THE INFLUENCE OF
TEMPERATURE AND SALINITY
ON THE CUTICULAR PERMEABILITY
OF SOME CORIXIDAE

by
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ABSTRACT

Most terrestrial, and many aquatic insects are made waterproof by a layer of lipid in or on the epicuticle. At a specific temperature, which is determined by their composition, these lipids undergo a phase transition which markedly increases the permeability of the integument.

The major purpose of this study was to assess the possibility that epicuticular wax transition could differentially affect the distribution of four species of water boatmen: *Cenocorixa bifida hungerfordi* Lansbury, *Cenocorixa expleta* (Uhler), *Cenocorixa blaisdelli* (Hungerford), and *Callicorixa vulnerata* (Uhler). The rates of water loss and cuticle temperatures of adult corixids were measured in a stream of dry carbon dioxide in steps of increasing temperature.

The temperatures at which transition occurred in these species were all approximately the same. Although they ranged from 30.3 to 32.6°C there were no major differences between the two genera, among congeners with different distributions, or between two coexisting congeners. This was true, however, only for corixids which had been acclimated to the same temperature: a positive correlation between transition temperature and acclimation temperature was demonstrated in *C. bifida*.

Both the short-term and long-term effects of transition on these insects were examined. Immersion of live *C. bifida* adults in water warmer than their transition
temperature did not appear to cause any irreversible changes in cuticular permeability. Survival tests at various temperatures showed that the survival time of C. bifida adults decreased with increased temperature. However, insects placed in warm water did not show any outward signs of osmoregulatory failure or loss of surface wax as a result of transition. In addition, C. bifida placed in water as warm as or several degrees warmer than their transition temperature survived much longer than the length of time that these insects would be exposed to these temperatures in the field.

The present study on transition effects suggests that the transition of epicuticular lipids does not affect the distribution of these corixids in the field.

However, it appears that salinity has a pronounced effect on the permeability of C. bifida adults. Individuals from fresh water habitats and highly saline ponds exhibited roughly equal cuticular permeability, but those from lakes of intermediate salinities were up to twice as permeable. This phenomenon was shown to be one of physiological acclimation, since individuals placed into distilled water showed a significant decrease in permeability after five days. It is possible that this influence of salinity on cuticular permeability may affect the relative dispersal success of the corixids which inhabit inland saline lakes.
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I. INTRODUCTION

A. THE PROBLEM

The large surface/volume ratio of insects and other arthropods necessitates control of integumentary water loss, and most terrestrial forms possess a remarkably impermeable cuticle in order to prevent desiccation (Neville, 1975). Many aquatic insects also require a relatively impermeable cuticle to minimize osmotic fluxes (Beament, 1961b; Foster and Treherne, 1976; Phillips and Bradley, 1977) or to prevent desiccation during aerial dispersal.

Kuhnelt (1928, as cited by Wigglesworth, 1945) was the first to show that the relative impermeability of insect cuticle was a property of its outermost part, the epicuticle. He found that removal or disruption of this layer resulted in rapid water loss by the insect. It is now known that an epicuticular lipid layer is responsible for this waterproofing (Beament, 1945, 1961a; Wigglesworth, 1945; Jackson and Baker, 1970; Ebeling, 1974; Jackson and Blomquist, 1976).

The fact that rising temperature can cause a drastic increase in the permeability of the epicuticle has been known for some time. When Gunn (1933) observed a sudden increase in the rate of water loss from a cockroach (Blattaria) between 30 and 35 C, he explained it in terms
of an increase in respiration. Ramsay (1935), however, found that this increase in evaporation rate also occurred with the spiracles blocked, and suggested that it was the result of the "melting of fat" in the cockroach cuticle.

Ramsay's work led to studies by V.B. Wigglesworth and J.W.L. Beament in the 1940's: Wigglesworth (1945) studied the effect of temperature on the permeability of many insects, and his results seemed to confirm the existence of a sudden increase in cuticular permeability at what he termed the "critical temperature". Beament's (1945) experiments with insect cuticular waxes spread on butterfly wing membranes showed that these waxes underwent a phase transition at a temperature closely corresponding to the insect's critical temperature. He concluded, therefore, that this transition of epicuticular lipids was the cause of the observed abrupt increase in permeability at the critical temperature.

A controversy soon arose over the existence of transition. Edney (1951) stated that the seemingly sudden increase in the rate of water loss was only the result of one's subjective interpretation of the evaporation/temperature plot. He pointed out that this increase could be accounted for simply by the exponential increase in the saturation deficit of air with increasing temperature, and when Holdgate and Seal (1956) eliminated the effects of increasing saturation deficit from their results, they found no indication of a sudden change in the rate of water loss.
Beament (1958) defended the transition theory by pointing out a number of inadequacies in previous techniques and analyses, and after performing experiments using sophisticated equipment, he found that a transition point was distinct, even after the saturation deficit was taken into account.

Beament (1961b) studied the permeability/temperature relationships of a number of aquatic insects, and found that the more impermeable forms have wax transitions similar to those exhibited by terrestrial forms, but in comparison with them, the transition temperatures were very low.

The question arises as to whether this phenomenon could exclude certain insects from warmer waters. Beament (1961b) gives evidence that this could be the case for the aquatic beetles *Dytiscus* and *Gyrinus*. The adults of these beetles have transition temperatures of about 24°C and die at temperatures only a degree or two warmer than this, seemingly from the effects of internal waterlogging. Beament (1961b) notes further that the related beetles *Agabus* and *Ilybius* have much higher transition temperatures and can withstand higher water temperatures than *Dytiscus* or *Gyrinus* can. In Australia, *Agabus* occurs in habitats much warmer than those where *Dytiscus* is found (Beament (1961b)).

It is possible, then, that related species may exhibit differences in transition temperature that could differentially affect their distribution. An investigation of transition and its effects would therefore be valuable.
In this study, I decided to investigate the transition phenomenon in several closely-related species of water boatmen (Corixidae). First, the possibility of intergeneric differences in transition similar to those observed by Beament, (1961b) in Agabus and Dytiscus were studied by comparing the transition temperatures of Cenocorixa blaisdelli (Hungerford) and Callicorixa vulnerata (Uhler), two coexisting species on the southwest coast of British Columbia. Second, to determine if transition could differentially affect the distribution of congeners, the transition temperature of C. blaisdelli was compared with those of two interior species: Cenocorixa bifida hungerfordi Lansbury and Cenocorixa expleta (Uhler). Third, the possible effects of transition on the coexistence of two closely-related species were determined by comparing the transition temperatures of C. bifida and C. expleta.

Oloffs and Scudder (1966) studied the transition phenomenon in C. expleta and found that the transition point lay between 28.5 and 33.8 °C cuticle temperature. Since water temperatures in some of the lakes that C. expleta inhabits can reach 30 °C several times in the course of a warm summer (Jansson and Scudder, 1974; Cannings, 1975), it appeared possible that the success of these bugs may be affected by the transition phenomenon.

Oloffs and Scudder (1966) also found that when dead C. expleta were placed in water warmer than their transition temperature, they showed a permanent increase in
permeability when returned to air at 20°C. This was not the same phenomenon described by Beament (1959), since pretreatment in warm air did not cause a permanent increase in permeability. They suggested that, at temperatures above transition, some of the wax is removed by water.

If this is the case, transition underwater may have more far-reaching consequences for these insects than merely an immediate increase in permeability. It could create osmoregulatory problems after periods of high water temperature as well as during them, and the corixids would be subject to desiccation if they undertook dispersal flights in air of only moderate temperature.

Corixids colonize lakes randomly each spring, as they seem unable to select appropriate water bodies before landing (Popham, 1964). Therefore, they may breed, or attempt to breed, in lakes which become too warm later.

