THE EFFECTS OF SOME CONSTANT AND SOME CHANGING CONDITIONS OF SALINITY ON THE DEVELOPMENT AND MORTALITY OF THE EGGS AND LARVAE OF THE PACIFIC HERRING CLUPEA PALLASII CUVIER

by

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF ARTS
in the Department
of
Zoology

We accept this thesis as conforming to the standard required from candidates for the degree of MASTER OF ARTS.

Members of the Department of
Zoology

THE UNIVERSITY OF BRITISH COLUMBIA

April, 1951

ABSTRACT

Methods were developed for the successful artificial fertilization and rearing of the eggs and larvae of the Pacific herring, Clupea pallasii Cuvier. Effects of various constant and changing conditions of salinity (0°/oo to 34.28°/oo) on the development, mortality and hatching of eggs and on the mortality of larvae were studied. Evidence of an optimum salinity, 11.55°/oo to 16.24°/oo, for development and survival of eggs is presented, although a wide salinity tolerance, 6.06°/oo to 34.28°/oo, for both eggs and larvae is evident. Eggs transferred to pond water (0°/oo salinity) during the first few days of development, perish within a few hours, but if transferred at a later stage, they will survive for at least two days. No apparent correlation exists between survival of eggs and magnitude of salinity change. The presence of two critical stages during embryonic development, the first at blastopore closure, the second prior to and during hatching, have been shown. An optimum survival of eggs was obtained on egg slide sections containing from 75 to 125 eggs.

ACKNOWLEDGMENTS

The author wishes to express his grateful appreciation to Dr. W. A. Clemens, Head of the Department of Zoology, and to Dr. W. S. Hoar, Professor of Zoology and Fisheries who supervised the work, gave willing assistance, provided the experimental equipment and criticized the final paper.

The writer is indebted to the B. C. Packers Company Ltd. for financial aid in the form of a scholarship, and to Dr. J. L. Hart, Director, Pacific Biological Station who kindly provided facilities for the impoundment of live herring; to Mr. A. Hourston, Mr. J. L. Lanigan, Mr. R. Isaacson, and other members of the staff of the Biological Station who devoted much time and effort to the capture and transport of live herring.

Valuable assistance has been received from Dr. P. A. Larkin of the Department of Zoology and B. C. Game Commission in the statistical analysis, and from Mr. G. J. Spencer, Department of Zoology, for his help in the photographic procedures.

Thanks are extended to those fellow students who at numerous times assisted in the carrying out of the experimental procedure.

Finally I would like to express thanks to my wife,

Doreen, for her valuable assistance and encouragement in the preparation of the manuscript, and to Mrs. D. P. Scott who so promptly and efficiently typed the manuscript.

TABLE OF CONTENTS

TABLE OF CONTENTS, Continued

INTRODUCTION

The Fisheries Research Board of Canada has been studying, for a number of years, the life history and fluctuations in abundance of the Pacific herring Clupea pallasii Cuvier. The aim of these studies is the establishment of a stable commercial fishery. In exploited areas the fishery depends, to a large extent, on three-year-old herring, the age at which they enter the spawning runs and commercial fishery. Earlier stages therefore, have received considerable attention. One feature emerging from these studies is the apparent independence of the size of the incoming year class as related to the amount of spawn from which it originated (Tester and Stevenson, 1949).

Fraser (1916) estimates the total mortality from egg to adult to be at least 99.99%. Hart and Tester(1934), in their studies of mortality on herring spawning grounds, conclude that the initial mortality of eggs due to "natural" causes is about 5% while that resulting from the ravages of birds and other animals accounts for no more than another 5%. If the above figures be accepted then, the greatest mortality takes place not on the spawning grounds but in the sea at some time between the larval and adult stages. However, Hart and Tester (1934) have also suggested that stormy weather might increase initial mortality considerably. It seems then, that any extreme environmental factor could

cause considerable mortalities during embryonic and larval stages of development.

Kelley (1945) showed that mortality of herring eggs under natural conditions could not have been due to respiratory difficulties caused by low CO_2 or high O_2 tensions. Ford (1929) carried out preliminary experiments on the effect of various temperatures and salinities on the European herring, Clupea harengus L. These experiments indicated a definite tolerance range in each case.

The following experiments were therefore undertaken in an attempt to indicate the effects of some constant and some varying salinities on the survival and development of the Pacific herring, Clupea pallasii.

Salinity is an environmental condition which remains remarkably constant in the open ocean but can vary considerably in inshore waters. As herring spawn inshore, often near river or stream outlets, it is suggested that the degree of salinity might, in certain localities, be a limiting factor in the survival of eggs and larvae. In the spring of 1949, on one of the larger spawning grounds of the North Pacific, (Queen Cove, Esperanza inlet) eggs were deposited in areas over which a considerable range of salinity was recorded, i.e. 8.21 $^{o}/_{oo}$ to 27.68$^{o}/_{oo}$. Limited observation indicated that these eggs seemed to develop and hatch normally.

METHODS

APPARATUS

Herring eggs and larvae have been reared as far as the yolk-absorption stage by several investigators (Ford, 1929; Kelley, 1945). However, as culture methods have not been standardized or described completely, preliminary rearing experiments were undertaken in 1950. During these initial experiments conditions of constant salinity and temperature were maintained. The apparatus was of such a design that the intensity of light at any one time was nearly constant over the whole tank.

Constant Temperature:- On the spawning grounds, during early development of the herring, temperatures varied between 5.8° C. and 10.0° C. In the rearing experiments a temperature of 8.5° C.(+ or - 0.2° C.) was maintained by incorporating both a heating and a refrigerating unit in the apparatus (Figure 1).

Constant Salinity:- Sea water, collected from English Bay, was adjusted to a salinity of 27.43°/oo (+ or - 0.5°/oo) by dilution with distilled water or by the addition of sea-salt. Two samples of sea water from Departure Bay, where herring were captured for stripping on February 22, 1950, showed salinities of 27.99°/oo and 26.02°/oo.

