HEAT DEATH AND ASSOCIATED WEIGHT LOSS OF THE OYSTER *CRASSOSTREA VIRGINICA* [1]

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High and low lethal temperatures have been ascertained for many organisms. In general, temperatures for heat death of terrestrial animals (insects, reptiles, birds, and mammals) are high, around 45°C. Temperatures causing heat death in moist air dwellers (earthworms, frogs) are lower than for inhabitants of dry air. Still lower temperatures are lethal for aquatic forms, some normally living in cold waters die of heat shock even below 30°C (Prosser et al., 1950).

Responses of oysters to changes in temperature have been noted by several investigators. The majority have dealt with the effects of temperature upon larval viability (Churchill, 1921; Seno, Hori, and Kusakabe, 1926; Clark, 1933) and spawning (Galtsoff, 1931, 1932; Hopkins, 1931a; Loosanoff, 1939; Nelson, 1928a, b; Stauber, 1950). Stauber (1950) described three physiological types of *Crassostrea virginica* on the basis of differences in minimum spawning temperature. The respective temperatures required for spawning in Long Island Sound, the Bideford River, Price Edward Island, Canada, and Delaware Bay were 16.4°, 20°, and 25°C.

Hopkins (1931b) determined the influence of temperature changes upon shell movements in Olympia oysters, *Ostrea lurida.* He concluded that changes in water temperature were more important than the actual water temperature in determining how long the shells remained open each day. Falling temperatures caused the shells to close while opening followed a temperature rise. Shells of oysters kept at 17°C were open a greater proportion of the day than the shells of oysters at the other temperatures tested. In 1935 Hopkins found *Crassostrea gigas* had an optimum temperature of 27-28°C for pumping activity.

Henderson (1929) determined the upper lethal temperature for several lamellibranchs. The oyster *Crassostrea virginica* survived a higher temperature than any of the other molluscs he used. His experiments showed that 48.5°C was lethal for the oyster when the temperature was raised at the rate of 12°C per hour.

Fingerman and Fairbanks (1956) demonstrated that body fluids were lost by oysters whose valves were kept apart by means of wedges.

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These investigators postulated that the oyster has very little ability to control the volume of its body fluids under conditions of stress. They concluded that oysters must be free to open and close their shells in order to control the volume of their body fluid. The present study was undertaken to test the hypothesis presented by Fingerman and Fairbanks (1956). Heat was chosen as the stimulus that would be used to place the oysters under stress.

Experiments and Results

First and second year specimens of the oyster *Crassostrea virginica* grown in Louisiana were maintained in the laboratory in aquaria containing water at a temperature of 22-23°C and with a salinity of 17 ‰. The water was continually recirculated and filtered through cotton, glass wool, and charcoal. An acclimatization period of three to 10 days in the stock aquaria was allowed before the oysters were used in an experiment.

Survival and body fluid loss of oysters heated at different rates.— As stated above, Henderson (1929) determined that 48.5°C was the lethal temperature for oysters heated gradually at the rate of 12°C per hour. The temperature of the water was 15°C at the start of his experiments. The oysters were examined frequently. He assumed that all oysters would gape when they were heated. When an oyster could not close its shells when tapped, it was returned to the 15°C aquarium for three to 12 hours and observed again in order to determine whether or not recovery had occurred, *i.e.* whether the shells would close when tapped. Henderson assumed that the thermal death temperature lay between the highest temperature from which recovery occurred and the lowest point from which no recovery was observed.

In the first series of experiments of the present investigation a technique similar to that employed by Henderson was used. In addition to determining survival of oysters heated gradually, measurements of weight loss upon heating were performed. Weight change was expressed as a percentage of the weight of the oyster body and shell fluid, exclusive of the shells themselves, at the beginning of each experiment. The original weight of the body and fluid in the shell cavity was calculated by shucking the oysters when they were removed from the constant temperature baths, finding the shell weight, and subtracting it from the total weight of shells, oyster body, and shell fluid determined at the start of the experiment. The oyster shells were blotted prior to weighing in order to remove excess moisture. In two experiments that will be described later notches were cut into the shells by means of a carborundum wheel and the shell fluid was shaken out. Weight changes of these oysters were expressed as a percentage of the original body weight.

