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THERMAL TOLERANCE OF THE ADULT ASIATIC CLAM CORBICULA MANILENSIS (MOLLUSCA:BIVALVIA)

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MASTER

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ABSTRACT

Knowledge of the thermal tolerance of Corbicula manilensis is important for antifouling at water intakes and protection below discharges. Groups of 20 clams were acclimated to temperatures ranging between 5 and 32°C and exposed (1) continuously to higher temperatures (24-39°C), (2) continuously to lower temperatures (2-20°C), or (3) for 30 minutes to temperatures between 37 and 43°C. Mortality was determined by lack of response to prying open the valves. Exposure to temperatures between 40 (5°C Acclimation) and 43°C (30°C Acclimation) for 30 minutes caused 100% mortality. For continuous exposures, upper tolerance limits (50%) were between 24 (5°C Acclimation) and 34°C (30°C Acclimation) and lower tolerance limits were between 2 (15°C Acclimation) and 12°C (30°C Acclimation). Relationships between acclimation and resistance temperatures were linear. The upper incipient lethal temperature was 34°C. Use of results at 24 and 48 hours (and 96 hours for long-term heat stress) yielded significant overestimates of tolerance limits.

MASTER

INTRODUCTION

Since first being observed in the U.S. in 1938,[1] Corbicula manilensis, the Asiatic clam, has increased rapidly in ecologic and economic importance. Most of the major drainage basins in the U.S. are now inhabited by Corbicula.[2-5] Where they are abundant, these clam populations are important in nutrient cycling and in energy flow (e.g., food for fish, ducks, raccoons and humans).[1,6] The species is a prime candidate for future freshwater aquaculture. Conversely, its ability to infest raw water intakes and canals and to clog power plant condensers has been recognized by the national press[7,8] as well as the scientific community.[1,9-11]

This interaction with raw water consumers and the concomitant necessity for controlling fouling have resulted in the major immediate impact of C. manilensis on man. Chlorination, usually continuous during some part of the reproductive period, has been the most usual method for reducing fouling by Corbicula.[11] However, the extreme toxicity of chlorine to freshwater organisms[12] and the possibility of creating toxic stable chloro-organics[13] suggest that this practice is not environmentally sound. Other chemicals such as general molluscicides have similar disadvantages.[11] Isom[11] suggested heat as a more environmentally sound alternative and presented preliminary evidence suggesting its efficacy. Evaluation of this potential method is possible only after a thorough examination of the thermal tolerance of these clams.

There have been two previously published studies on aspects of the thermal tolerance of C. manilensis.[11,14] Habel[14] acclimated clams

to about 23°C and exposed them for four days to temperatures between 29 and 38°C. He found the upper incipient lethal temperature for this acclimation to be 34°C. Isom[11] acclimated clams to 10°C and exposed them to temperatures ranging from 21 to 50°C. All exposures greater than 43°C killed the clams within minutes. Our studies were begun to extend Isom's preliminary work and to obtain thermal tolerance data that could be reasonably extrapolated to real situations.

MATERIALS AND METHODS

The Asiatic clams, C. manilensis, used in these tests were collected at Mile 23 on the Clinch River, Tennessee. Clams used in most tests ranged from 24 to 35 mm in longest dimension [shell length (SL)]; most were about 30 mm. In the cold tolerance tests some groups of clams 22-24 mm in shell length were used for comparison. Clams in the larger size group are about 4 years old, while those in the smaller size group are about 2 years old.[15]

The clams were held in 190-liter aquaria supplied with constantly flowing well water and with temperatures maintained at ±0.5°C. Acclimation was carried out at a rate of less than 1°C per day to temperatures of 5, 10, 15, 20, 25, 30 and 32°C. Clams did not survive attempted acclimation to 2 or 35°C. All clams were held at the acclimation temperature for at least one week prior to testing and, as far as possible, all acclimation temperatures were tested simultaneously. Clams were thus held in the laboratory for 4 to 5 weeks prior to test initiation.