While attempting to determine the transition temperature of some field-caught bugs, I found that these insects were far more permeable than those that I had raised in the laboratory. Since the field-caught bugs had come from a saline pond and the laboratory ones had been reared in dechlorinated tap water, I hypothesized that the observed difference in the permeability of these insects was the result of the difference in salinity of their respective habitats. With this in mind, further studies were undertaken to determine if salinity and permeability were indeed correlated in some way.
B. EPICUTICULAR LIPIDS AND THE TRANSITION PHENOMENON

Epicuticular lipids are generally thought of as a thin layer spread over the surface of the cuticle (Beament, 1961a), but there has been a great deal of controversy over the exact structure and composition of this layer. Beament (1945) suggested that the lipid was an orientated monolayer bound to the cuticulin substrate, and later hypothesized that it consisted mainly of long-chain alcohols (Beament, 1955, 1961a). Gilby and Cox (1963), however, found that paraffins, not alcohols, made up the bulk of the lipid layer of cockroaches (Beament's experimental animal). Fatty acids, aldehydes, and esters made up most of the remainder of the lipids. Since then, work on numerous other insects has shown that the composition of epicuticular lipids is quite diverse (reviewed in Hackman, 1974; Neville, 1975, pp. 98-104; Jackson and Blomquist, 1976). Alcohols are rarely found, however, except in some specialized waxes (Chibnall et al., 1934; Hackman, 1951; Bowers and Thompson, 1965).

Gilby and Cox (1963) doubted that the lipid mixture present in cockroach cuticle could form a tightly-packed monolayer, and recent work by Lockey (1976) has shown that a film of cockroach lipid is unstable during compression and does not form a tightly-packed monolayer. An effective lipid monolayer could only exist, therefore, if there were some unusual property of the lipids of the cuticulin substrate. Lockey (1976) suggests that the crucial layer of lipid is located in the outer region of the cuticulin.
In his electron microscope studies of lipids in the epicuticle of *Rhodnius*, Wigglesworth (1975) has found that the lipid does not occur as a discrete layer, but is bound in a fragile non-lipid silver-staining membrane. Detailed studies such as this have not been made in other insects, however, so we cannot generalize from this observation.

Since the exact structure of the epicuticular lipid layer is not known, the mechanism of transition is still the subject of speculation.

Beament (1964) suggested that long-chain polar lipids are optimally packed at an angle of $24.5^\circ$ to the vertical. At the critical temperature, thermal agitation breaks the Van der Waals forces binding the chains together and causes them to assume a mean vertical position, resulting in an increase in permeability to water.

Locke (1965) proposed an explanation for transition based on the presence of wax filaments in the epicuticle. His electron micrographs of the epicuticle show these filaments passing through the cuticulin layer and connecting with the surface wax layers. Locke suggests that these filaments consist of long-chain lipids in the middle phase of a lipid-water liquid crystal. At the transition point, this crystal structure could change either to a complex hexagonal phase or to a reversed middle phase, and water would be allowed to escape. Locke accepts the idea of a lipid monolayer preventing water loss, but suggests that the monolayer consists of lipids in a liquid-crystal.
Filshie (1970), however, found that these filaments could not be removed by lengthy extraction with lipid solvents, and states "it is unlikely that the filaments themselves are composed entirely of wax precursors". Wigglesworth's (1975) electron microscope studies confirm, however, that epicuticular lipids are transported through these canals.

Davis (1974b) postulated that the low permeability of the cuticle is the result of lipids in a special arrangement "probably dictated by the molecular arrangement of the lipoproteins in the cuticulin layer". Davis suggests that at transition the lipids undergo a phase change from the solid crystalline state to the liquid crystalline state. Neville (1975) favours this last hypothesis.

Regardless of its mechanism, however, epicuticular lipid transition appears to be a real physico-chemical phenomenon which may have a pronounced effect on some insects.

C. THE CORIXIDS

Four species of corixids were investigated in this study, with the greatest emphasis on *Cenocorixa bifida hungerfordi* Lansbury. *C. bifida* is an inhabitant of temporary and permanent ponds throughout much of temperate western North America, (Jansson, 1971). Although it is found elsewhere, its distribution is closely linked with semi-arid regions, where it can be found in freshwater or moderately saline lakes and ponds (Jansson, 1971).
Scudder (1969a,b) found that *C. bifida* cannot tolerate salinities greater than 20,000 umhos/cm surface conductivity.

*Cenocorixa expleta* (Uhler) coexists with *C. bifida* in ponds of intermediate salinity (5990–20,000 umhos/cm), but can tolerate salinities up to 33,000 umhos/cm (Scudder, 1969b). It is absent, however, from fresh water habitats (Scudder, 1969a,b), and is therefore more restricted in general distribution than *C. bifida* (Scudder, 1969a,b).

*Cenocorixa blaisdelli* (Hungerford) is a coastal species, favouring temporary or semi-permanent ponds within 2km of the Pacific Ocean (Jansson, 1971). Although Jansson (1971) never found this corixid in waters more saline than 215 umhos/cm, Reynolds (pers. comm.) has taken it in the brackish waters adjacent to Witty's Lagoon near Victoria, B.C.

The *Callicorixa* species studied keyed to *Callicorixa vulnerata* (Uhler) in Hungerford (1948), and furthermore, it was true *C. vulnerata* according to key characters supplied by Jansson (pers. comm.). This species ranges along the west coast of North America from California to the Alaskan Peninsula (Hungerford, 1948). In British Columbia it is found along the Pacific coast and throughout the southern interior (Scudder, 1977). It is a species characteristic of temporary pools and ponds, and as such, is a frequent flyer (Scudder, pers. comm.).
II. MATERIALS AND METHODS

A. TRANSITION POINT DETERMINATIONS

1. The corixids and their care.

Corixids were collected in the field using aquatic sweep nets and were transported to the laboratory in Thermos jugs half-filled with water. *Cenocorixa blaisdelli* and *Callicorixa vulnerata* were collected from a small but permanent pond at McCleery Golf Course in Vancouver, B.C. *C. blaisdelli* were also taken from a small temporary pond on the University of British Columbia campus. *Cenocorixa bifida* was obtained mainly from Long L. on Becher's Prairie near Riske Creek, B.C. However, some samples were taken from Round-up and Barnes Lakes (Phalarope and Box 4 Lakes respectively in Scudder (1969a,b)) on Becher's Prairie, and from LE3 and LE5 on the Green Timber Plateau west of Clinton, B.C. Figures 1 and 2 give the locations of these lakes. *Cenocorixa expleta* was taken from Barnes and Round-up Lakes.

In the laboratory, the corixids were maintained in round plastic trays (8cm deep X 24cm in diameter) half-filled with dechlorinated water. The bugs were fed frozen brine shrimp every other day and the water in the trays was changed if and when it became putrefied. To prevent the rotting of excess food, the water in the culture trays was continuously aerated. With the exceptions noted below, all bugs used in transition point tests were kept in constant
FIGURE 1

The Springhouse and Green Timber Plateau collection sites:

A. A portion of the Cariboo and Chilcotin Plateaus of British Columbia. The locations of Figures 1B and 1C are outlined.

B. The Springhouse collection sites.

C. The Green Timber Plateau collection sites.

D. The location of Figure 1A in British Columbia.
FIGURE 2

The Becher's Prairie collection sites.
temperature chambers at 20 C under a 16hr light: 8hr dark light regime.

In the case of C. blaisdelli and C. expleta, the bugs were allowed at least one week to acclimate to these laboratory conditions before they were tested. Only flying form C. expleta were used.

All the individuals of Callicorixa vulnerata and Cenocorixa bifida used in transition experiments had hatched from eggs laid in the culture trays. The larvae were raised under the same conditions as the adults were maintained. After they had emerged, the adults were held for at least one week before testing, so that their cuticles could harden.