Henderson's criterion for death was gaping by oysters three to 12 hours after removal from the test tanks. The results of some preliminary experiments revealed that Henderson's criterion for death was not valid because all oysters killed by heat do not necessarily

gape at any time during the course of an experiment or during the subsequent 12 hours. The oyster may be killed while the adductor muscle is fully contracted with the result that no gape is evident. According to the criterion of Henderson such oysters would be counted among the survivors. In view of this fact, after a heat exposure the oysters used in the present investigation were returned to the holding tanks for three to seven days to determine accurately the number of oysters that had been killed during the course of an experiment. After this period of time the shells of oysters killed with their adductor muscle contracted gaped because of muscle decay. Oysters with gaping shells were not considered dead until they showed no signs of movement when handled and none of the oysters with closed shells were considered dead until they showed some sign of putrefaction such as gas bubbles and tissue disintegration. In several cases fungal growth was also evident. Oysters that had died with their adductor muscle fully contracted began to gape due to the action of the ligament when the muscle disintegrated.

In each experiment of this series oysters were removed from the stock aquaria, blotted, weighed, and then placed into water baths at 24°C. The water was then gradually heated to 45-55°C. For each experiment a different rate of temperature rise was used. One group was heated at the rate of 0.74°C per hour, another at 4.5°C per hour, and a third at the rate of 13.2°C per hour. At each of the preselected temperatures 10 oysters were removed from the water baths, drained, and weighed in order to determine the weight loss that had occurred during the exposure period, and then replaced in the aquaria for three to seven days when the number of survivors was determined.

Oysters that died as a result of exposure to heat generally did so within five days and almost certainly within seven days after termination of the exposure. The survivors of high sublethal temperatures remained in a weakened condition with shells agape and gave no closing response to shadows but did respond to handling or tapping on the aquarium by closing the shells for a short time. These weakened suvivors apparently regained strength gradually over a period of two to three weeks after exposure because they became better able to close their shells rapidly and to keep them closed for longer periods of time. The gape also became more restricted and definite closing responses to shadows could be observed. Finally the survivors appeared normal.

With two exceptions that will be described later, no attempt was made to exclude the weight of the shell fluid from the oysters prior to their use in these experiments because of the possibility than any physical stimulus necessary to accomplish this would result in compensatory secretion of additional shell fluid by the body that would result in the introduction of another variable. Presumably the shell fluid lost by an oyster would represent a constant percentage of its original body weight, although not necessarily the same percentage for all oysters, and would therefore interfere minimally with the com-

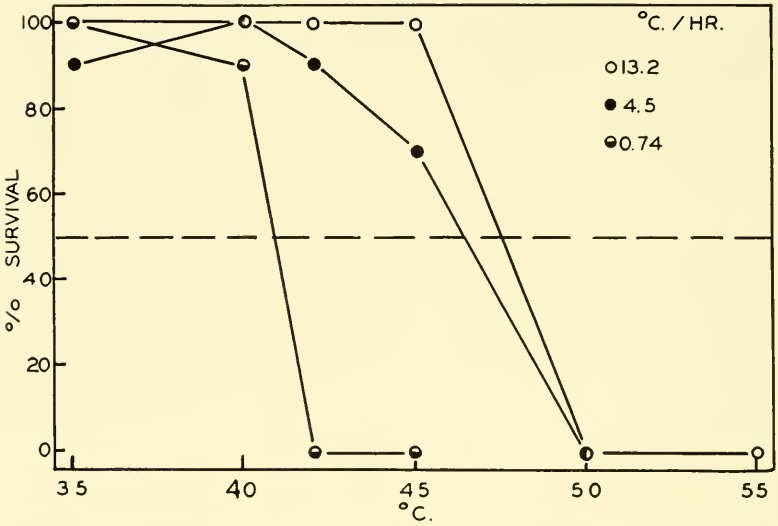

Figure 1. Percentage survival at temperatures reached at three different rates of increase.

parative aspect of the results.