REARING

Eggs of the Clupeidae have, as a rule, the form of

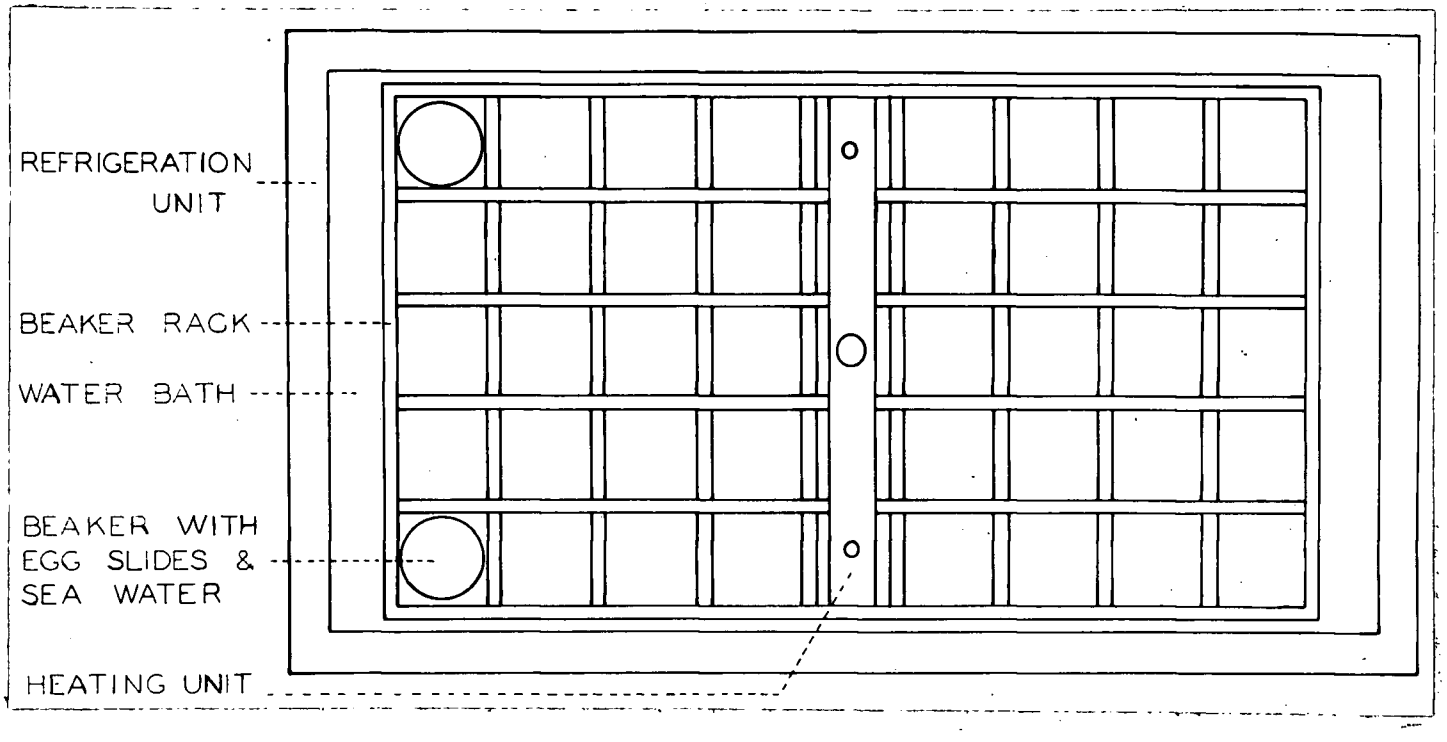


Figure 1. Plan view of the constant temperature bath showing the beaker-retaining rack and heating unit. The refrigerating unit measures $4\frac{1}{2}$ x $6\frac{1}{2}$ feet.

small, translucent, glassy spheres, possessing a strong hard shell or chorion (like transparent horn). They may cling together in a spongy mass, or they may form a film of transparent pellets on stones, algae, shells or debris. Eggs which cling together like those of the herring are coated with a tenacious mucous. As they fall through the water, they are fertilized by the milt of the male which clouds the water. On reaching the bottom, the external coat hardens so that the eggs adhere to one another and to the substrate. This property of adhering to the substrate lends itself rather conveniently to the laboratory rearing of herring eggs, as eggs stripped from a ripe female readily adhered to a glass slide, thus facilitating their handling and observation.

FERTILIZATION

Herring eggs were artificially fertilized in the following manner:

1. eggs were stripped onto one side of a glass slide so that they formed rows, one or two layers in thickness;

2. the egg slide was placed in a container of sea water of the desired salinity and temperature;

3. milt was then stripped into the container;

4. after remaining in the milt and sea water

solution for about ten minutes, the egg slides were transferred, unwashed, into 600 ml. rearing beakers containing about 400 cc. of sea water, and

5. the rearing beakers were placed in the constant temperature bath by means of a wooden rack (Figure 1), and the water aerated continuously.

OBSERVATION OF THE EGGS

An estimate of the survival of herring eggs reared under laboratory conditions was the main concern of preliminary experiments; consequently, daily mortalities were recorded. The chorion and vitelline membranes are widely separated in dead eggs, hence live and dead eggs were readily distinguished (Figure 5). In addition the chorion, formerly elastic, becomes soft and is readily punctured. For observation, slides were removed from the rearing beakers, placed in petri dishes containing sea water of the same temperature and salinity, and observed with a binocular microscope.

PRELIMINARY EXPERIMENTS

OBJECT OF PRELIMINARY EXPERIMENTS

In 1950 experiments were undertaken to determine:

1. the survival of eggs taken artificially, fertilized in the field and then transported to the laboratory;

2. the fertility of eggs and sperm and the survival of eggs taken and fertilized from dead fish which had been stored on ice for a short period;

3. the survival of eggs taken artificially and fertilized from live fish in the laboratory;

4. to provide a description of the external changes in the early development of the herring, and

5. the effect of various diets on the survival of larval herring.

RESULTS OF PRELIMINARY EXPERIMENTS

Experiment 1:- Effects of transportation.

Mature herring for this experiment were seined from Departure Bay, Nanaimo, B.C., on February 22, 1950. Fertilization was carried out immediately after capture. The egg slides were then placed in ordinary slide boxes which, in turn, were immersed in two-gallon jars of sea water. Ice, packed around the base of each jar, held the temperature of the sea water within the range of 7.5° C. to 8.5° C. during transport. In this manner eggs were transported to the University of British Columbia, the trip lasting about five hours. After reaching the laboratory the egg slides were transferred to rearing beakers and then to the constant temperature bath.

Figure 2 shows that 6,558 eggs were fertilized on

February 22 and at the end of the twelfth day, by which time all surviving eggs had hatched, there were 123 larvae, a survival of 1.9%.

Experiment 2:- Survival of eggs taken and fertilized from stored fish.

In this experiment a number of mature male and female herring were iced and shipped to the laboratory. Following 16½ hours storage, the fish were stripped and the eggs fertilized as in the above procedures. Following fertilization, the egg slides were placed in the constant temperature bath and daily observations of mortality noted.