During acclimation and testing, clams were fed a powdered mixture of Biorell tropical fish flakes and trout chow. Food addition to each aquarium was calculated to make at least 0.01 gm dry weight of food available to each clam per day. The amount of food added was based on (1) maximum growth rate of Corbicula calculated from data of Heinsohn,[16] (2) physical characteristics of the aquarium system (water turnover time, number of clams in the tank, etc.), (3) a filtration rate of about 1 liter/hour (Mattice and Nodvin, unpublished data) and (4) an assumed 50% assimilation.

Though each of the types of tests was begun and ended in the same way, some of the details differed. Each experimental group consisted of 20 clams held in screened 4-liter plastic containers. Tests were initiated by direct transfer of the containers and clams without water from the acclimation tanks to the test temperatures. The combinations of acclimation and test temperatures are given in Table 1. Controls were maintained in the acclimation tanks. The tolerance tests included exposing clams to:

1. long-term stress higher than acclimation temperature (long-term heat stress),
2. long-term stress at temperatures colder than acclimation temperature (long-term cold stress), and
3. 30-minute shocks at temperatures above incipient lethal temperature (heat shock).

In the latter experiments the shock temperature was maintained at ±0.2°C except during the initial 2 or 3 min when variation was ±0.4°C. Following the 30-min shock, clams were immediately transferred back to the original acclimation temperature. In all the experiments mortality was tested at varying intervals; usually several times the first day, twice a day for the first week and at increasing intervals as the mortality rate decreased. When the rate of control deaths equalled the rate of experimental deaths, observations were terminated. This period of observation extended from three to five weeks.

Death of the clams was determined in two ways. If the clam was open, the body was gently prodded ("gape test"). If the clam was closed, a knife was carefully inserted between the valves and gently twisted

("twist test"). If no closing response could be observed following either test, the clam was considered dead. In questionable cases, the clam was isolated at the appropriate temperature for more careful observation in the next scheduled test.

Most tolerance limits and comparisons were based on median survival times. Calculation of these median survival times at given test temperatures was accomplished by assuming a normal distribution of percent mortality. In this procedure, percent mortality (on a probit scale) is plotted against the log of death time.[17] In cases where control deaths had also occurred, the number of control deaths was subtracted from the experimental deaths to give a corrected percent mortality at each time interval. A line was fitted by eye through the points to estimate the time to 50% death (e.g., Fig. 1). The same graphic methods were used to determine the test temperatures causing 100% mortalities in the heat-shock experiment. In the 5°C acclimation group 15% of the controls died in less than 2 hr. This early mortality was ignored in estimating the test temperature causing 100% mortality. The experiment was ended when the corrected percent mortalities remained the same for two weeks. At this point we assumed that the effect of temperature on mortality was ended.

Since it is common to report temperatures yielding 50% mortality after 24, 48 and 96 hr, an analysis was made to compare the validity of the results obtained after each time interval. Regression lines were fit across acclimation temperatures for the incipient lethal temperatures obtained for the 24-, 48-, and 96-hr and final time intervals. The regressions were compared to determine whether or

not a single linear regression could adequately be used to describe the relation for each of the different exposure times and the total effect. This was done by comparison of the residual sums of squares and the calculation of an F statistic.[18]

RESULTS

Long-Term Heat Stress

At each acclimation temperature, a decrease in exposure temperature increased resistance time (Fig. 2). Although resistance times ranged over 2 or more orders of magnitude, decrease in test temperature ultimately reached a temperature which failed to cause 50% mortality no matter how long it was applied. This limit is indicated by the oblique line at the right of Fig. 2. This line cuts through times of about 300 to 500 hrs. Approximately one-third of the points plotted in Fig. 2 are times longer than 96 hr; i.e., 50% mortality does not occur in 96 hr. Extremely rapid mortality of all clams, including controls at 15°C acclimation, indicated the effect of a second factor beside temperature. Since this seemed due to a problem in developing the twist test, these results have not been presented here.

Incipient lethal temperatures (50% mortality) based on 24-, 48- and 96-hr testing intervals do not yield equivalent results to those obtained when the total effect of temperature is realized, i.e., when control and experimental death rates are equal (Fig. 3a). Each of the 24-, 48- and 96-hr regressions was significantly different from the total effect ($P < .01$).