The results of this series of experiments are presented in Figures 1 and 2. Figure 1 represents the percentage survival at temperatures attained by means of three different rates of increase. The dashed horizontal line represents the 50 percent mortality level. In some experiments the time required to kill 50 percent of the oysters was determined from inspection of the figures whereas in others the temperature at which 50 percent of the oysters was killed was determined from inspection of the figures. Fifty percent values served as bases of comparison of the results of different experiments. Use of the time required to kill 50 percent of the organisms in an experiment as determined by inspection of a plot of the data is a standard technique in pharmacology and has been used by several investigators in studies of temperature tolerance in fishes, *e.g.* Brett (1952).

As is evident from Figure 1, the slower the rate of temperature increase, the lower was the temperature required to kill 50 percent of the oysters. With rapid temperature increase (13.2°C per hour) 50 percent were killed at 47.5°C, 100 percent at 50°C; whereas with the slowest temperature rise (0.74°C per hour) 50 percent were killed at 41°, 100 percent at 42°C. With a 4.5°C per hour increase, the 50 percent mortality level was reached at 46.5°C; there was no survival at 50°C.

The computed lethal temperatures are not the temperatures at which the tissues died but are the computed temperatures of the water bath. A time lag in penetration of the heat into the oyster body was evident due to the time required for heat to penetrate the

shells. The lag should obviously be greatest with the most rapid rate of increase of temperature, whereas with the slowest rate temperature changes of the oysters should keep pace more readily with temperature changes of the water bath.

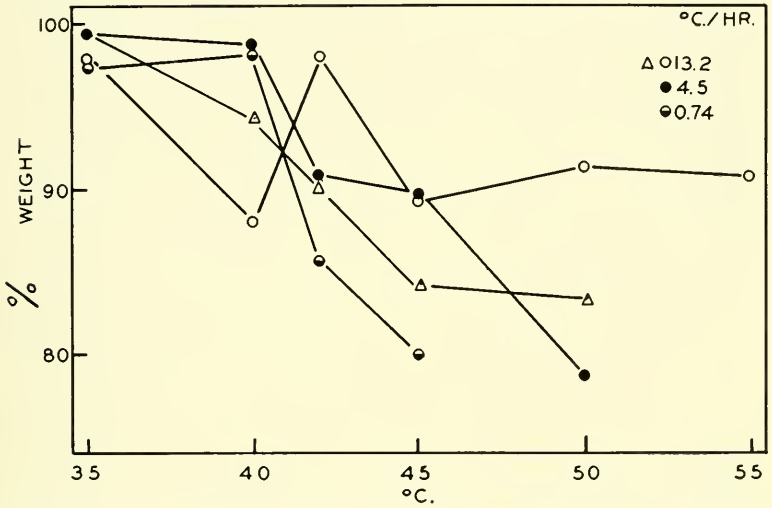

Figure 2. Percentages of original weight remaining after exposure to temperatures reached at three different rates of increase. See text for explanation of "original weight".

In Figure 2 are presented the weight losses of the same oysters represented in Figure 1. The triangles in Figure 2 do not apply to this experiment. Their significance will be described below. Apparently loss of weight is associated with heat death. The critical point in weight loss appears to be about 41°C where the weight loss of the rapidly heated oysters leveled off at approximately 10 percent of the original weight. At about 41°C differences in survival among the three groups of oysters were also manifested. The weight loss was beyond doubt due to loss of body fluid and shell fluid by the oysters. Body fluid must have been the primary source of weight loss. Some mucus and wastes may also have been expelled but in quantities insignificant in comparison with the fluids lost.

The following experiment was performed in support of the contention that most of the weight loss is due to body fluid. Notches were cut into the shells of 40 oysters and the free shell fluid drained. The oysters were then weighed, placed in a water bath, and heated at the rate of 13.2°C per hour. Ten oysters were removed when the temperature reached 40°, 42°, 45°, and 50°C respectively. The free fluid was then shaken out, the shells were blotted, and the oysters were weighed. The oysters were then shucked and the shells were weighed in order to determine the original body weight so that weight changes

could be expressed as percentages of the original body weights. The data were presented in Figure 2 (triangles). As is evident from inspection of the figure, the notched oysters lost a greater percentage of weight than the intact oysters heated at the same rate. There is, therefore, no doubt that heating oysters induced a loss of body fluid.