Commencing with 1,254 eggs on February 23, 111 eggs hatched by the end of the twelfth day, a survival of 8.9% (Figure 2).

Experiment 3:- Survival of eggs taken and fertilized from living fish, following which no transportation of the fertilized eggs took place.

Through the courtesy of Mr. D. Outram of the Pacific Biological Station, live herring were transported from Departure Bay to the laboratory in Vancouver. Eggs were stripped and fertilized from these fish as in the above experiments. Following fertilization, the egg slides were placed in the constant temperature bath and were not touched until the following day. Figure 2 shows that through this treatment there is a survival of 53.7%, i.e.

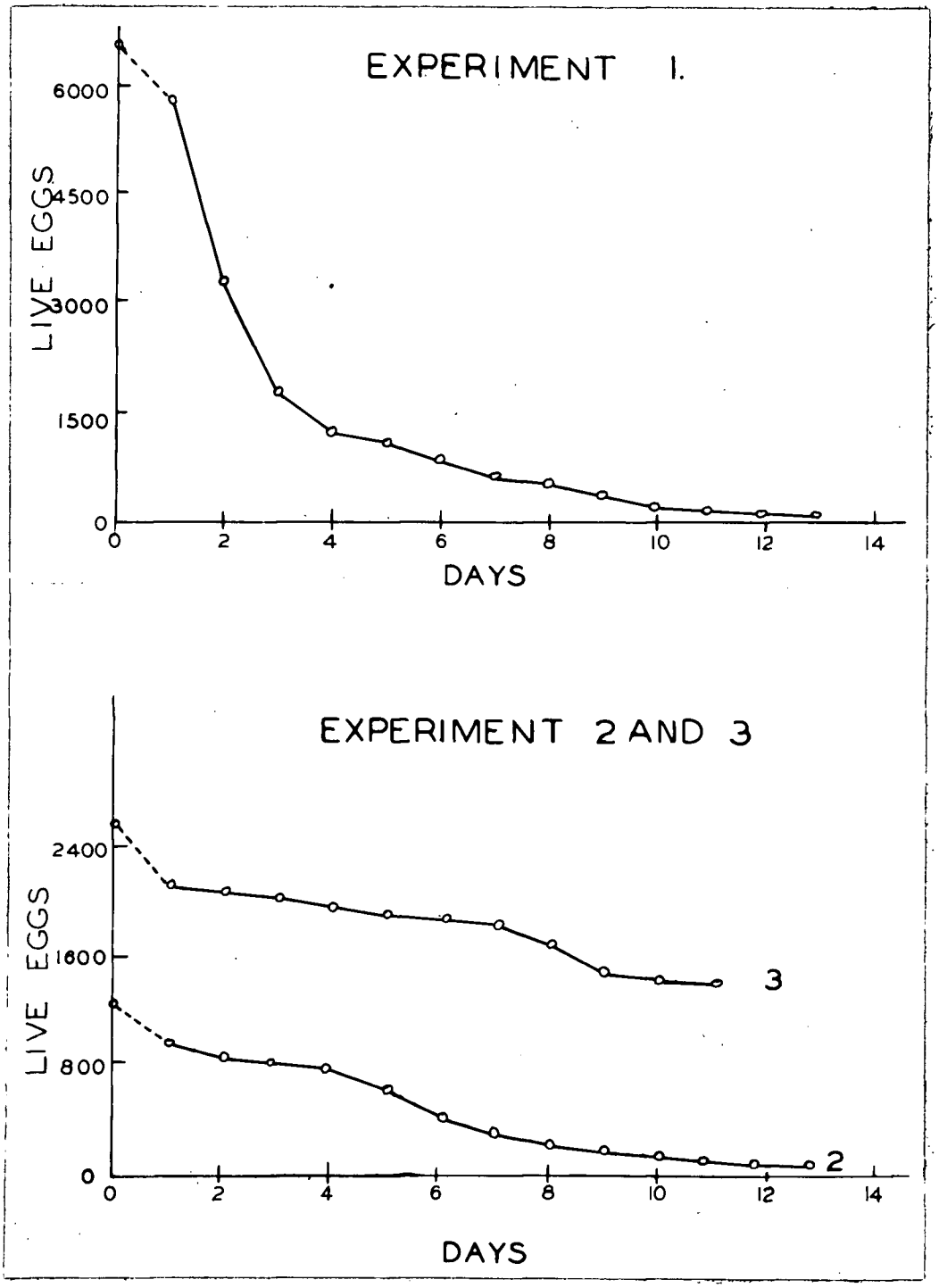


Figure 2. Survival of herring eggs in experiments 1,2, and 3, during development from fertilization to hatching.

1,426 eggs hatched from an initial 2,656 (Figure 2). Figure 2 was derived from the data given in Table I (Appendix).

DISCUSSION OF RESULTS OF EXPERIMENTS 1, 2 and 3.

Regression coefficients were calculated for the data in Table I, and the significance between coefficients tested by using the formulae given by Simpson and Roe (1939: 277 - 280). The statistical analysis indicates that no significant difference exists between the coefficients of experiments 1 and 2. In other words, the survival of eggs in these two experiments cannot be considered to differ. However, in the case of experiments 1 and 3, a significant difference at 0.02 probability was indicated (Table II, Appendix).

On the basis of the above results, it is evident that experiment 3 produces the greatest survival of eggs.

Figure 3 shows the data from Figure 2 plotted on semi-log paper. Figure 3 indicates that the lines as plotted seem to fit the data better than if one straight line had been fitted to the scatter diagram. Perhaps the explanation for the occurrence of several inflections in the survival curves for these experiments lies in the possibility of there being a differential susceptibility of the embryo to external influences. This susceptibility is dependent upon the stage of development. Apparently there are three points of inflection, these inflections occurring at approximately