Three groups of C. bifida were treated in a different manner to the rest of the corixids. Some were transferred to 25 C while still fourth or early fifth instar larvae. After these larvae emerged as adults, a portion of them were kept at 25 C until they had matured, while the rest were returned to 20 C after their cuticles had hardened. A third group, which had emerged as adults at 20 C, were moved down to 10 C after they had matured. The corixids in these last two groups were allowed at least one week to acclimate to the new temperatures before being tested.

2. Transition point determinations

The temperature of cuticular lipid transition in these insects was determined using the basic techniques described by Beament (1958, 1961a). Generally, the method was to
suspend an insect in a dry CO₂ atmosphere and measure its rate of water loss over time. Figure 3 shows the experimental apparatus used.

a. Apparatus

Carbon dioxide from a pressurized cylinder was released slowly through a two-stage regulator. Flowing through tygon tubing, it entered a copper heat-exchanging coil 1.6m long submerged in a water bath. It then flowed through a glass desiccator tube (16.5 cm long X 3.2 cm in diameter) packed with 8-mesh calcium sulphate (Drierite). From here, the CO₂ passed through a Gilmont RG F1500 flow meter. The volume of gas flowing through the system was too small to be accurately measured by this flow meter, but every effort was made to keep the flow relatively constant. The CO₂ then entered the glass flow-through tube in which the insect was suspended, and escaped through the bottom of this tube. Figure 4 gives the details of this portion of the apparatus.

The experimental temperature was controlled by the water bath. This consisted of two gallons of water in a cylindrical PYREX brand jar (22.2cm outside diameter X 25.4cm high). The water bath temperature was controlled by a Haake EL2 constant temperature circulator. Water was pumped through surgical tubing into the jacket of the double-walled flow-through tube. After flowing up and around the water jacket, the water left by the upper port and returned
FIGURE 3

The experimental apparatus used in the determination of transition temperatures:

A. Electrobalance
B. Flow-through tube (see Figure 4)
C. Micromanipulator with thermocouple leads
D. Ice bath: cold junction for thermocouple
E. Electrobalance control unit
F. Time derivative computer
G. Two-pen recorder
H. Thermocouple potentiometer
J. CO$_2$ cylinder
K. Water bath
L. Copper heat-exchanging coil
M. Constant temperature circulator
N. Desiccator tube
O. Flow meter
FIGURE 4

Detail of flow-through tube (B in Figure 3). Diagram is to full scale.
to the bath (Figure 4). The water bath itself sat in a rectangular plastic tray 30 X 36 cm X 15 cm deep, which served to catch overflow water from the bath. This tray is not shown in Figure 4.

Both CO₂ and cuticle temperature were measured using a chromel/alumel thermocouple made from 29 s.w.g. wires. This was mounted on a micromanipulator so that the thermocouple could be easily moved within the weighing tube. The chromel and alumel leads were connected to copper leads in an ice bucket, which served as a 0 C reference. A Doran Mini Thermocouple Potentiometer was used to determine the output of the thermocouple.

The weighing apparatus consisted of a Cahn RG Electrobalance and a modified Cahn "Little Gem" Thermogravimetric Analysis Kit. As shown in Figure 4, the experimental bug was hooked under the head and suspended from the balance arm by a 0.10mm nichrome wire. Output from the balance's control unit followed two routes: firstly, it went directly to a lmv two-pen recorder (Omniscribe, Houston Instrument). Secondly, it went to a Cahn Time Derivative Computer (Mark II). This unit translated the changing weight signal of the balance into a rate of change signal. Its output also went to the recorder.

b. Procedure

Corixids to be used in transition point determination were handled as little and as carefully as possible in order to minimize damage to the external lipid layer. They
were removed from the culture trays with a plastic mesh scoop and transferred to small scintillation vials partly filled with distilled water. The air in the vial was then replaced with CO₂ and the vial was capped. The corixid usually came to the surface after a few minutes, began to jerk, and then floated motionless on the surface of the water. It was left in the vial from two to five hours before the test began. After being removed from the vial, it was lightly dried on tissue paper and then suspended on the hook of the hangdown wire. The lower flow-through tube was then raised over the bug and was connected to an upper tube by means of a retaining collar. The corixid was now in a dry CO₂ atmosphere approximately the temperature of the water bath. After waiting 15 minutes for the surface water of the corixid to evaporate, the temperature of the CO₂ and of the corixid's cuticle was recorded. The cuticle temperature was taken by gently moving the thermocouple tip forward until it touched some point on the ventral surface of the insect's abdomen. Following the temperature reading, the temperature was raised as quickly as possible to the next level. This was accomplished by pouring boiling water into the water bath. It was not possible to raise the temperature precisely to a predetermined level with this procedure, but an approximate level could be easily reached. The next reading was taken as soon as the rate of weight change trace had levelled off. This procedure was repeated until readings
had been taken at all the desired temperatures. Figure 5 shows a tracing from the recorder of a test with a female C. blaisdelli.

c. Analysis

The original wet weights of the corixids were determined by extending the weight loss trace back to the time that the bug was placed on the balance. This procedure eliminated the weight of external water.

The time derivative computer, when calibrated, gave a value of the rate of water loss in mg/hr. To eliminate the effect of the bug's size on its rate of water loss, this value was divided by an approximation of the bug's surface area to give a value in mg/cm²/hr. The surface area approximation was derived using the general formula

\[ A = \frac{M^2}{3} \cdot k \]

where: \( A \) = surface area
\( M \) = mass
\( k \) = a constant.

In order to derive \( k \) for adult C. expleta, Oloffs and Scudder (1966) dismembered several bugs and measured the surface area of the separated parts under a microscope with an eyepiece graticule. They reported a mean value of \( k \) for this species of 10.8 (when mass is measured in grams and surface area in square centimeters). This value of \( k \) was used in this study for all estimations of surface area, since all species studied were about the same shape.

The effect of increased temperature upon evaporation rate had to be dealt with as well. This effect was elimi-
Reproduction of a recorder trace showing the loss of weight of a female *Cenocorixa blaisdelli* in steps of increasing temperature (left ordinate). The trace increasing in steps with time is the rate of weight loss trace from the time derivative computer (right ordinate). The values above each of these steps indicate the cuticle temperature at that point, while the values beneath indicate the CO$_2$ temperature.
nated by dividing the water loss per unit area by the saturation deficit of water at the experimental temperature. In a dry atmosphere, such as the one used in this study, the saturation deficit of water is equal to its vapour pressure at that temperature.

We thus arrive at an index of cuticle permeability which is the insect's evaporation rate (mg/hr) divided by its surface area (cm$^2$) and by the saturation deficit of water (mm Hg).

Regression analysis was used to determine a mean transition temperature with confidence limits. The data set (e.g. Fig. 6) was divided in two at a certain temperature (several degrees below what appeared to be the inflection point) and a pair of regressions were plotted. A sequence of regression pairs were then calculated with the data divided between sequential data points along the temperature axis until a pair of best fit was found. The intersection of this pair defined the transition point. Ninety-six percent confidence limits were placed on the transition temperature by plotting the 80% confidence limits of each of the two regression lines and finding the two extreme intersections of the confidence limits (the probability of these lines intersecting at or beyond these confidence limits is the product of the probabilities of the individual regressions being outside them; i.e. 0.20 x 0.20 = 0.04).
B. PRETREATMENT TESTS

The basic procedure of these tests was to "pretreat" corixids in water of varying temperatures, then measure their permeability at a constant low temperature.

The individual C. bifida used in these tests were members of the first summer generation. They were collected from Round-up and Barnes Lakes (Figure 2), and transported to the laboratory and maintained there at 20 C as described previously.

Four different pretreatment temperatures were used: 25, 30, 32, and 35 C. Three to five corixids were tested at each of these temperatures. The bugs were taken from their 20 C culture tray and placed directly into water held constant at the treatment temperature. The aquarium used in this test is described in the next section.

After three minutes, the corixids were taken out of the aquarium and put into individual vials, and were killed as described previously. Their evaporative water loss at 20 ± 1 C was then determined using the apparatus and procedure as described for the transition temperature determinations, but only one reading was taken.