Survival and changes in body fluid volume of oysters placed at constant temperatures.—The second series of experiments utilized an abrupt rather than a gradual thermal change. Oysters that had been in the stock aquaria no less than three days were taken, blotted, weighed, and put into constant temperature baths containing estuarine water at one of a series of constant temperatures for different exposure periods. The water baths were set at 35, 40, 42, and 45°C. At the end of the exposure period the oysters were removed, blotted, and reweighed. After the second weighing the oysters were returned to the stock aquaria. One hour later those that had been in the 42° and 45°C water baths were weighed again and returned to the stock aquaria for three to seven days when the number of survivors was determined. The percentage survival as well as percentage of the original weight was calculated for these oysters also. The term "original weight" refers to the weight of the oyster body and shell fluid at the start of the experiment.

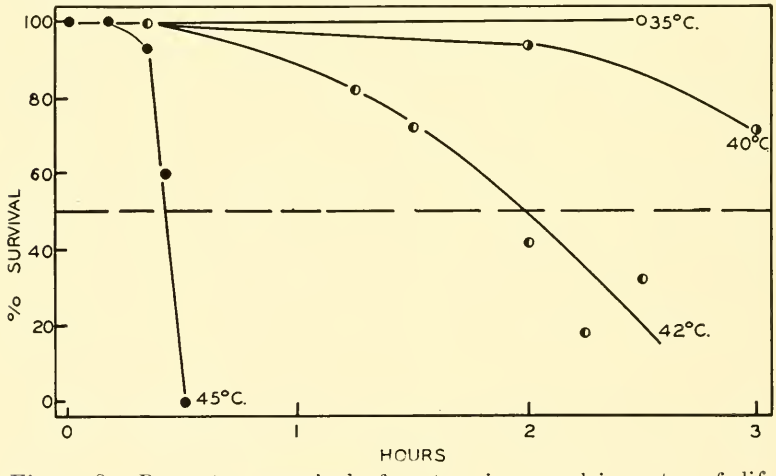

Figure 3. Percentage survival of oysters immersed in water of different temperatures.

Figures 3, 4, and 5 present the results of these experiments. Figure 3 shows the percentage survival in water of different temperatures. Figure 4 presents the weight losses in different temperatures. The triangles in Figure 4 do not refer to this experiment. Figure 5 gives the weight changes of the oysters 60 minutes after they were removed from the water baths and immersed in water at 22-23°C. The per-

centages in Figure 5 are based upon the weight of the oyster body and shell fluid when the oysters were removed from the water bath. The points in the figures represent from 13 to 43 oysters. The average number in Figure 3 is 24.2; in Figure 4, 27.2; and in Figure 5, 30.3. The 50 percent mortality level is represented in Figure 3 by the dashed horizontal line.

As is evident from Figure 3 no heat death occurred among oysters exposed to 35°C. After three hours of exposure to 40°C 29 percent of the oysters died. At 42°C 50 percent mortality was observed after approximately two hours of exposure; increased exposure duration resulted in progressively higher mortality rates. The 50 percent mortality level was reached after only 25 minutes at 45°C; complete mortality occurring with five additional minutes of exposure to 45°C. Therefore, the higher the temperature of the water bath, the faster the oysters were killed; the time of exposure being important in determining the lethal temperature. The same percentage of the oysters could have been killed by short exposure to high temperature or long exposure to a lower temperature.