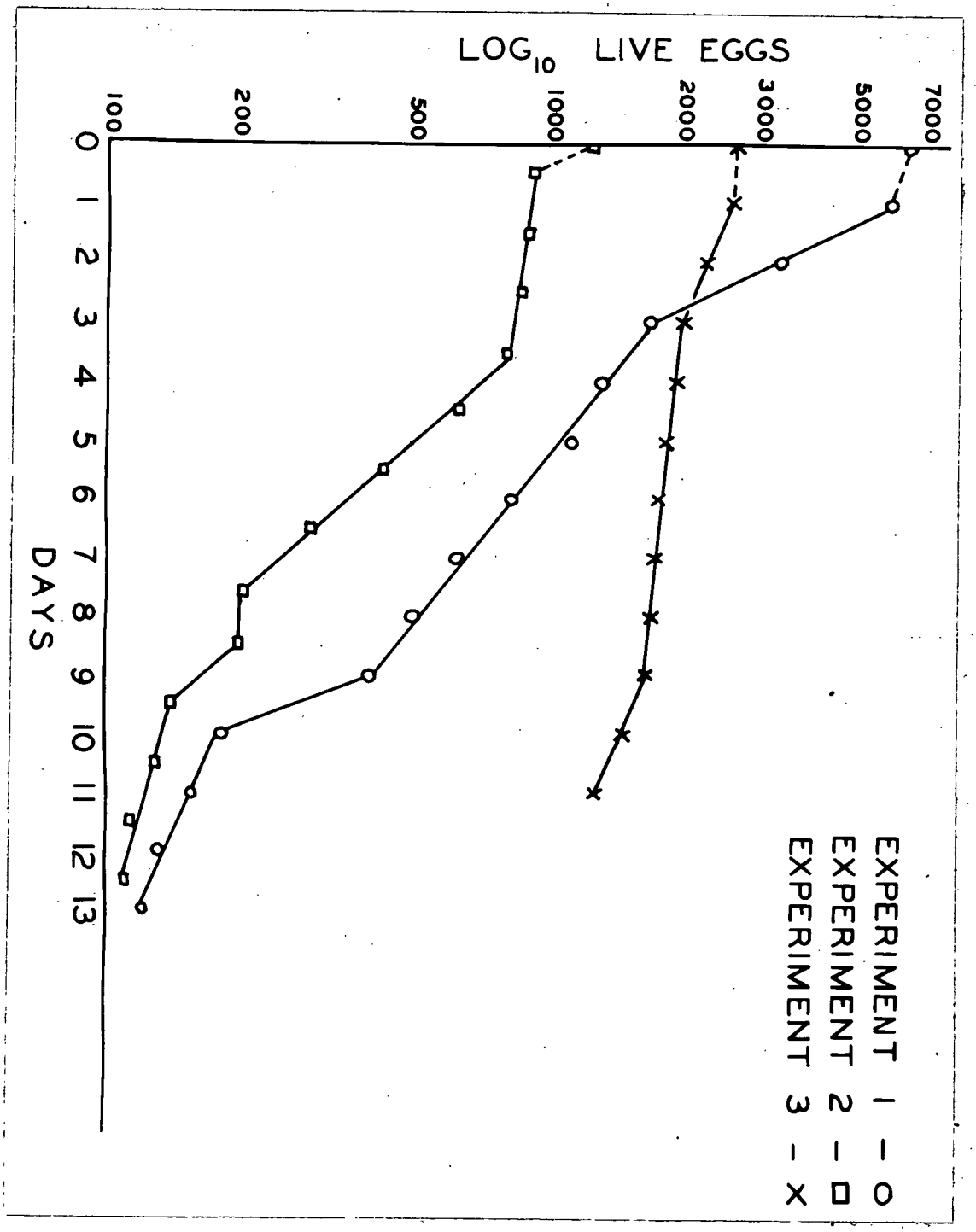


Figure 3. Semi-$\log_{10}$ plot of the survival of herring eggs in experiments 1, 2 and 3, showing the points of inflection at various stages of development.

the same time in each of the three experiments (Figure 3). A number of investigators have observed that the susceptibility of fish eggs to external influences changes during the incubation period.

Battle (1944), in her studies on the embryology of the Atlantic salmon, Salmo salar Linnaeus, concludes that the periods of greatest mortality during development would seem to be during cleavage and blastoderm formation, up to blastopore closure, and at hatching. Hein (1907 and 1911) is quoted by Battle (1944) as having observed in the case of eggs of the brown trout, Salmo fario, subjected to various types of adverse conditions, a gradual decrease in susceptibility to the closure of the blastopore and a subsequent increase just prior to hatching. Battle (1929) found that the lethal temperature of the developing eggs of the four-bearded rockling, Enchelyopus cimbrius, increases from fertilization to a maximum just prior to the closure of the blastopore, then decreases before rising to a second high value immediately preceding hatching. Worely (1933) states that for the mackerel: "apparently the stages of germ ring formation and epiboly and the somite multiplication stages are critical periods in the development of the embryo". Hindroth (1942) states that "the critical point in the life history of the salmon Salmo salar L. is the last egg stage".

Finally, Hayes (1949) found that salmon eggs are most likely to die either about the time of blastopore closure or shortly before hatching. He states that the former critical period may be due to rupture of the vitelline membrane, the latter to failure of sufficient oxygen to reach the embryo through the egg capsule.

In the case of herring eggs, the first inflection appears at the end of the first day of development. This period is represented by a dotted line in Figure 3. The mortality is made up of eggs which died subsequent to fertilization plus non-fertilized eggs. It would seem then that the first inflection does not really represent a "critical period" in the survival of herring eggs, as the differences in percent loss during the first day (Figure 4) can probably be explained on the basis of the number of eggs which died as a result of non-fertilization. In experiments 1 and 3, in which eggs and sperm were taken from living fish, the survivals at the end of the first day were 89.4% and 80.3%. In experiment 2, in which eggs and sperm were obtained from stored fish, the survival at the end of this period was 73.4%. The differences in percent survival at the end of the first day might be explained on the basis of sperm motility and egg viability, i.e. eggs taken from live female herring would certainly be more viable than those taken from refrigerated females, and also

the sperm cells from living male herring, when viewed under the microscope, are seen to be much more active than those taken from stored fish.

Figure 3 indicates that during days one to three in experiments 1 and 3 there is a pronounced increase in mortality, the period corresponding to blastopore formation and closure. In experiment 2 the mortality rate appears to decrease over the same period. This observation does not appear so anomalous when it is considered that in the latter experiment, a higher mortality was indicated during the first day. Thus, eggs which would have perished during days two to three probably died during the first day of development, yielding an apparent decrease in mortality in the following day or two.