C. TEMPERATURE SURVIVAL TESTS

In this experiment, the length of time required for each corixid to die at a given temperature was measured.

C. bifida from Long Lake (Becher's Prairie) were used, and these bugs had been divided into two groups: one group acclimated at 10 C for at least two weeks before testing,
the other group held at 20°C. At each experimental temperature, both groups were tested at the same time. Ten corixids made up each group, but occasionally one managed to escape and the test finished with only nine bugs accounted for. The sex ratio was kept as close to 1:1 as possible.

Four experimental temperatures were used: 27, 30, 33, and 36°C. At 27°C, no corixids acclimated to 10°C were tested.

The tests took place in a 5 gallon aquarium which was divided into three sections: one end was separated from the rest of the aquarium by fine plastic screening and the other section was divided lengthwise into two sections by coarser plastic screening. A Haake E12 constant temperature circulator was positioned at the end enclosed by the fine screening. This screening reduced the turbulence caused by the heater pump while allowing water to circulate freely. Another sheet of coarse screen covered the aquarium to prevent corixids from flying out.

The corixids were taken from their culture trays and put into the aquarium at 20°C. The temperature was then raised by approximately 0.75°C/min until the experimental temperature was reached.

A corixid was considered "dead" when it could no longer swim in a coordinated manner.

For the test at 36°C, the bugs were watched constantly, while at 33°C, the corixids were observed continuously for
the first hour, and hourly thereafter. During the tests at 27 and 30 C, the bugs were checked at least twice daily, and deaths were recorded as having occurred in the middle of the 12-hour periods. When the corixids were checked more often than every 12 hr., deaths were recorded as having occurred in the middle of the previous period of absence.

To find out if previous acclimation temperature has a significant effect on the survival of these corixids, the results of bugs acclimated to 10 and 20 C were compared using one-tailed t-tests. The hypothesis tested was that bugs acclimated to 10 C would not survive as long as those acclimated to 20 C.

D. SALINITY EFFECTS

The C. bifida used in these tests were collected from SP8, Westwick, and Boitano Lakes near Springhouse, B. C. (Figure 1b); from Sapper (Box 22), Barkley (Opposite Box 4); Long, and Barnes (Box 4) Lakes on Becher's Prairie (Figure 2); and from LE3 and Long Lake on the Green Timber Plateau (Figure 1c) (names in parentheses are those used by Scudder (1969 a, b)). They were transported to the laboratory in refrigerated styrofoam tubs (6cm high, 10cm outside diameter at the top, 7.5cm outside diameter at the base) half-filled with lake water. In the laboratory the corixids were held in these tubs at 5 C until they were tested. Their rate of water loss at 20 ± 1 C was determined by the procedure used in the pretreatment tests.
One difference, however, was that they were killed in vials containing lake water, rather than distilled water.

The conductivity of the lake water was determined at the time of testing by use of a Radiometer CDM2 conductivity meter, and corrected to 25°C.

E. RESPONSE TO SALINITY CHANGE

To investigate the possibility that individual *C. bifida* could change their cuticular permeability in response to a change in salinity the following experiment was undertaken.

Twenty *C. bifida* collected from Long Lake (Becher's Prairie) in the first week of September 1977 were used for this test. Ten of these bugs were placed into distilled water and ten remained in their own lake water. All the bugs were kept in styrofoam tubs (5 corixids per tub) at 20°C for 5 days. No food was given to the bugs during this period. Their rates of water loss at 20 ± 1°C were then determined following the procedures outlined previously.

Since it was hypothesized that the corixids in distilled water would be less permeable than those in lake water, a one-tailed t-test was used to determine the significance of the results.
III. RESULTS

A. TRANSITION POINT DETERMINATIONS

i) General results.

Table 1 gives the mean masses and approximate surface areas of the various groups of corixids tested.

The rates of water loss of the corixids studied here are comparable to values obtained in other investigations. Table 2 compares some of the values found in the literature to the data obtained in this study.

In general, individual bugs showed a great increase in permeability above a certain temperature. A sharp transition point was not evident in many cases, however; instead, the water loss traces curve upwards over a range of 2 or 3°C. Figure 6 illustrates the permeability/temperature relationship for individual *C. bifida*. No individual curves for the other sets of corixids tested are illustrated, but with regard to the properties of individual traces, those shown in Figure 6 are representative.

One can see that there was considerable variation in the rates of water loss for individual bugs: at 20°C some corixids showed water loss rates twice those of other bugs. The overall permeability/temperature relationship was fairly consistent, however. Below transition most bugs showed a constant gentle increase in water loss rate (about 0.0017 mg/hr/cm²/mm Hg/°C).
TABLE I

The mean weights and approximate surface areas of the corixids used in the transition temperature determinations.

TABLE II

The rate of water loss at 20°C of corixids in this study compared with values reported in the literature.
<table>
<thead>
<tr>
<th>Species</th>
<th>temperature conditions</th>
<th>sex</th>
<th>N</th>
<th>mean mass (mg)</th>
<th>S.E.</th>
<th>mean surface area (cm²)</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cenocorixa bifida hungerfordi</td>
<td>emerged at 20 C and acclimated to 20 C</td>
<td>♂</td>
<td>5</td>
<td>16.76</td>
<td>0.47</td>
<td>0.707</td>
<td>0.013</td>
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<td></td>
<td></td>
<td>♀</td>
<td>5</td>
<td>22.65</td>
<td>0.40</td>
<td>0.865</td>
<td>0.010</td>
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<tr>
<td></td>
<td>emerged at 20 C and acclimated to 10 C</td>
<td>♂</td>
<td>7</td>
<td>15.74</td>
<td>0.25</td>
<td>0.678</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>♀</td>
<td>5</td>
<td>20.80</td>
<td>0.50</td>
<td>0.817</td>
<td>0.026</td>
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<td></td>
<td>emerged at 25 C and acclimated to 20 C</td>
<td>♂</td>
<td>5</td>
<td>14.84</td>
<td>0.18</td>
<td>0.652</td>
<td>0.011</td>
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<td></td>
<td></td>
<td>♀</td>
<td>3</td>
<td>19.20</td>
<td>0.08</td>
<td>0.775</td>
<td>0.003</td>
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<tr>
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<td>emerged at 25 C and acclimated to 25 C</td>
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<td>0.44</td>
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<td>18.92</td>
<td>--</td>
<td>0.767</td>
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<td>C. blaisdelli</td>
<td>acclimated to 20 C</td>
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<td>11.16</td>
<td>0.17</td>
<td>0.539</td>
<td>0.005</td>
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<td></td>
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<td>7</td>
<td>13.21</td>
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<td>0.603</td>
<td>0.019</td>
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<tr>
<td>C. expleta</td>
<td>acclimated to 20 C</td>
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<td>16.71</td>
<td>--</td>
<td>0.706</td>
<td>--</td>
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<td></td>
<td></td>
<td>♀</td>
<td>4</td>
<td>24.52</td>
<td>1.18</td>
<td>0.911</td>
<td>0.029</td>
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<tr>
<td>Callicorixa vulnerata</td>
<td>emerged at 20 C and acclimated to 20 C</td>
<td>♂</td>
<td>9</td>
<td>12.51</td>
<td>0.26</td>
<td>0.520</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>♀</td>
<td>1</td>
<td>15.35</td>
<td>--</td>
<td>0.545</td>
<td>--</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>rate of water loss (mg/cm²/hr)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corixa punctata</td>
<td>2.1</td>
<td>Holdgate (1956)</td>
</tr>
<tr>
<td>Corixa sp.</td>
<td>1.2-1.8</td>
<td>Beament (1961b)</td>
</tr>
<tr>
<td>Cenocorixa expleta</td>
<td>3.5</td>
<td>Oloffs &amp; Scudder (1966)</td>
</tr>
<tr>
<td>C. expleta</td>
<td>3.8</td>
<td>this study</td>
</tr>
<tr>
<td>C. bifida</td>
<td>1.4</td>
<td>this study</td>
</tr>
<tr>
<td>C. blaisdelli</td>
<td>1.6</td>
<td>this study</td>
</tr>
<tr>
<td>Callicorixa vulnerata</td>
<td>2.2</td>
<td>this study</td>
</tr>
</tbody>
</table>
FIGURE 6