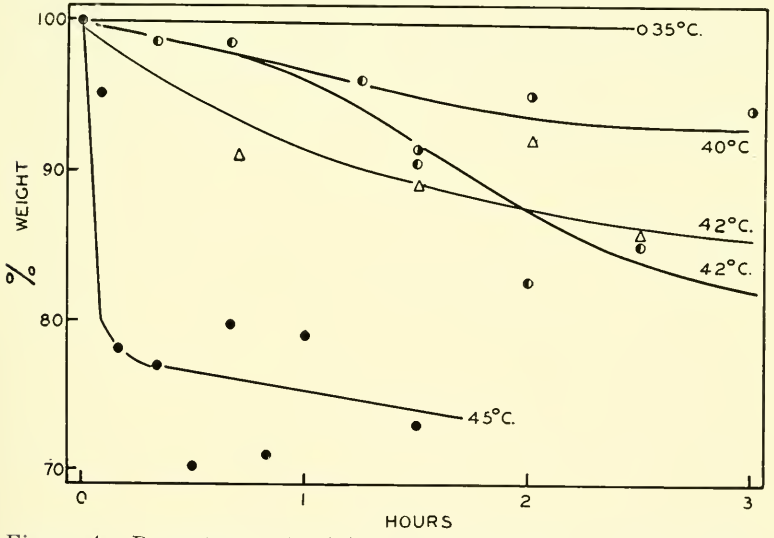

Figure 4. Percentages of original weight remaining after exposure to different temperatures.

The percentage weight loss determined at the time the oysters were removed from the water baths can be determined from Figure 4. Inspection of the families of curves shown in Figures 3 and 4 reveals a striking similarity between them that is highly suggestive of a relationship between exposure time, temperature, survival, and weight loss. Oysters placed in estuarine water at 35°C for 2.5 hours lost only 0.5 percent of their body weight and none died. Oysters kept

at 40°C lost six percent of their original body weight after three hours; 29 percent died. Oysters maintained at 42°C lost weight at a still more rapid rate, reaching a 15 percent loss of original body weight after 2.5 hours; the 50 percent mortality level was reached after 117 minutes of exposure. Oysters in 45°C water lost weight very rapidly, approximately 25 percent of the original body weight was lost in five minutes; 50 percent died after 25 minutes of exposure.

The highest temperature used (45°C) in this series of experiments caused the greatest weight losses and the most rapid death. For the same exposure period greater survival and less weight loss were evident at 42°C than at 45°C. For any given percentage survival, *e.g.* 50 percent, oysters that died following exposure to 42°C lost less weight than oysters killed by exposure to 45°C. Therefore, heat was the primary cause of death and not loss of weight because the oysters were killed after losing different amounts of their original weight. Heat death was probably due primarily to heat inactivation of vital enzymatic processes and structural protein denaturation (Prosser et al., 1950). Weight loss was probably merely a secondary cause of death. More weight was lost by oysters kept at 45°C than at 42°C probably because of rapid destruction of membrane semipermeability with a concomitant loss of body fluid; whereas at 42°C death occurred more slowly due to slower inactivation of the vital mechanisms including those whereby tissue fluids are kept within the body.

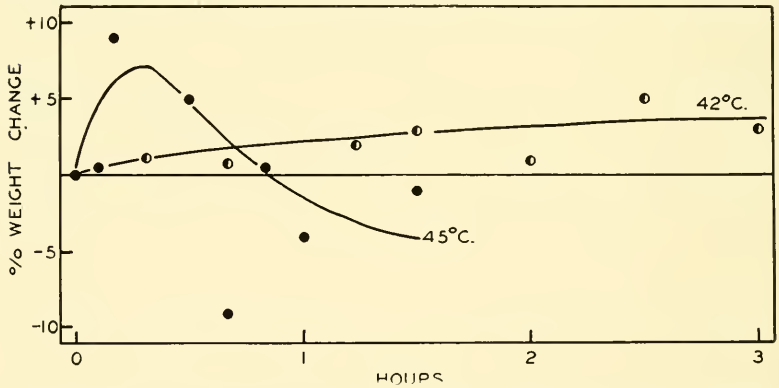

Figure 5. Weight changes of oysters 60 minutes after they were removed from the water baths and immersed in water at 22-23° C. Percentages are based upon the weight of the oyster body plus shell fluid when first removed from the water bath.