In all experiments the third inflection appears during the eighth to tenth day, the period immediately prior to and during hatching. The important feature about these inflections in the various experiments seems to be that they occur at approximately the same time (the same stage of development) in each experiment. These times correspond to germ ring formation, blastopore formation and closure, and finally hatching. The critical periods, then, in the development of the herring egg occur at the same time as the critical periods were found to occur in other teleostean embryonic development. It might be possible in future

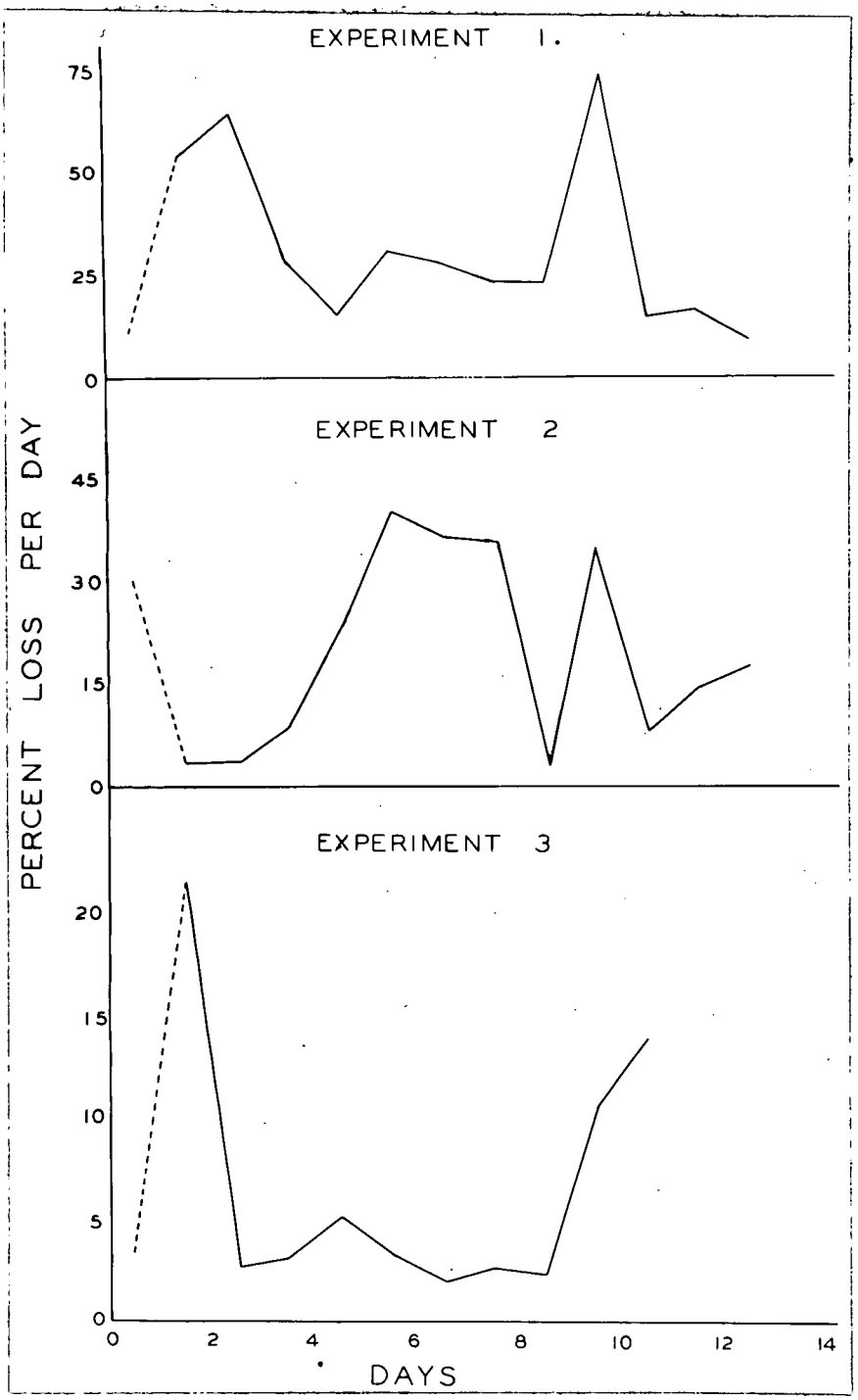


Figure 4. Graphs indicating the percentage loss per day of herring eggs during experiments 1, 2 and 3.