The rate of water loss (with the effect of increasing saturation deficit removed) of individual Cenocorixa bifida as a function of cuticle temperature.
RATE OF WATER LOSS (mg/cm²/hr/mm Hg)

CUTICLE TEMPERATURE (°C)
The results shown here are actually conservative estimates of the increase in rate of water loss with temperature. As a bug loses water at a constant temperature, its rate of water loss decreases. Two male C. bifida were tested at 30 C: their water loss rates decreased by 0.092 and 0.066 mg/cm\(^2\)/hr/mm Hg/per cent original weight lost (Figure 7). Since corixids usually lost about 20 per cent of their original weight in the transition point tests, the last rate of water loss value obtained would be about 20\(^{0}/\) per cent 0.08mg/cm\(^2\)/hr/ per cent original weight lost, or 1.6 mg/cm\(^2\)/hr too low. At 40 C, this would correspond to an underestimate of about 0.03 mg/cm\(^2\)/hr/mm Hg.

Although all the permeability data given here have been analysed and presented with reference to cuticle temperature, the temperature of the carbon dioxide surrounding the corixid was also measured. If the water bath was below room temperature, the corixid's cuticle temperature was usually equal to or slightly (up to 1 C) higher than the CO\(_2\) temperature. As the experimental temperature increased, the cuticle temperature became increasingly cooler relative to the CO\(_2\) temperature. At 43 C CO\(_2\) temperature, the cuticle was approximately 40-41 C.

ii) Males vs. Females.

Figure 8 shows that there was no apparent difference between male and female C. bifida with regard to their cuticular
FIGURE 7

The rate of water loss at 30 C of two male *C. bifida* as a function of the percentage of their original wet weight remaining.
FIGURE 8

The rate of water loss of male (closed circles) and female (open circles) \textit{C. bifida} as a function of cuticle temperature.
RATE OF WATER LOSS  
(mg/cm²/hr/mm Hg)

CUTICLE TEMPERATURE  (°C)
permeability/temperature relationship. Therefore, no attempt was made to separate males and females in the analysis of the transition point data.

iii) Differences among species.

The results of the transition point tests are summarized in Figures 9 to 15. The transition temperatures of the three Cenocorixa species and Callicorixa vulnerata were quite similar to each other. Figure 16 shows that although their transition temperatures ranged from 30.25 to 32.60 C, they all lay within the confidence limits of each other. C. bifida and C. vulnerata, which were raised under the same laboratory conditions, were especially similar.

The results for C. expleta in this study are comparable with those derived from the data of Oloffs and Scudder (1966).

iv) Differences among temperature classes.

A different story appears, however, when we compare the transition temperatures of C. bifida individuals kept under different laboratory conditions (Figure 17). Corixids which emerged at 20 C and then acclimated to 10 C had much lower transition temperatures than those acclimated to 20 C. Similarly, C. bifida adults which emerged at 25C and acclimated to 25 C had significantly higher transition temperatures than bugs which were acclimated to 20 C. The data from corixids which emerged at 25 C and were acclimated to 20 C show high variation, so nothing concrete can be concluded from them. It appears, though, that these bugs had a lower transition
FIGURE 9

Rate of water loss as a function of cuticle temperature for *C. bifida* reared at 20°C and acclimated to 20°C. Points used in the calculation of the lower regression are represented by circles: points used in the calculation of the upper regression are represented by squares.
FIGURE 10

Rate of water loss as a function of cuticle temperature for C. blaisdelli acclimated to 20C. Symbols as in Fig. 9.
FIGURE 11

Rate of water loss as a function of cuticle temperature for C. expleta acclimated to 20 C. Symbols as in Fig. 9.
FIGURE 12

Rate of water loss as a function of cuticle temperature for *Callicorixa vulnerata* reared at 20°C and acclimated to 20°C. Symbols as in Fig. 9.
FIGURE 13

Rate of water loss as a function of cuticle temperature for *C. bifida* reared at 20 C and acclimated to 10 C. Symbols as in Fig. 9.
FIGURE 14

Rate of water loss as a function of cuticle temperature for C. bifida reared at 25 C and acclimated to 25 C. Symbols as in Fig. 9.
FIGURE 15

Rate of water loss as a function of cuticle temperature for C. bifida reared at 25 C and acclimated to 20 C. Symbols as in Fig. 9.
FIGURE 16

The transition temperatures (as defined by regression intersections) of the various corixid species studied. Vertical bars indicate the 96\% confidence intervals of the intersections.

a. C. bifida
b. C. blaisdelli
c. C. expleta
d. C. expleta (derived from Oloffs & Scudder, 1966)
e. C. vulnerata
FIGURE 17

The transition temperatures of the various temperature classes of *C. bifida*. Vertical bars indicate the 96% confidence intervals of the regressions.

a. Reared at 20 C and acclimated to 10 C.
b. Reared at 20 C and acclimated to 20 C.
c. Reared at 25 C and acclimated to 20 C.
d. Reared at 25 C and acclimated to 25 C.
CUTICLE TEMPERATURE °C

a

b

c

d
temperature than those that were acclimated to 25 C.

B. PRETREATMENT TESTS

There were no significant differences in the rates of water loss of corixids pretreated in water at various temperatures (Figure 18).

C. TEMPERATURE SURVIVAL TESTS

The results of this experiment are summarized in Figure 19. The survival time of the corixids decreased with increased temperature, and appeared to decrease more quickly above 30 C.

The bugs at both 33 and 36 C showed no outward signs of osmotic influx of water, and there were no indications of wax removal (i.e. there was a high contact angle with water, and the plastrons remained functional).

At 36 C, the survival time of corixids acclimated to 10 C was significantly lower than that of corixids acclimated to 20 C (t = 2.457, p less than 0.05). At 30 and 33 C, however, the survival times of the two groups were not significantly different (t = 0.52, p greater than 0.05; t = 0.17, p greater than 0.05 respectively).

D. SALINITY EFFECTS

The transpiration rates of corixids from lakes of a wide range of salinities indicate that permeability and salinity are indeed correlated (Figure 20). Cenocorixa bifida from SP8, Sapper, Barkley, and Westwick Lakes (all less than 2000 umhos/cm surface conductivity at 25 C) lost
FIGURE 18

Rate of water loss at 20°C of *C. bifida* pretreated in water of various temperatures. Vertical bars indicate 1 SE above and below the mean. Numbers below these bars are the sample sizes.
Temperature tolerance of *C. bifida*. Mean survival times of insects acclimated to 20°C are represented by squares, and those of insects acclimated to 10°C are represented by circles. Vertical bars indicate 1 SE above and below the mean. Numbers below these bars are the sample sizes. The ordinate is a logarithmic scale.
FIGURE 20

Rate of water loss of *C. bifida* as a function of the salinity of their native lakes. Vertical bars represent 1 SE above and below the mean. Numbers above the bars give the sample size.
RATE OF WATER LOSS
mg/cm²/hr

CONDUCTIVITY
μmhos/cm x 10³ at 25°C
water at rates less than \(3.5 \text{ mg/cm}^2/\text{hr}\), while those from Long Lake on Becher's Prairie (8000 umhos/cm) lost water at approximately \(5 \text{ mg/cm}^2/\text{hr}\). Corixids from Boitano Lake (5500 umhos/cm) showed intermediate rates of water loss. As salinity increases further, the trend of increasing permeability reverses: \textit{C. bifida} from Long Lake on the Green Timber Plateau (17,000 umhos/cm) were as impermeable as those from Barkley and Westwick Lakes. LE3 (10,300 umhos) and Barnes Lake (13,500 umhos) corixids showed intermediate rates of water loss.