As is evident from Figure 5 oysters were able to take back fluids in direct proportion to the amount of weight lost. When oysters were placed in 22-23°C estuarine water after being in 45°C estuarine water for 10 minutes they rapidly regained a large amount of fluid. However, when oysters had been in 45°C water for more than 40 minutes, instead of taking back fluids they continued to lose weight.

At 42°C where death and weight loss were not as rapid as at 45°C, they were able to continue taking back fluids over a considerable period of time. They, however, did not return to their original weight.

To test further the hypothesis presented above that the weight loss was primarily due to escape of body fluids the following experiment was performed. Notches were cut in the shells of 40 oysters, the shell fluid was drained, the shells blotted, and the entire oyster weighed. The oysters were then placed into a water bath maintained at a constant temperature of 42°C. Ten oysters were removed periodically, the shell fluid was drained, shells blotted, and the entire oyster was weighed. The oysters were then shucked and the weight of the shells was determined. Weight changes were calculated as a percentage of the original body weight and presented in Figure 4 (triangles). As evident from inspection of this figure the results obtained with notched and intact oysters maintained at 42°C were comparable. During the first hour of immersion notched oysters lost more weight than intact ones but the difference between the weight loss of the two groups was not as evident during the next 90 minutes of immersion. Obviously, heat induced a loss of body fluids. This

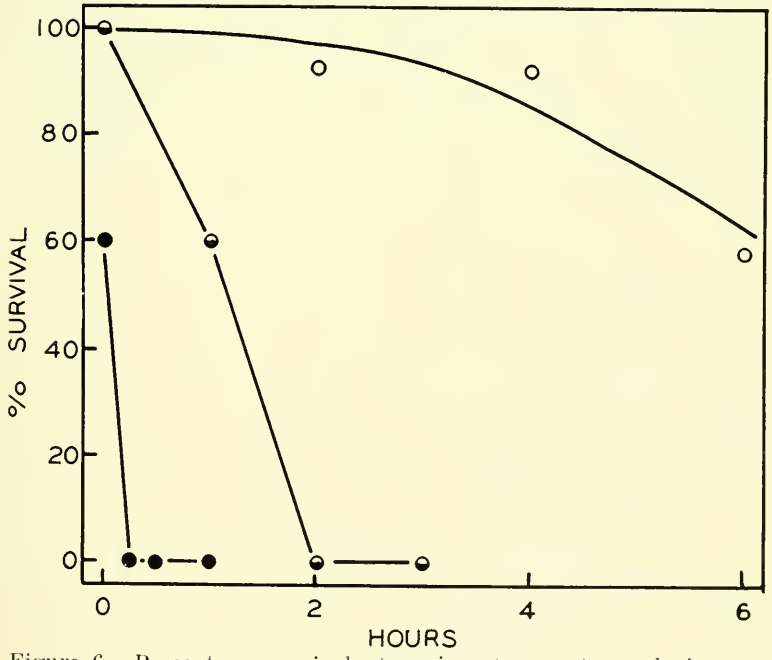

Figure 6. Percentage survival at various temperatures during exposure of oysters to constant temperatures of 40°, 42°, and 45° C. Oysters were immersed in water at 24° C and heated at the rate of 4.5° C per hour to the respective test temperatures. Symbols: circles, 40° C; half-filled circles, 42° C; dots, 45° C.

loss of body fluid must have been responsible for the vast proportion of the weight losses shown in Figures 2 and 4.

Survival of oysters gradually heated and then maintained at a constant temperature.—In the final series of experiments oysters were taken from the stock aquaria and placed in a water bath at 24°C. The water in the bath was then gradually heated at the rate of 4.5°C per hour. When the temperature of the bath reached 40°C the temperature of the water was maintained constant. The experiment was repeated with constant temperatures of 42° and 45°C. Twelve oysters were removed from the water bath when the temperature reached the desired level and at selected intervals thereafter. The oysters were then placed in the stock aquaria for three to 10 days when the number of survivors was determined. The results of this series of experiments are shown in Figure 6. The percentage survival at zero time represents the percentage surviving after the temperature of the water bath had attained the desired level. The results of this series of experiments were essentially the same as shown in Figure 3, *i.e.* oysters were killed more rapidly at higher temperatures. Gradual increase of temperature to 42°C and 45°C, in contrast to direct immersion, caused a more rapid death. For example, in this experiment 50 percent of the oysters were killed approximately three minutes after the water had reached 45°C whereas 50 percent were not killed until after 25 minutes of exposure when oysters were directly immersed in water of 45°C. Evidently much of the lethal effect was produced while the water was heating from 42°C to 45°C since all the oysters survived when removed from the water bath when the temperature had reached 42°C.