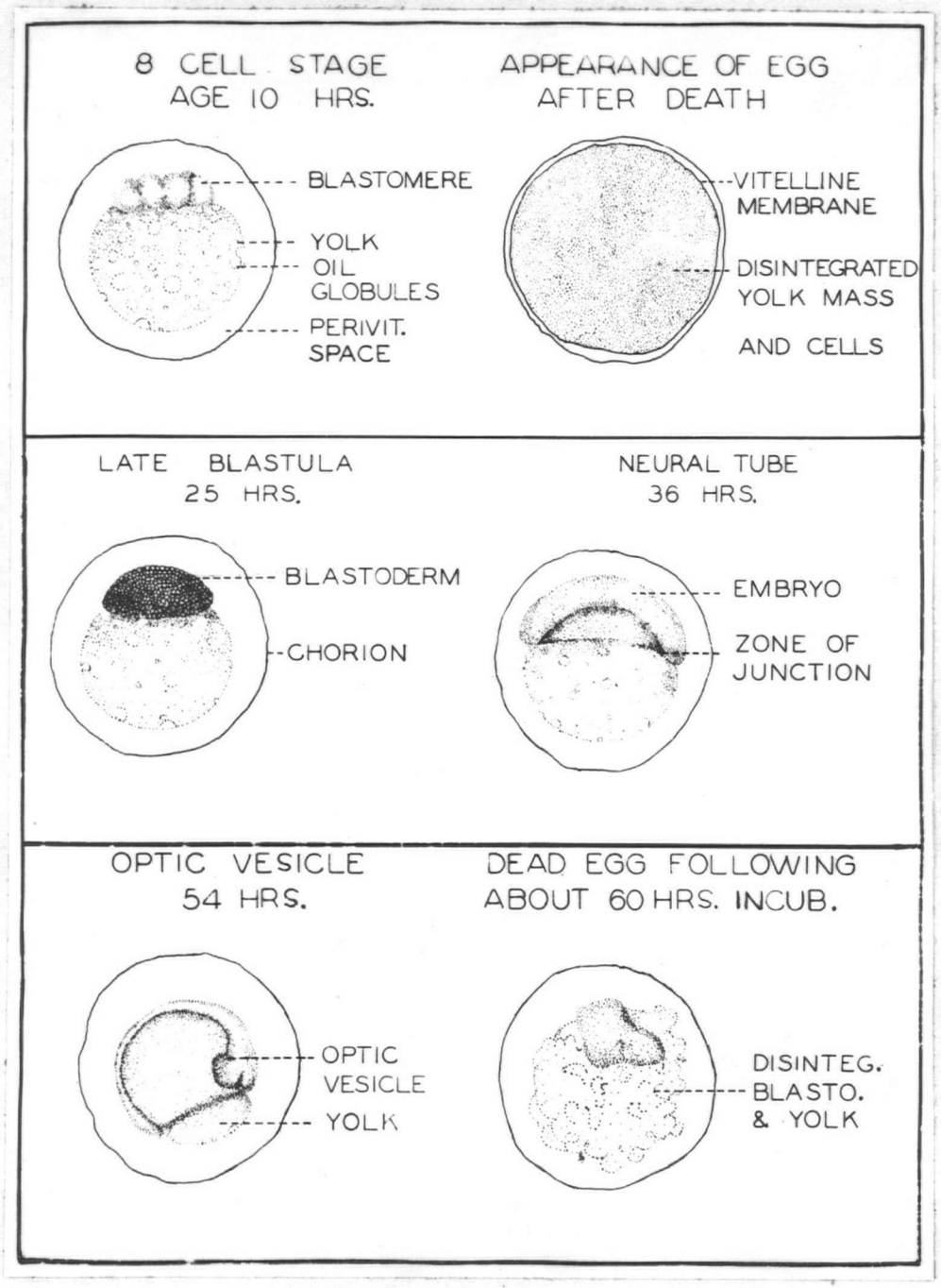


Figure 5. External appearance of the embryo of the Pacific herring during development up to 54 hours of incubation at 8.5° C. and 27.43°/oo salinity.

experiments to increase survival of herring reared in the laboratory by exercising care, or by leaving the eggs entirely untouched, during the indicated critical periods.

DEVELOPMENT OF THE HERRING UP TO HATCHING

Figures 5 and 6 illustrate the external development of the Pacific herring up to the time of hatching at a temperature of 8.5° C. and a salinity of 27.43°/oo. Eight hours after fertilization the first cleavage plane becomes apparent and by the end of the first day gastrulation has commenced. Neuralation commences around 30 hours and is completed before 50 hours - corresponding to the closure of the blastopore. Towards the end of the second day, the primordia of the eyes (the optic vesicles) are seen. During the latter part of the third day, epiboly has proceeded to such an extent that the yolk mass has been completely overgrown by ectoderm. Unpigmented eyes are prominent in the four-day-old embryo. At the end of the fourth day, the embryo has elongated so that it extends about three-quarters of the way around the yolk sac. During the fifth day of development, body somites and pectoral fins make their appearance. On the fifth or sixth day, the body of the embryo begins to twitch spasmodically. The ninth day is probably best characterized by the commencement of a violent thrashing about of the embryo,

especially the tail region. The thrashing tail seems to be the means by which the shell is ruptured during the latter part of the ninth or tenth day to produce hatching. All living eggs had hatched by the twelfth day and although a few were seen to hatch "head first" the majority of the larvae hatched "tail first".

FEEDING EXPERIMENTS

Under laboratory conditions, larval herring have apparently never been hatched and carried beyond the yolk-sac-absorption stage, the stage at which they would have been foraging independently for several days under natural conditions.

In 1874 experiments were carried out at Kiel, Germany, whereby herring were artificially fertilized and incubated under the supervision of a special commission. The young larvae were kept successfully only until the yolk sac was absorbed in the sixth day. The United States Fish Commission in 1878 hatched herring at Gloucester, Mass., and in 1883, Professor Ewart, Mr. J. T. Cunningham and Dr. J. Gibson carried out further hatching experiments in Edinburgh (Prince, 1907).

In none of the above experiments were herring larvae kept alive beyond the absorption of the yolk sac.

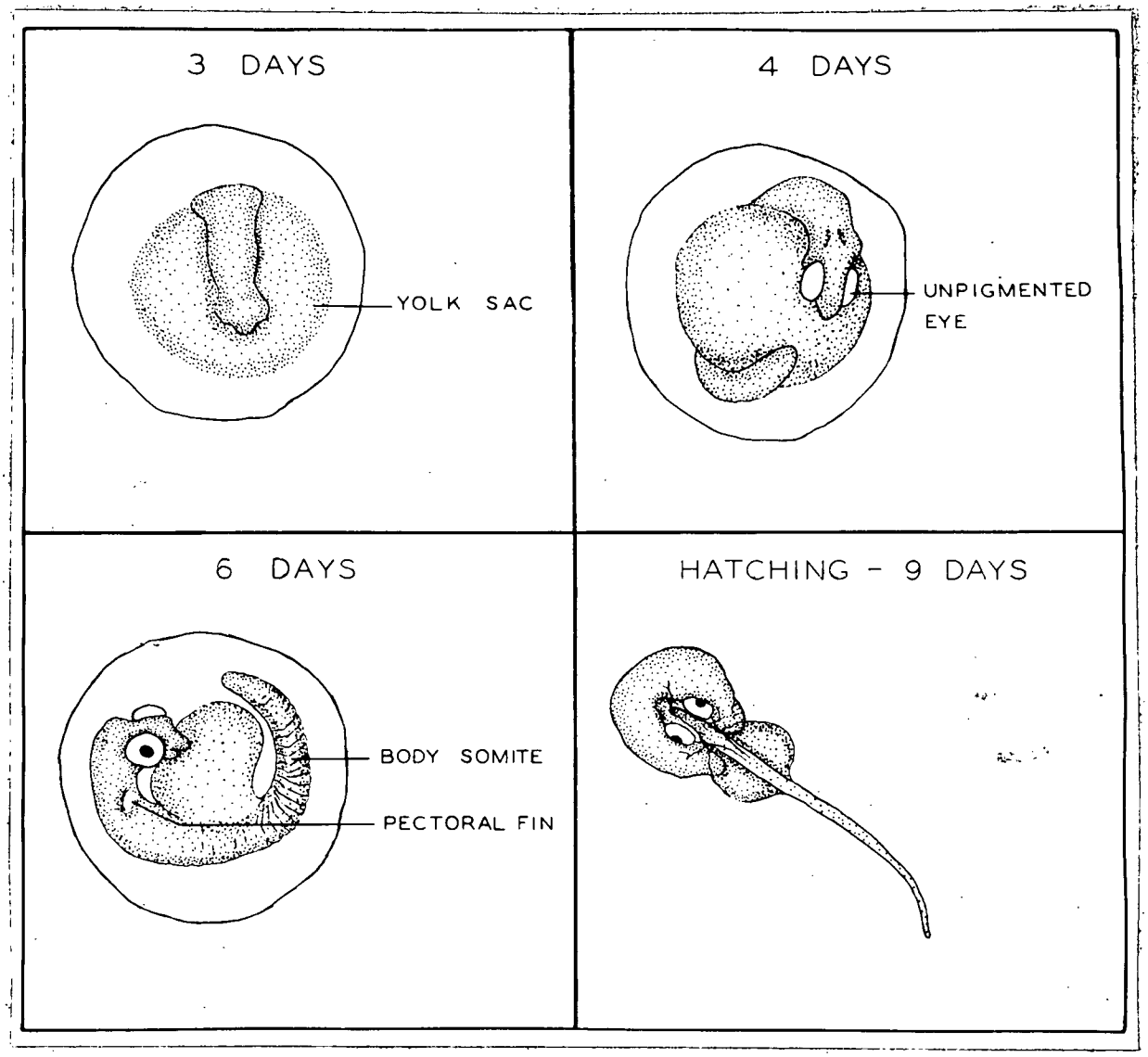


Figure 6. External appearance of the embryo of the Pacific herring during development from the third day to hatching.