E. RESPONSE TO SALINITY CHANGE

After remaining in their Long Lake (Becher's Prairie) water (8400 umhos/cm) for five days, the eight control bugs tested had a mean rate of water loss at 20°C of \(3.56 \text{ mg/cm}^2/\text{hr}\). The seven bugs which had spent five days in distilled water showed a mean rate of \(2.72 \text{ mg/cm}^2/\text{hr}\), which was significantly lower (p less than 0.05) according to a one-tailed t-test.
IV. DISCUSSION

A. TRANSITION POINT DETERMINATIONS

1. Techniques.

The general technique followed in this study was that described by Beament (1958, 1961a). In his discussion of this method, Beament (1958) mentions several critical factors in the detection of the transition temperature of an insect.

First, the permeability/temperature curves of individual insects should be used: individual variability can obscure a sharp transition if composite curves are plotted. Oloffs and Scudder (1966) demonstrate well this phenomenon in their work with *Cenocorixa expleta*. Second, Beament (1958) claims that cuticle temperature, not air temperature should be used in the analysis of the rate of water loss. Since evaporation from a surface causes a decrease in temperature at that surface, the cuticle temperature will be lower than the temperature of the surrounding air. If an abrupt increase in evaporation occurs at transition, the cuticle temperature would lag behind the air temperature even more. Thus, the use of air temperature in calculating the relative permeability of the insect could obscure a transition point.

In opposition to this, Oloffs and Scudder (1966) showed that transition points in *C. expleta* were detectable using either air or cuticle temperatures. However, Beament (1958)
points out that, although a knowledge of air temperature at transition may be ecologically important, it is the temperature of the cuticle that is essential in any physico-chemical interpretation. In addition, these corixids probably never encounter air temperatures warm enough to cause epicuticular lipid transition. They only fly during the day in the spring (Scudder, pers. comm.; Smith, pers. comm.), a time when diurnal temperatures are not extreme (Cannings, 1973; Jansson and Scudder, 1974; Smith, 1977). In the summer and early autumn they fly during the evening (Scudder pers. comm.), again avoiding extreme temperatures (Jansson and Scudder, 1974, Cannings, 1975). Probably the only situation in which transition could occur in these insects, then, would be as they swim through shallow water during hot summer days. Since their cuticle temperature is equal to that of the surrounding water, I decided to measure cuticle, rather than air temperature, in the determination of the transition points of these insects.

Beament (1959) also stresses that handling of experimental insects must be minimized, for any disturbance of the external wax layer can also obscure a sharp transition point. Most other researchers in this field have measured the rate of water loss from insects at a known temperature by determining their weight before and after exposure for a given period of time. This involves handling the insect
to some extent between readings, and the period during transfer to the balance could introduce errors as well. Also, one wants to minimize the duration of the experiment so that the drying of the insect changes its rate of water loss as little as possible.

Beament (1958) solved these problems by building a balance into the experimental system, while Edney and McFarlane (1974) suspended cockroaches in the experimental chamber from a balance above. Since it appeared to be the simplest solution to the problems mentioned above, I chose to use a below-the-balance continuous weighing procedure similar to that of Edney and McFarlane (1974). Instead of hanging the insect in a relatively large chamber suspended in a water bath, however, I decided to use a much more compact system. With the apparatus shown in Figures 3 and 4, access to the insect is much improved, and thermocouple positioning is a simple operation. The major reason for using a narrow tube for an experimental chamber, however, is that it would be impossible to accurately weigh an insect the size of a corixid in a chamber in which air was being stirred by a fan. Cahn and Peterson (1967) found that with flowing gas systems, turbulence was much greater in tubes with diameters greater than 16 mm. In fact, I found that with a tube 16 mm in diameter, the signal noise (caused by turbulence buffeting the insect) was so great that the trace could not be analysed. In a chamber one would expect
this noise to be greater still.

With their apparatus, Edney and McFarlane (1974) used continuously rising temperatures to examine changes in the rate of water loss with temperature. Although this method reduces experimental time considerably (and thus minimizes the insect's water loss), it has several disadvantages, namely:

i) Cuticle temperature cannot be measured at the same time weight loss is being recorded. Edney and McFarlane (1974) established a relationship between air and cuticle temperature during trial runs, but could predict cuticle temperature to within only 1°C.

ii) The rise in temperature must be kept constant among different runs, because the cuticular response may differ at various rates of temperature increase. This constancy is also necessary to predict cuticle temperature from a known air temperature.

iii) Perhaps the greatest disadvantage of this procedure is that the rate of water loss of the insect may lag behind the rising temperature. This lag would cause an underestimate of the relative permeability of the cuticle, especially at the higher temperatures. In fact, this phenomenon could be the reason why Edney and McFarlane (1974) failed to detect a sharp transition in the cockroaches that they were studying.

In short, then, I decided to use a step-by-step procedure (rather than having the temperature rise continuously) for the following reasons:
1) Cuticle temperature could be easily measured at the end of each rate of weight change reading.

2) Any lag phenomena were eliminated.

3) The length of time that the insect was exposed to a desiccating atmosphere could be kept to a reasonably short period of time (about 1 hr) by raising the water bath temperature as quickly as possible between temperature levels.

A frustrating problem that sometimes occurred with this procedure regarded the flow of CO₂. When a fresh tank of CO₂ was in use, the extreme pressure difference across the regulator made it very difficult to produce a smooth, even flow of gas. A "fluttery" flow of CO₂ caused the insect to sway in a jerky fashion on the hangdown wire, and the recorder traces were rough.

At higher temperatures, the measurement of CO₂ temperature was also a problem. The temperature next to the water jacket could be 1 C or so greater than that of the CO₂ in the center of the tube. Thus, depending on the exact position of the thermocouple tip with respect to the tube wall, the CO₂ temperature could vary. Since I was not really concerned with atmospheric temperature, I made no effort to correct this problem. In the future, however, it might be advisable to measure atmospheric temperature with the tip of the thermocouple positioned directly above the insect. This problem could also be eliminated by keeping the CO₂ warm so that it is not appreciably cooler than the water jacket as it enters the flow-through tube.
2. Analysis -- sources of error.

a) $k$, the area constant.

Holdgate (1956) states that the measurement of surface area (using this calculation) was one of the chief causes of inaccuracy in his work. Oloffs (1964) gives the range of $k$'s he calculated for an unknown number of $C. \text{expleta}$ as 9.3 to 12.3. If this range were used as the confidence limits of $k$ for corixids, the surface area calculation would contain an error of roughly $+14^\circ/o$. This could account for a good portion of the variability in the transition data.

It is obvious that this method of surface area estimation does not take into account any convolutions of the cuticular surface. Glynne-Jones (1955) found that the epicuticle of the honey bee may have an area 10 times its projected surface area, and using krypton adsorption, Lockey (1960) demonstrated that the elytra of several insects had true areas 6.7 to 8.2 times their projected surface areas. Thus, the permeability index that is derived using an estimate of projected area could be an overestimate. On the other hand, extensive hair piles may trap air and thus reduce the saturation deficiency of the air adjacent to the cuticle surface. This would cause the permeability index to be underestimated.

However, despite the problems involved in obtaining an index of permeability accurate enough to be useful comparatively, the index derived using the area constant ($k$) can certainly be used to compare the cuticular permeabilities of insects as morphologically similar as corixids.
b) The decrease in rate of transpiration with desiccation.