Long-Term Cold Stress

Results of exposure to long-term cold stress indicated that a decrease in acclimation temperature enabled the clams to withstand lower test temperatures. Few combinations of acclimation and test temperature resulted in 50% mortality. Clams could not be acclimated

to 2°C. The variation in the data used to calculate 50% mortality levels was greater than for the long-term heat stress data because of the difficulty in determining death at the lower temperatures. Smaller clams (22-24 mm SL) exhibited similar mortality to larger clams, when both were acclimated to 20°C and exposed to 2°C (Table 2). Neither group experienced mortality at 7.5°C. Minimum time to 50% mortality for any of the test temperatures was 200 hr.

Short-Term Heat Shock

In the heat-shock tests, the upper lethal temperature (50% mortality) varied depending on whether it was assessed at 24, 48 or 96 hr or when experimental and control death rates were equal (Fig. 3b and c). Although production of 100% mortality was the object of the shock temperatures, the 50% level was used for comparisons because of the increased accuracy of estimate. Data for 1973 and 1974 are similar, but the 96-hr regression for the 1974 data was not significantly different ($P > 0.25$) from the total effect. All other times were significant: for the 24-hr regressions from both 1973 and 1974 and the 48-hr regression from 1974, $P < .01$; for the 48-hr regression from 1973, $P < .025$; and for the 96-hr regression from 1973, $P < .05$.

Summary of Thermal Tolerance

The upper and lower incipient lethal temperatures (50% mortality) derived from the long-term heat and cold stress define a temperature tolerance range for adult C. manilensis (Fig. 4). The incipient lethal temperatures are shifted upward by acclimation to warmer temperature

and downward by acclimation to cooler temperature. The upper ultimate incipient lethal temperature is about 34°C, and the lower ultimate incipient lethal temperature is about 2°C.

The temperatures which produce 100% mortality after a 30-min exposure also increase with acclimation to warmer temperatures. The 100% mortality levels obtained in 1973 and 1974 were not significantly different ($P > .75$). The upper lethal temperature for 30-min exposure was 43°C but was only 3°C less at the 5°C acclimation temperature.

DISCUSSION

The value of continuing observations over a long time period (until control and experimental death rates are equal) was consistently demonstrated in our measures of thermal tolerance. Habel[14] carried out heat-stress experiments on Corbicula for slightly over 96 hr following acclimation to approximately 23°C. His 50% mortality level agreed very closely with our 96-hr results at similar acclimation temperature (25°C), but was 3.5°C higher than our total effect level for that acclimation group. Thus, short observation periods result in an overestimate of tolerance. This overestimate would be expected to be greatest during long-term exposures. Dickie[19] noted that median mortality levels of the giant scallop (Placopecten magellanicus) at 120-hr exposures were about 0.8°C lower than those at 48-hr exposures. Our data demonstrate that the period of observation can have a much greater effect on determination of upper incipient lethal temperature for Corbicula than for the scallop.

Because of the length of time (2-4 months) required for acclimation and testing, the feeding regime is important. The nutritional state of the clams can apparently alter the results of resistance tests quite significantly. The results of our long-term heat stress were considerably different from those obtained by C. C. Coutant (unpublished M.S.), who found that clams acclimated to 15 and 20°C were less resistant to heat stress than those acclimated to 5 and 10°C. He concluded that this reversal of the usual order resulted from the increased maintenance requirement of clams at higher temperatures and from starvation. The clams Coutant used were collected

from a potentially nutrient-poor environment (a water supply tank) and were not fed while in the laboratory. Our results show the usual increase in resistance with increase in acclimation temperature. The most reasonable explanation for the difference is the effect of the nutritional state of the clams.

Part of the variability in our data, particularly at low temperatures, was undoubtedly due to difficulty in accurately determining death. The criterion for death was a lack of response, but response included a range from immediate closure of the valves to a very slight movement of the mantle. At the cold temperatures (10°C or less) the response could be extremely slow. It was at these low temperatures that the twist test was most valuable because the clams usually remained closed. On the other hand, at these temperatures the twist test may have slightly stressed the clams. A more accurate criterion for death would have been helpful, but probably would not have significantly affected the 50% mortality levels.

