were removed from an incubator population and weighed. These were split into two more or less equal groups by weight; each group was placed in a Gooch crucible unit over 75% R.H. and confined at 35°C. Every 2 days the lice were removed and weighed. One group was subjected to a drop in humidity of 2·5% R.H. every 2 days. The control group remained in 75% R.H.

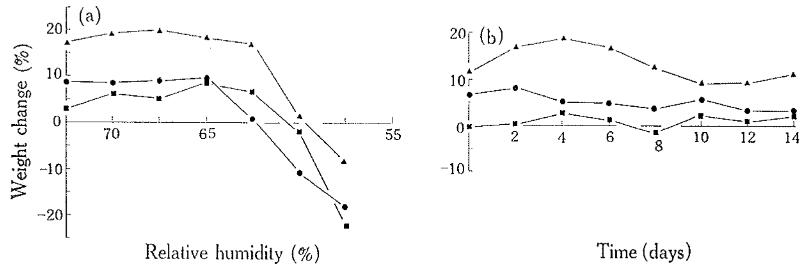

Fig. 6.—Weights of *G. colchici*: (*a*) subjected to 2·5% drops in humidity from 75 to 55% R.H. at 35°C; (*b*) maintained at 35°C. ▲ Females with ovum undergoing resorption. ● Females with fully developed ovum. ■ Male.

Figure 6 shows the weight changes of typical animals. In the experimental group, every animal held its weight steady until a humidity of 65% R.H. was reached, at which point two females died. A further female died in 62·5% R.H. One female and two males died in 57·5% R.H., followed by another female and two males in 55% R.H. All the remaining animals died in 52·5% R.H. The rate of weight loss immediately preceding death was not as rapid in these lice as in those subjected to 5% humidity drops in the previous experiment.

Three of the control animals (two females and one male) died during the course of this experiment, the remainder remained constant in weight.

An interesting feature of these results is the excessive increase in weight, often up to 20% of W_0, shown by females starting the experiment with an ovum undergoing resorption [Fig. 6(*a*)]. As was noted in the previous experiment, the weight of fecund females increased by up to 10%, and that of males by up to 5%.

IV. Discussion

Since the water relations of these insects have been determined on the basis of weight changes, the obvious discrepancy between weights of animals removed from birds and taken from the incubator requires explanation. As was demonstrated, incubator females are heavier and have a more restricted weight range than those from a bird. This is explicable by the fact that adults removed from a bird are at all stages of development from the newly moulted to fully reproductive. In the incubator very few lice are reared successfully, so that the number of newly moulted animals

is low; animals taken from the incubator therefore consist mainly of adults removed from the bird and stored *in vitro*. Hence, a non-fecund animal taken from a bird is almost certainly newly moulted and capable of further development, whereas a similar louse removed from the incubator is likely to be mature. This accounts for the fact that non-fecund animals from the host have low weights, whereas the weight range of similar animals taken from the incubator does not differ from that of any other incubator female. As the "bird" population matures, its weight range comes to approximate to that of the incubator population. A similar trend is seen in the case of males.

This explanation gains support from the weight gains of animals switched from a bird to the incubator. The largest increases in weight are recorded by non-fecund females, and are often in excess of 15% of the initial body weight, compared to those of mature females, which average less than 5%.

With regard to the water relations of these insects, the curves obtained for the rate of water loss with temperature, by both living and dead insects, conform closely to those obtained for other insects, as first demonstrated for *Periplaneta americana* (Ramsay 1935), and since for many other species (Beament 1945; Wigglesworth 1945). All insects examined exhibit the phenomenon of "critical temperature", above which water loss accelerates, but below which water loss is less severe. In the case of adult females of *G. colchici*, it occurs at about 40°C.

The rate of water loss was less in the living than the dead insect, and it would thus appear that the living animal is able to exert some control over water loss. It was thought that this ability might function more efficiently at temperatures centring upon 35°C, thus accounting for the dependence of these animals on temperatures in the 30–40°C range. If this were so, it should be reflected in the rates of water loss of animals confined at 35°C and 0% R.H. compared with those over the rest of the temperature range. In fact, as has been shown, under these conditions all non-moulting animals lose 35–40% of their body weight as water in 24 hr, and die within 36 hr. Only in moulting animals is this rate of water loss reduced, to about half of the non-moulting rate.

None of this evidence suggests that, in desiccating conditions, the regulation of water loss by *G. colchici* is more efficient at 35°C than at other temperatures in the 30–40°C range.

On the other hand, the water balance of these lice is maintained if the humidity is above a certain level, between 52·5 and 62·5% R.H. An individual louse confined above its threshold humidity maintains its body weight at a steady level. When confined at a humidity below the threshold, even if by only a few percent, the animal loses water at a rate similar to that obtained in completely desiccating conditions. Maintenance of water balance, therefore, is seen clearly to be not a function of the efficiency of restricting water loss, but a dependence upon the presence of water vapour above a certain concentration.

The causes for this dependence on water vapour above a threshold level for maintenance of water balance in *G. colchici* form the basis for the final paper in this series.

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