Several workers (Wigglesworth, 1945; Edney, 1951; Loveridge, 1968) have found that the rate of water loss from some arthropods gradually decreases as the animal dries out. Bursell (1957) points out that this decrease also occurs in the tsetse fly (Glossina morsitans), but here it is not caused by a change in the cuticle, but instead by spiracular regulation. However the Agriotes larvae that Wigglesworth (1945) studied were dead and had their spiracles blocked before transpiration measurements were taken. Also, since the major spiracles in corixids are situated in enclosed spaces (Parsons, 1970, 1976), and since the corixids used were dead, it is unlikely that spiracular regulation could account for this phenomenon in the present study. Oloffs (1964) did not find any evidence of this decrease in the rate of transpiration in his tests with C. expleta, but his procedure did not allow him to test this with individual bugs.

Bursell (1955) suggests that this phenomenon might be because of the increase in concentration of body fluids, which lowers the activity of water on the inside of the membrane. Wigglesworth (1945), on the other hand, attributes it to the drying of the cuticle itself which could decrease the permeability of the endo- and exocuticle.
King (1944) shows this to be the case in keratin membranes. It seems to me, however, that this latter mechanism would give a noticeable decrease in permeability only at temperatures above transition, when the wax layer may not be the limiting factor in the diffusion of water. In cases of extreme desiccation, the actual availability of water for diffusion through the cuticle would cause a decrease in the rate of water loss (Wigglesworth, 1945).

c) The regression analysis.

The regression analysis could have contributed to the error (as seen in the confidence limits of the transition temperatures) in two ways:

i) If the corixids showed transition points at approximately the same temperature, but varied widely in their permeability below transition, the confidence limits of the regression overestimate the variability of the transition temperature itself. A good example of this can be seen in the set of C. bifida which emerged at 25 C and were acclimated to 20C. Most of the corixids in this group showed a transition at about 32-35 C, but the confidence limits calculated from the regressions encompass 27.7 C to 41 C.

ii) These regressions are used with the assumption that the permeability/temperature relationship is linear below and above transition. Beament (1961a) states that below transition hard wax has a linear temperature coefficient of permeability, whereas above transition there is a "quasi-
exponential" relationship between temperature and permeability. Nevertheless, he interprets experimental data as two straight lines meeting at the transition point.

With my data, exponential functions did not explain more of the variability observed, so I continued using linear regression in the analysis of the data.

3. The existence of transition.

A few years after Beament (1945) and Wigglesworth (1945) had proposed epicuticular wax transition as an explanation for their permeability/temperature data, a controversy arose over its existence. Edney (1951), Mead-Briggs (1956), and Holdgate and Seal (1956) all argued that the increase in the rate of water loss with rising temperature was exponential. Beament (1958) defended the transition theory by demonstrating the importance of using results from individual insects and using cuticle, rather than air temperatures in the analysis of these results.

Recently, however, Hackman (1974) cites unpublished data of A. R. Gilby and says that "More recent work on several species of insect confirms the fact that temperature and water loss are related exponentially". Also, Edney and McFarlane (1974) failed to find a sharp increase in the rate of water loss of Periplaneta americana with rising temperatures. This failure, however, is probably a result of their techniques, as was discussed previously.
To ensure that my results showed two separate permeability states of the cuticle, rather than one related exponentially to temperature, I plotted the rate of water loss (per unit saturation deficit) on a logarithmic scale versus temperature (Figure 21). This clearly shows that there is a discontinuity in the original data. If the relationship was a simple exponential, the points in Figure 21 would lie in a straight line.

4) The transition temperatures of the various corixid species.

The transition temperatures of a group of closely-related species, especially those of coexisting species, have never really been studied before. In the past, workers have either concentrated on one species (e.g. Davis, 1974a; Hadley, 1970; Loveridge, 1968; Oloffs and Scudder, 1966), studied two species from different habitats (e.g. Edney and McFarlane, 1974) or surveyed a broad spectrum of insects (Wigglesworth, 1945; Beament, 1945, 1959, 1961b). Beament (1961b) found a great variety of temperature/permeability relationships in aquatic insects. The two closely-related dytiscid genera Agabus and Ilybius, however, both had transition temperatures of about 34 or 35 C, whereas their more distant relative Dytiscus had a transition temperature of only 24 C (Beament, 1961b).

Therefore, the fact that the transition temperatures of the four species investigated in this study all fell
FIGURE 21

The rate of water loss of *C. bifida* (from Fig. 13) plotted on a logarithmic ordinate as a function of cuticle temperature.
within 2 or 3 C of each other is not an unexpected result. These corixids are all congeners, with the exception of *Callicorixa vulnerata*, which is a co-member of the Tribe Corixini with the three *Cenocorixa* species. (Hungerford, 1948). In addition, they are inhabitants of ponds with approximately the same summer temperature regimes (Jansson & Scudder, 1974).

It appears, then, that these species do not differ in their susceptibility to the transition phenomenon. Whether its effects on each species differ is another question, and one which was not investigated in this study.

The factors influencing permeability, and transition in particular, as well as the effects of transition on the well-being of corixids were studied in *Cenocorixa bifida* alone.

v) The influence of acclimation temperature on transition temperature.

There was a definite positive correlation between acclimation temperature and the transition temperatures of the corixids (Figure 17). Although this has not been noted in previous studies with insect cuticular lipids, Fraenkel & Hopf (1940) and Munson (1953) showed that at higher acclimation temperatures, the body lipids of insects were more saturated and had higher melting points. In addition, work on the cuticular lipids of plants has shown that there tends
to be a shift to greater chain length of cuticular alkanes at higher temperatures (Wilkinson & Kasperbauer, 1972; Giese, 1975; Hass, 1977). This increase in chain length would result in a higher transition temperature (Chapman and Leslie, 1970).

Temperature could also interact with diet to produce changes in cuticular lipids (although this would not be the case in this study). Blomquist and Jackson (1973) report that a considerable portion of the n-alkane constituents of the epicuticle of the grasshopper *Melanoplus sanguinipes* are derived directly from the diet. Branched hydrocarbons, secondary alcohols, and ketones, however, are synthesized. It is possible, then, that the prey of corixids produce lipids which are more saturated and are longer-chained at higher acclimation temperatures. If a portion of these dietary lipids are incorporated directly into the epicuticular lipid layer, the acclimation temperature would cause an increase in the insect's transition temperature via its diet.

There appears to be differences in the effects of the temperature at adult emergence and the temperature at which the corixid was acclimated. For example, the transition temperatures of *C. bifida* which had developed and emerged at 25 C and had then been acclimated to 20 C appears to be still 3 C or so higher than those of *C. bifida* which had emerged at 20 C and had been held at 20 C. Whether or not this is a permanent difference cannot be determined from
these data alone. It might be that the change in the lipids takes a longer period of time than was allowed in this experiment. On the other hand certain lipids may not change at all, giving the resulting lipid composition an intermediate transition point. Few lipids may have to change to produce a noticeable change in the transition temperature, for certain lipids have synergistic effects on transition (Chapman and Leslie, 1970).

The question of why there is a change in the composition of their epicuticular lipids is still a matter for speculation. Does this hedge against the possibility of transition occurring? Or, as Beament (1962, 1976) suggests, does this merely maintain the proper mobility of the wax, so that damage to the cuticular surface can be easily repaired?

Whether or not the former is the case will probably depend on what effect transition has on these insects. This is discussed in the following sections.

vi) Transition underwater.

The results of the pretreatment experiment indicate that there was no permanent increase in cuticular permeability following immersion in water warmer than the corixid's transition temperature. This is in direct opposition to the results of Oloffs and Scudder (1966), which show a 25°/o increase in the rate of water loss from a corixid pretreated in warm water. This difference could have resulted from one of the following:
a) The variability of my data is rather great, and could have obscured a trend. On the other hand, the variability in Oloffs and Scudder's data is not given: if it is large, the trend seen in their data may be false.

b) The corixids used in this study were placed in the pretreatment water while alive, whereas those used by Oloffs and Scudder were killed before they were immersed. Living insects may somehow be able to prevent the loss of wax or quickly replace it as it is lost.

c) The epicuticular wax layers of C. bifida and C. expleta may differ enough to give opposing results in this experiment.