The potential for stressing the clams with the twist test was demonstrated by the results of the long-term exposures to high temperature. Clams at the 15°C acclimation temperature were the first ones tested in this way. It was obvious from observation of the clams that the twist test had been applied too vigorously and the clams forced open in a manner that injured them. Further tests were carried out more carefully, with pressure being applied only to the extent that resistance was obvious; however, the data at 15°C acclimation was faulty and had to be discarded.

The long-term studies presented here have practical applicability to assessment of power-plant impacts. The instantaneous temperature

changes to which the clams were subjected at the start of each of our tests may not be representative of the usual conditions near power-plant discharges. However, rapid changes in the temperature of discharge waters can occur. A recent shut-down in Alberta, Canada[20] resulted in a 16.9°C drop within 30 min. Certainly very rapid increases of temperature can occur at start-up or during fluctuations in the spatial location of the discharge plume as well.

This study is a first step in the overall examination of C. manilensis and its interaction with power plants. Except for the tests on 2-year-old clams (22-24 mm) exposed to long-term cold stress, tests reported here were carried out on 4-year-old adults (24-35 mm). No significant difference was found in tolerance, but further testing of different age groups will be necessary since it is the veliger larval stage which infests and fouls various types of intake structures. The heat-shock tests indicate the feasibility of using hot water (e.g., at 43°C for 30 min) to remove adult clams small enough to pass through the condenser tubing. Either reversal of water flow, where that is possible, or a feedback loop to recycle discharge water to the intake for short periods of time could provide lethal temperatures. Removal of clams before they can clog condensers or intake screens would prevent the costly shutdowns necessary for mechanical cleaning, while avoiding the long-term toxicity problems involved in the use of chlorine. However, results obtained using adult clams can only provide general guidelines for the potentiality of preventing fouling by the veligers. Final decision regarding the efficacy of using heated water to prevent larval fouling is dependent upon studies of larval thermal tolerance.

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Table 1. Acclimation and test temperature ranges used for measuring thermal tolerance of Corbicula

Acclimation Temperature °C	Long-term High Temp °C[1]		Long-term Low Temp °C[2]		High Shock Temp °C 1973[3]		1974[4]	
	Low	High	Low	High	Low	High	Low	High
5	24	36	2	5	32	42	37	43
10	24	36	2	5	36	44	37	43
15	--[5]	--[5]	2	10	40	46	37	43
20	24	36	2	10	40	48	37	43
25	26	39	2	15	42	50	37	44
30	30	39	2	15	40	46	37	44
32	34	39	not tested		not tested		37	44

[1]Test temperatures were at 2°C intervals except for tests at mid-range (i.e., 30,31, 32°C).

[2]Test temperatures used included 2,5,7.5,10 and 15°C where appropriate.

[3]Test temperatures were at 2°C intervals except in the 15 and 20°C acclimation groups for which 1°C test intervals were used.

[4]Test temperatures were at 1°C intervals from low to high temperature listed.

[5]Data are not presented; see text for explanation.

Table 2. Acclimation and incipient lethal temperature and times to 50% mortality determined for clams exposed to long-term low temperatures

Acclimation Temperature °C	Incipient Lethal	Time to 50% Mortality (hr)		
		2°C	5°C	7.5°C
5	~2	—	—	—
10	~2	—	—	—
15	~2	—	—	—
20	est. 4	290	—	—
25	est. 7	510	560	—
30	est. 9	220	420	—
205	2 < x < 7.5	200	—	—

Figure 1. The results of one of the long-term high temperature tests. Percent mortality (in probits) is plotted against log time for groups of clams acclimated to temperatures of 5, 10, 20, 25, 30, and 32°C and exposed to 36°C. No line is drawn for the 5°C data because of the variability.

PERCENTAGE
2% 5 10 15 20 30 40 50 60 70 80 85 90 95 98%
PROBABILITY
X 3 LOG CYCLES
KEUFFEL & ESSER CO.
46 8080
MADE IN U.S.A.
PROBIT
98% 90% 80% 70% 50% 40% 30% 20% 10% 5%

Figure 2. A summary of the time in hours to 50% mortality (on a log scale) plotted against test temperature. Lines are calculated regressions for data at each acclimation temperature. The dashed line is a construct which was drawn to the right of the lowest test temperature which would cause 50% death at a given acclimation temperature.