McHugh and Boyd (1948) successfully reared two marine species of the family Atherinidae, the grunion, Leuresthes tenuis and the bay smelt, Atherinops affinis littoralis, past the critical stage of yolk sac absorption. In their experiments, eggs were fertilized by the dry method, well mixed and allowed to stand for about one minute. The excess milt was then washed off and the eggs placed in beakers containing a liter of sea water. On hatching the larvae were transferred to 5-gallon aquaria. Freshly hatched Artemia salina nauplii were introduced into the aquaria well before the yolk sac was absorbed.

In 1950 an attempt was made to raise herring larvae beyond the yolk sac absorption, this absorption being completed by the seventh day after hatching. Three diets were used including:

(a) ground Daphnia pulex eggs;

(b) 75% beef liver plus 25% skim milk (by weight)

and

(c) Artemia salina nauplii.

RESULTS OF FEEDING EXPERIMENTS

Diet (a):- On March 10, 25 freshly hatched herring larvae were placed into a 600 ml. beaker. Daphnia pulex

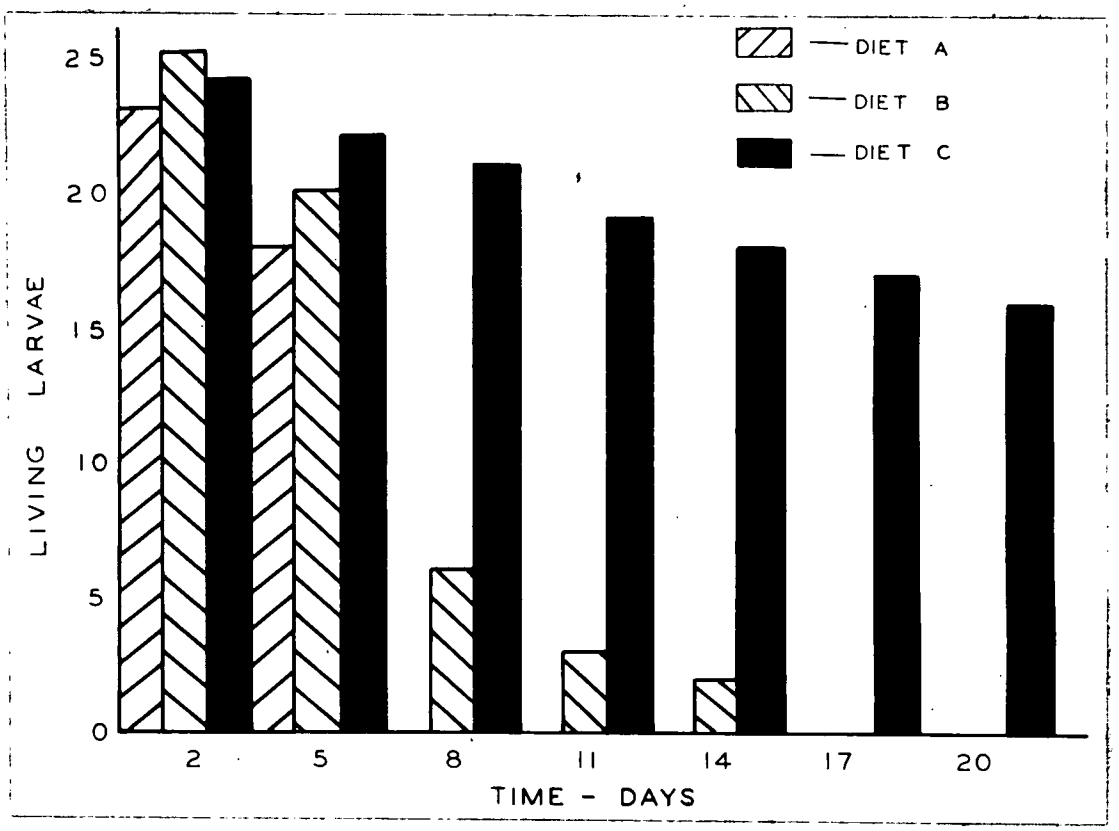


Figure 7. Survival of herring larvae fed on various diets. Diet A - Daphnia pulex eggs, Diet B - Liver-skim milk, and Diet C - Artemia salina nauplii.

eggs were fed to the larvae after the third day. Within eight days all of the larvae had succumbed. As the yolk sac was completely absorbed by the seventh day it would seem probable that the larvae died of starvation (Figure 7).

Diet (b):- This experiment was commenced on March 10 with 25 newly hatched larvae. On the fourth day, daily feedings of the diet of liver - skim milk was begun. All but three of the larvae had died within eight days; two survived for fifteen days at which time the experiment was terminated (Figure 7). Figure 7 is taken from Table III, Appendix.

Diet (c):- Commencing on March 10 with 25 recently hatched herring larvae, an experiment on the survival of the larvae fed on the nauplii of the brine shrimp was undertaken. Eight days after hatching, herring larvae were observed actively pursuing brine shrimp larvae. In many of the herring at this time, nauplii were plainly visible in the intestine. Active feeding continued until March 30 (20 days), at which time 16 larvae were still alive. In twenty days the herring larvae had grown an average of 4.8 mm., the size at hatching being about 6.0 mm. and the size at the termination of the experiment varying from 7.8 to 12.3 mm. (Table IV, Appendix).

CONCLUSIONS FROM PRELIMINARY EXPERIMENTS

1. The five-hour transportation of fertilized eggs, outlined previously, results in a survival of 1.9% at the time of hatching.

2. Survival is increased slightly but not significantly if stored fish are used for the experiments; after being refrigerated for 16½ hours, the fertilized eggs from these fish showed a survival of 8.9%.

3. When eggs and sperm are taken from living herring, following which no transportation of the fertilized eggs takes place, a survival of 53.7% is realized.

4. There appear to be at least two critical periods which occur during the development of the herring embryo. These critical periods coincide with blastopore closure, 2 - 3 days, and with the period of hatching, 9 - 11 days. Also there appears to be a period of inflection occurring at the end of the first day, but this inflection is not so pronounced as the other two.