One can say, however, that in the experiment done in this study, nothing occurred which permanently altered the permeability of the corixids to a greater degree than could be attributed to normal variability. This means, of course, that transition can only affect a living corixid while the temperature is greater than that of transition.

vii) The effect of temperature on survival.

Figure 19 shows that there is a continual decrease in survival time with increasing temperature. The apparent change in the slope of the logarithmic function between 30 and 33 C may or may not be significant. It is possible that this change results from a detrimental effect of transition. However, if transition was the factor causing death, I would expect the effect to be much more immediate: the bugs kept
at 33 C survived for a mean period of 17h, and many of them survived a day or even longer. This is not the speedy death described by Beament (1962) for Dytiscus adults, which he claims to be the result of cuticular wax transition.

In their natural habitat, these corixids would be exposed to temperatures above transition for only an hour or two at the most (Jansson and Scudder, 1974; Cannings, 1975). This fact, coupled with the observation that most of the corixids removed from the tests at 33 and 36 C recovered quickly after being placed in cooler water, makes it appear that these bugs do not suffer any immediate drastic effects as a result of transition.

The fact that these corixids usually die shortly after breeding (Scudder, 1975) might explain why their survival time at 27 C is shorter than might be expected from the other data. The bugs at 27 C, as well as one or two at 30 C, bred and laid eggs during the course of the tests.

The comparison of the survival of bugs acclimated to 10 and 20 C is an interesting one. It seems that acclimation temperature makes little difference in survival time at lower temperatures (30 and 33C), where survival is lengthy and the shock of the initial increase in temperature is least. At 36 C, however, the 10 C bugs were clearly more quickly affected.

B. SALINITY AND PERMEABILITY

The cuticular permeability of C. bifida was greatest in those bugs which inhabited lakes of intermediate salinity
(Figure 20): C. bifida adults which lived in higher salinity water or fresh water had more impermeable cuticles. It was demonstrated that these differences in permeability represented a process of physiological acclimation, as the cuticular permeability of Long Lake (Becher's Prairie) corixids decreased significantly while they were in distilled water for five days.

The data indicates that C. bifida "relaxes" its cuticular permeability as the osmotic gradient between itself and its environment decreases. Scudder et al. (1972) found that the haemolymph osmotic pressure of C. bifida is fairly constant at lower salinities, whereas at higher salinities the haemolymph osmotic pressure of C. bifida increases with increasing salinity. This increase begins at salinities of about 10,000 umhos/cm at 25 C. Thus, the corixids begin to reduce cuticular permeability at approximately the point where it becomes difficult to maintain a constant haemolymph osmotic pressure.

Subjective observations indicate that the peak in cuticular permeability occurs in the salinity range in which C. bifida does best. This species can reach extremely high densities in lakes such as Long (Becher's Prairie) (8110 umhos/cm at 25 C), Round-up (10,700 umhos/cm*), and LE5 (7133 umhos/cm*), but is usually sparse in lakes like Barkley (1225 umhos/cm) (Conductivities marked by an asterisk are from the September 1976 data of Smith (1977); unmarked values are from the samples used in the permeability tests of this study).
The relationship between salinity and the cuticular permeability of aquatic insects has been studied very little. Nicolson and Leader (1974) and Phillips and Bradley (1977) found that salt water mosquito larvae have cuticles which are 3 to 10 times less permeable than those of fresh water insect larvae. Osmotic regulation in *Ephydra cinerea* is known to be aided by a very impermeable cuticle (Nemenz, 1970). However, I know of no reports of changes in cuticular permeability in response to salinity changes. Phillips and Bradley (1977) state that the cuticular permeability of *Aedes campestris* larvae does not change during adaptation to extreme hyposmotic and hyperosmotic conditions.

The effect of salinity on permeability found in this study could have a pronounced impact on the dispersal of these corixids. Unless their cuticular permeability is reduced before flight takes place, corixids flying out of intermediate salinity lakes (in which they are so abundant) would desiccate at twice the rate as those leaving fresh water lakes. In addition, if these more permeable bugs land in a relatively fresh pond (which are much more common than saline ponds on Becher's Prairie (Smith, 1977)), they would experience a sudden osmotic influx of water.

C. GENERAL DISCUSSION

Beament (1960, 1961b, 1962, 1976) has suggested that low transition temperatures may limit the distributions of some aquatic insects. In this study I have attempted to determine whether or not epicuticular lipid transition could limit the distribution or dispersal of some water boatmen. These bugs
avoid the possibility of transition occurring during dispersal by flying in the evening during the late summer (Scudder, pers. comm.). Spring dispersal takes place during the day (Scudder, pers. comm.; Smith, pers. comm.), but maximum air temperatures at this time do not generally exceed 25°C (Cannings, 1973; Jansson and Scudder, 1974).

What, then, is the probability of transition occurring underwater? The results indicate that the transition points of these corixids are all at the extreme upper range of water temperatures they are likely to experience in this part of their range. The highest temperature recorded by Jansson and Scudder (1974) for the littoral regions of Becher's Prairie and Springhouse lakes was about 30°C, and Cannings (1975) recorded shallow water temperatures in the range of 30 and 30.5°C in White Lake, a shallow saline pond near Okanagan Falls, B.C.

In addition, *C. bifida* individuals are able to increase their transition temperature at high water temperatures, making it even more unlikely that transition of their cuticular lipids will occur naturally. There is also the possibility that these corixids could avoid shallow water warmer than their transition point by seeking cooler microhabitats. Cannings (1975) found experimental evidence for this in *C. expleta*, but not in *C. bifida*.

If transition of the cuticular wax of these insects does occur in their natural habitat there are indications
that it would not have any obvious adverse effects on them. First, the pretreatment tests indicated that no noticeable permanent increase in permeability occurred as a result of transition underwater. Second, these corixids would probably only be exposed to temperatures above transition for a very short period of time, at least in this part of their range (Jansson and Scudder, 1974; Cannings, 1975). The survival test at 33°C showed that _C. bifida_ individuals lived many times longer than the natural exposure would be. In the field, acclimation responses would probably greatly increase this survival time. Third, the corixids tested at 33 and 36°C showed no outward signs of decreased osmoregulatory ability or loss of external wax.

Since it seems unlikely that transition would occur in the field, and since it appears that these bugs suffer no short-term adverse effects from lipid transition, I must conclude that epicuticular lipid transition is not relevant to the differential distribution of these species or to the coexistence of _C. expleta_ and _C. bifida_.

The effect of habitat salinity on the cuticular permeability of _C. bifida_ was only superficially studied here, but the results of this study tied in very well with the permeability/temperature results. The important general feature that emerged from both of these studies was the very dynamic nature of the control of cuticular permeability. Wigglesworth (1945) stated that a constant rate of water loss
was typical of each species and of each developmental stage of the species, but Holdgate and Seal (1956) and Beament (1959) showed that the permeability and permeability/temperature relationship of pupal cuticles changed markedly with the age of the pupae. Although Silhacek et al. (1972) and Harris et al. (1976) did not study permeability, they showed that the cuticular hydrocarbon patterns of the stable fly and house fly also varied considerably with the age of the individual. In these cases, however, the compositional changes appeared to be related to the pheromonal role of a portion of the cuticular hydrocarbons. Still, changes such as these could alter the permeability and permeability/temperature relationship of the cuticle. Davis (1974a) showed that the cuticular permeability and transition temperature of the rabbit tick *Haemaphysalis leporispalustris* (Packard) change markedly during the life of the animal, both between and within developmental stages. More importantly, he was able to correlate these changes with changes in composition and amount of epicuticular lipids (David, 1974b). Until now, however, there has been no evidence that insects or other arthropods have the ability to alter the permeability of their epicuticular wax layer in response to environmental changes.
LITERATURE CITED