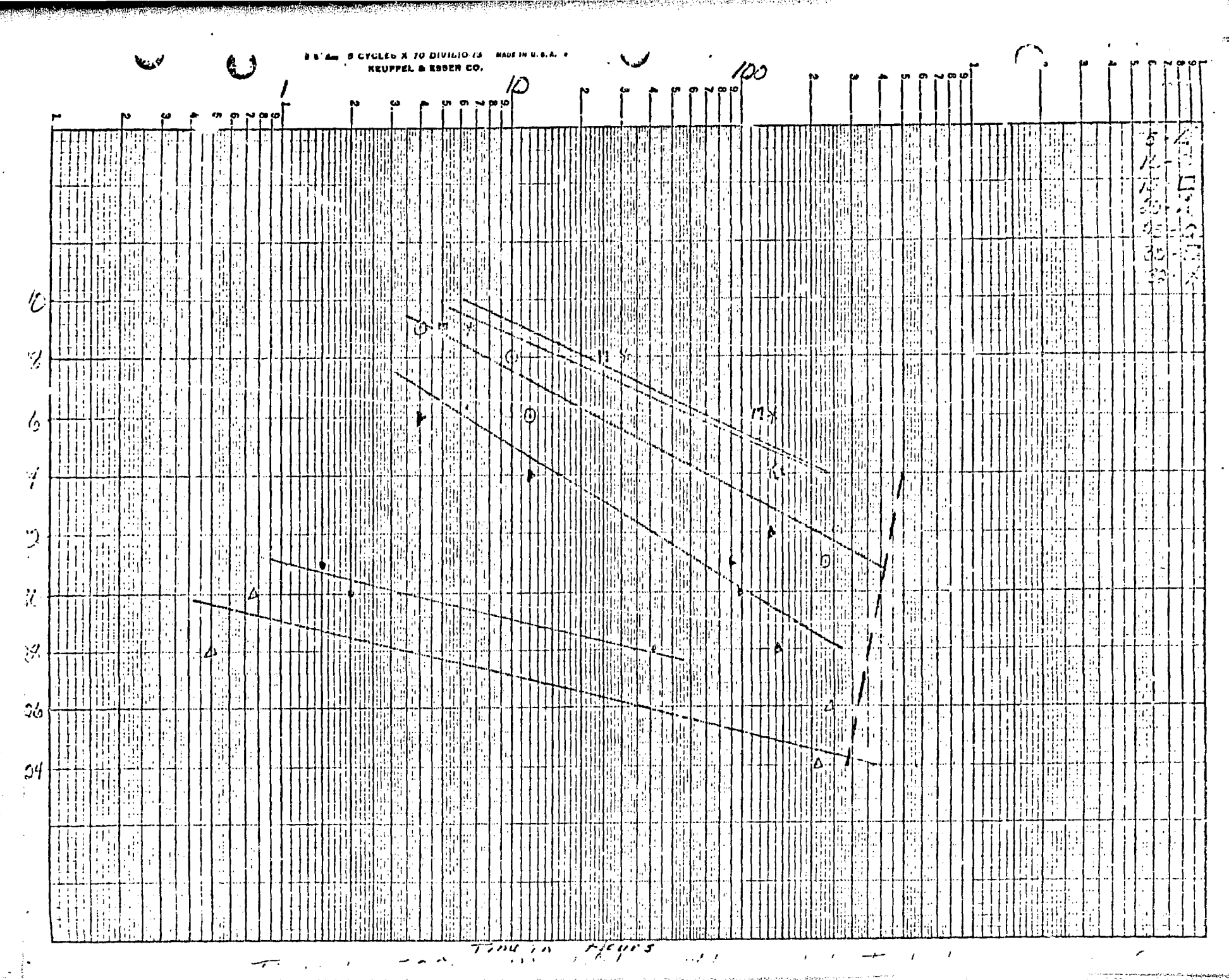
KEUFFEL & ESSER CO.
1
10
100
10
8
6
4
3
2
1
36
24
TIME IN HOURS

Figure 3. A comparison of the upper lethal temperatures (50%) obtained from observations made at 24, 48 and 96 hr with that obtained by continuing observations until experimental and control mortality rates are equal. Regression lines for each time interval are shown across the various acclimation temperatures for (a) long-term heat stress and for short-term heat shock in (b) 1973 and (c) 1974.