General Discussion

The results of these experiments support the hypothesis of Fingerman and Fairbanks (1956) that bleeding is one of the responses of oysters exposed to injurious stimuli. Apparently oysters have not evolved protective mechanisms whereby they can prevent excessive losses of body fluids when exposed to harmful stimuli inasmuch as under natural conditions they can close their shells and isolate themselves from injurious agents in their environment. However, when they are unable to withstand the harmful stimuli as in the experiments reported above and those of Fingerman and Fairbanks (1956) the generalized response appears to be a loss of body fluids.

Fingerman and Fairbanks (1956) demonstrated that wedging open the valves of an oyster, a mechanical stimulus, caused this generalized bleeding. Likewise heat, a physical stimulus, evokes a generalized loss of body fluids. Presumably noxious chemical stimuli could evoke the same generalized bleeding response.

Henderson (1929) found 48.5°C was the upper thermal death temperature. However, as is evident from the present results, death will occur at lower temperatures if oysters are exposed for long periods of time. Furthermore, Henderson considered the lethal temperature

was that temperature between the highest one from which all the oysters recovered and the lowest temperature from which no recovery was observed. He heated his oysters at the rate of 12°C per hour and found 48.5°C was the lethal temperature according to his criteria. The rate of 13.2°C per hour used in the present investigation is comparable to the rate employed by Henderson. Fifty percent of the oysters heated at the rate of 13.2°C per hour were killed at 47.5°C, a value quite similar to the value determined by Henderson. However, oysters from a cooler climate such as those of Henderson would be expected to be killed at a lower temperature than Louisiana oysters. He obtained his specimens from Buctouche, New Brunswick, where the mean water temperature is lower than that of the oyster producing waters of Louisiana, the source of the oysters used in the present investigation. Hathaway (1927) reported that lethal temperatures are lower for organisms from cooler environments. The unexpected result obtained by Henderson is probably due to the criteria for death that he employed. He considered oysters to be dead only if the shells gaped within 12 hours after exposure to high temperatures. However, in the present investigation the observation was made that some oysters will die with their adductor muscles fully contracted. Such oysters do not begin to gape until three to seven days after exposure to high temperature. Furthermore, with a high rate of rise of temperature fewer oysters are killed with their valves agape than with a low rate of temperature rise. According to the criteria of Henderson oysters that had been killed with their valves shut were considered alive giving a higher lethal temperature than the correct one, thus accounting for the unexpectedly high value obtained for the New Brunswick oysters.

Some of the data presented above appeared difficult to interpret and inconclusive without the aid of certain inferential tests. The chi-square test for common distribution seemed to be an appropriate non-parametric test (Walker and Lev, 1953) that could be applied with minimum assumptions to the data of Figure 2 and portions of the data of Figures 1 and 4.

Since the observed weight losses of the four different distributions represented in Figure 2 were all recorded at comparable temperatures and the notched oysters appeared to have lost no more weight than any of the intact oysters it was assumed that the oysters subjected to any one rate of temperature increase would have lost no more at higher temperatures than those recorded. No survival beyond the calculated time of maximum weight loss at these temperatures and rates (fig. 1) supports this assumption in indicating an early death precluded further weight loss. Five hypotheses were tested with the chi-square test at the five percent level of significance. The first hypothesis tested, involving the data of Figure 2, was that the weight losses of intact oysters subjected to different rates of increasing temperature, 0.74°C per hour, 4.5°C per hour, and 13.2°C per hour,

all have a common distribution. The hypothesis was rejected ($P < 0.0005$).