F.A.B.a.

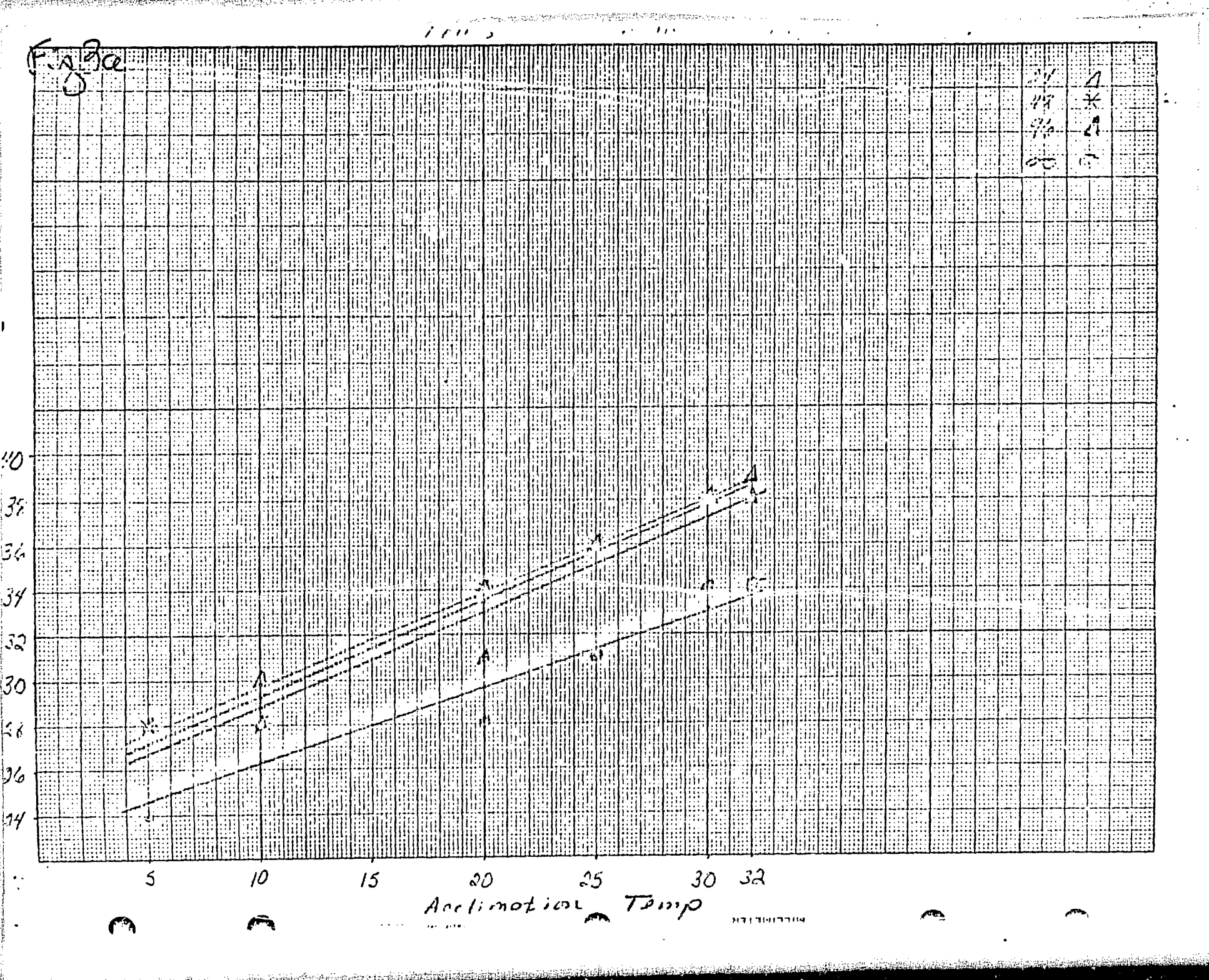

Fig 3b


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ACCLIMATION TEMPERATURES

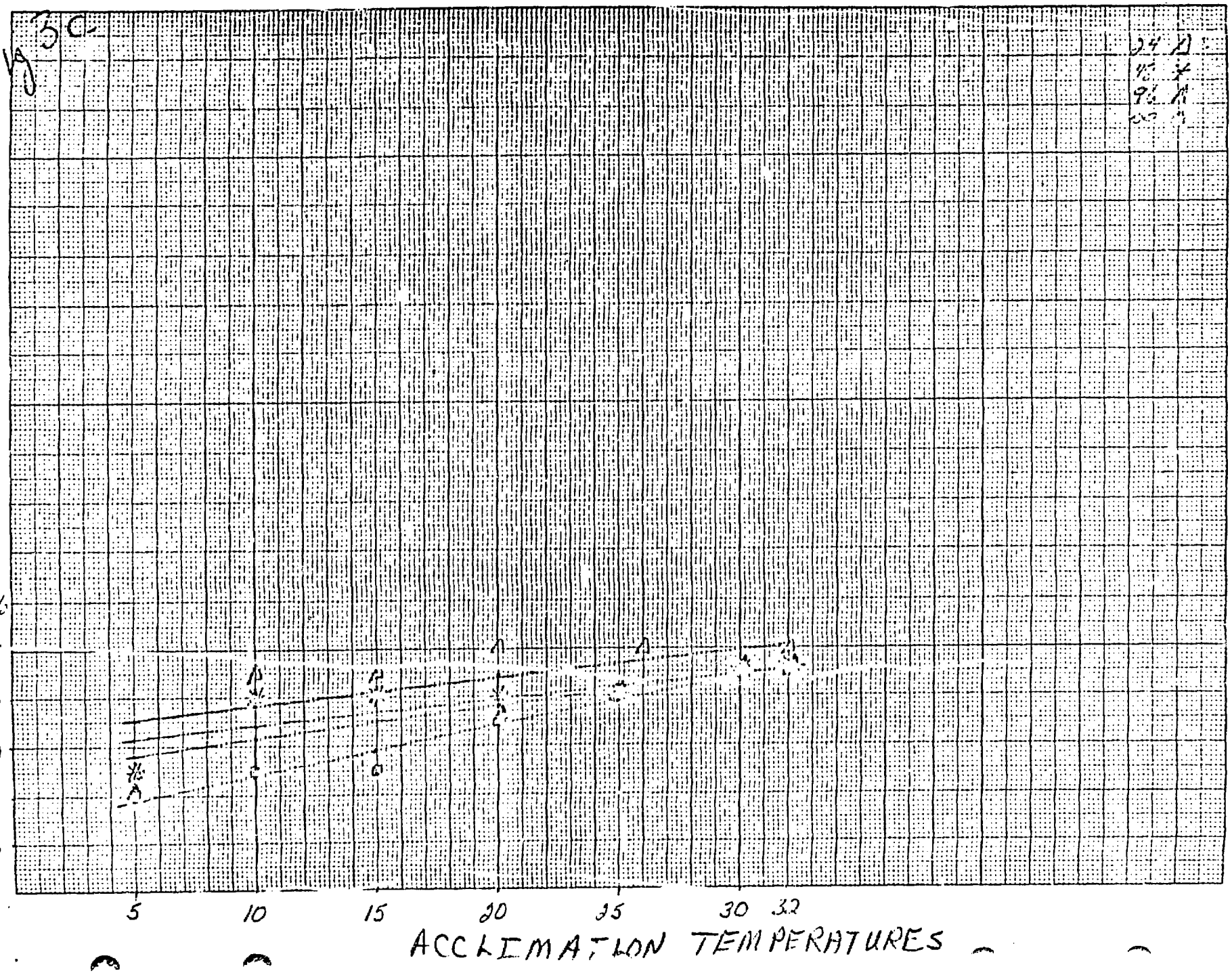
Fig 5C
TEST TEMPERATURE
46
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40
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25
30
32
ACCLIMATION TEMPERATURES

Figure 4. Summary of the thermal tolerance of Corbicula manilensis. Long-term upper and lower thermal tolerance limits (50%) and temperatures resulting in 100% mortality after 30 min are shown. Upper and lower incipient lethal temperatures were about 34 and 2°C, respectively.

Fig 4

Δ - 1973
* - 1974