The second hypothesis tested, also involving the data of Figure 2, was that the weight losses of intact oysters subjected to two different rates of increasing temperature, 4.5° per hour and 0.74°C per hour, and the weight loss of notched oysters subjected to the rate of 13.2°C per hour all have a common distribution. The hypothesis was accepted ($P = 0.645$).

The third hypothesis tested involved the data of Figure 4 that presented the weight losses of intact oysters subjected to direct immersion in estuarine water at 42°C and notched oysters immersed directly into estuarine water at 42°C. The hypothesis was that the two conditional weight loss distributions are the same. The hypothesis was accepted ($P = 0.081$).

Acceptance of the second and third hypotheses tested shows that the observations do not support a conclusion that the weight losses of notched oysters are different from the weight losses of intact oysters. The variability of weight losses of the notched oysters (fig. 4) appears to be as great as that of the intact oysters. In Figure 2 the data for notched oysters show only slightly less variability in weight loss distribution than do the intact oysters with the exception of the intact oysters subjected to 13.2°C per hour increase in temperature.

Acceptance of the second hypothesis tested shows that the observed weight loss distributions of oysters subjected to the rates of 4.5°C per hour and 0.74°C per hour do not support a conclusion that weight losses associated with these two rates are different from each other or different from weight losses by notched oysters subjected to 13.2°C per hour. Under these circumstances then the conclusion may be reached (from the sample evidence and the inferential tests) that rate of increase in temperature as such has very little influence over extent and rate of weight loss. Apparently, therefore, temperature and time only determine rate and extent of weight loss.

The fourth hypothesis tested involved the three conditional distributions of percentage survival represented in Figure 1. The hypothesis was that oysters subjected to the three different rates of increasing temperature, 13.2°C per hour, 4.5°C per hour, and 0.74°C per hour, have a common conditional distribution of percentages of survival. The hypothesis was rejected ($P < 0.001$).

The fifth hypothesis tested involved the two conditional distributions of percentage survival, 4.5°C per hour and 13.2°C per hour, represented in Figure 1. The hypothesis was that oysters subjected to the two different rates of temperature increase, 13.2°C per hour and 4.5°C per hour, have a common conditional distribution of percentages of survival. The hypothesis was accepted ($P = 0.600$).

Summary

1. Experiments were designed to determine the upper thermal limit for the oyster *Crassostrea virginica* and to determine if loss of body fluid is associated with heat death.

2. All oysters survived temperatures below 35°C with no significant weight loss. Above 41°C appreciable death and weight loss occurred among the oysters. The slower the rate of temperature increase the lower was the temperature at which 50 percent of the oysters were killed. However, no significant correlation was evident between rate of temperature rise and weight loss.

3. Oysters can be killed by short exposure to high temperatures or long exposure to lower temperatures.

4. Oysters exposed to different temperatures for the same period of time lost more weight at higher temperatures than at low temperatures. Weight losses have been interpreted as due primarily to loss of body fluid.

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Abstract

Oysters were taken directly from stock aquaria that contained estuarine water (salinity 17 $^{0}/_{00}$) at 22-23° C, weighed and placed for different periods of time in constant temperature baths maintained at 35, 40, 42, and 45° C. They were then removed, reweighed, placed in the stock aquaria for one hour, weighed again, and returned to the stock aquaria for several days when the percentage survival was determined. All oysters survived 35° C with no significant weight loss. Twenty-nine percent of the oysters exposed to 40° C for three hours died, 50 percent were killed in 117 minutes at 42° C, and 50 percent were killed in 25 minutes at 45° C. Evidently oysters can be killed by short exposure to high temperature or long exposure to a lower temperature. Oysters exposed for the same interval lost more weight at higher temperatures. Oysters were able to take back fluid in direct proportion to the amount lost during the exposure period. In the second series of experiments oysters were gradually heated from 24° to 45-55° C. The slower the temperature increase, the lower the temperature required to kill 50 percent of the oysters. With rapid temperature increase (13.2° C per hour) 50 percent were killed at 47.5° C, whereas with slow temperature rise (0.74° C per hour) 50 percent were killed at 41° C. Evidently, the lethal temperature varies with the conditions of the experiment.