5. Larvae were successfully reared beyond the yolk-sac-absorption stage through the utilization of a diet consisting of the nauplii of the brine shrimp, Artemia salina.

SALINITY EXPERIMENTS

Following rearing procedures developed during the preliminary experiments in 1950, studies were undertaken in 1951 on the effects of constant and varying salinity on egg and larval development and mortality.

PREPARATION OF SOLUTIONS

A series of rearing solutions varying between 0°/oo and 34.28°/oo salinity were prepared in 48 litre glass containers. These solutions were made up by the addition of either sea-salt or pond water to English Bay sea water of a known salinity. After rough concentrations or dilutions a precise determination of the salt content of each solution was made. One of the solutions was pond water taken from a small fish pond on the University campus. The salinity titrations yielded the following results for the eight solutions; 0°/oo, 6.06°/oo, 11.55°/oo, 16.24°/oo, 22.30°/oo, 26.13°/oo, 30.26°/oo and 34.28°/oo. Portions of these prepared solutions were then used during the experiment.

PROCEDURE

Constant Salinity Experiment:- On February 26, 1951, live mature herring, seined from the vicinity of Departure Bay, B.C., were transported from the Pacific Biological Station in Nanaimo to the University of British Columbia. Four fry-tins holding 7 herring per tin were used during

transportation. In addition to the water in each tin being agitated frequently, ice was added periodically so that the water temperature never rose above 7° C. Transported in this manner, all herring were alive and active upon reaching the University.

For the constant salinity experiments eggs were fertilized at 8.5° C. in water of a salinity identical with that to which they would be transferred. For example, in the 0°/oo salinity solution experiment, milt from one herring was stripped into a tray of pond water at 8.5° C., the eggs from one female were then stripped onto a number of glass slides and fertilized in the milt solution for approximately ten minutes. Immediately following fertilization the egg slides were transferred, unwashed, to four 600 cc. beakers, two slides to each beaker. These beakers were immersed in the constant temperature bath shown in Figure 1. The 4 beakers, used for each of the 8 different experimental salinities, were not scattered randomly over the bath but were placed in one row to simplify observation and record keeping. This procedure was justified as light intensity over the bath varied, at any one time, by less than 12 foot candles, and temperature, although apparently not as constant as in 1950, remained within the range of 8.5° C. $\pm$ 0.5° C. Water in each beaker was aerated continuously and changed every other day. The above procedure was carried

out for each test solution; thus the whole experiment consisted of 32 beakers each containing 2 egg slides, or 4 replications for each of the 8 salinities. Observations on development and mortality were made by examining each slide every second day.

Varying Salinity Experiment:- Eggs for this experiment were stripped onto glass slides and treated as in the preceding experiment. In this case, however, each slide was scored with a diamond in order that the egg slide could be readily broken into three pieces. Sixteen marked slides were placed into each of the eight salinities, 4 slides to each beaker and left for at least two days. From then on, at intervals of approximately 48 hours, eggs were transferred from one salinity to another and left in that secondary salinity for approximately 48 hours. The transfers from each salinity were placed in a fifth beaker in each salinity row, this beaker also containing water of the same salinity as the other 4 beakers in that row. For example, in the case of the 22.30°/oo salinity, 1/3 egg slide sections were taken from one of the beakers in that row and transferred to the fifth beaker in each of the other seven salinities. A 1/3 slide section was also removed from the beaker from which the other sections were obtained and transferred, as a control, to the fifth beaker (of the same salinity) in that particular row. Each slide section con-

tained from 10 to 200 eggs. In the above manner 56 slide sections were moved every second day; theoretically there should have been a transfer of 64 slide sections, but all the eggs placed in the pond water (0°/oo salinity) row died during the first day. After about 48 hours in the secondary salinities, each slide section was examined and observations made on development and mortality. As development is rapid in the case of the herring, it was felt that if eggs survived for two days in the changed environment they could be considered as having become acclimatized to the new set of conditions.

Except for a few slides which were once more transferred to other salinities, the egg slides from the original transfer were discarded after examination.

Larval Experiments:- As soon as eggs from slides held in each of the various salinities throughout the period of egg development began to hatch, the larvae were transferred from the beakers to 3 litre rearing jars containing water of the same salinity as that in which the larvae hatched. The jars were immersed in a hatchery trough and held at a temperature of approximately 7° C. Aeration was not continuous as correspondence from Mrs. K. Herlinveaux of the Biological Station suggested that larvae tended to "feed" on the air bubbles, this apparently producing high mortalities. It was found during the larval

experiments that vigorous aeration for a few minutes every second day produced no harmful effects but was adequate for the oxygen requirements of the larvae. Water in the rearing jars was changed once a week. Two to three days after transfer to the rearing jars cultures of recently hatched Artemia salina nauplii were introduced as food for the larvae. Regular observations were made on mortality and development.

RESULTS - CONSTANT SALINITY EXPERIMENT

For ease in comparison of the egg survivals in various salinities, numbers surviving at each observation were converted to a percentage of the initial number of viable eggs. As the numbers of eggs involved in each experiment exceeded 800, reduction to percentages did not necessitate weighting of the means.

During the experiment there appeared to be a differential mortality among the egg slides held in the same salinity. As shown by Table VI, the percentage survival of eggs on individual slides in any one salinity show considerable variation. For example six of the eight slides in one of the salinities at a particular stage of development might show a survival of about 70%, whereas the remaining two slides, in a different beaker, had only a 20% survival. Figure 8 represents the frequency of occurrence

of percent survival groups during the last three observations before hatching commenced. There seems to be a more or less well defined trend which indicates a high frequency of high and low mortalities with a relatively low frequency of occurrence of the intermediate survivals. This would seem to indicate that there is a high percentage survival on egg slides until some factor, perhaps the production of toxic substances from dead eggs, appears and quickly kills practically all of the eggs on the slides in a particular beaker. In the analysis of the data, slides exhibiting this apparently excessive mortality were treated along with the rest of the egg slides. Perhaps smoother trends would have resulted if these slides had been ignored.

Survival in Different Constant Salinities:- Table VI shows the percentage survival for all egg slides in each salinity at the indicated stage of development. The data were tested for significance by the multiple classification treatment given by Snedecor (1946: 275-280).

This analysis indicates that:

(a) interaction is not significant, i.e. various constant salinities do not have different effects at any particular age;

(b) there is a significant difference (P 0.01) in the percent survival as age increases, and

(c) survivals which differ significantly (P 0.01)

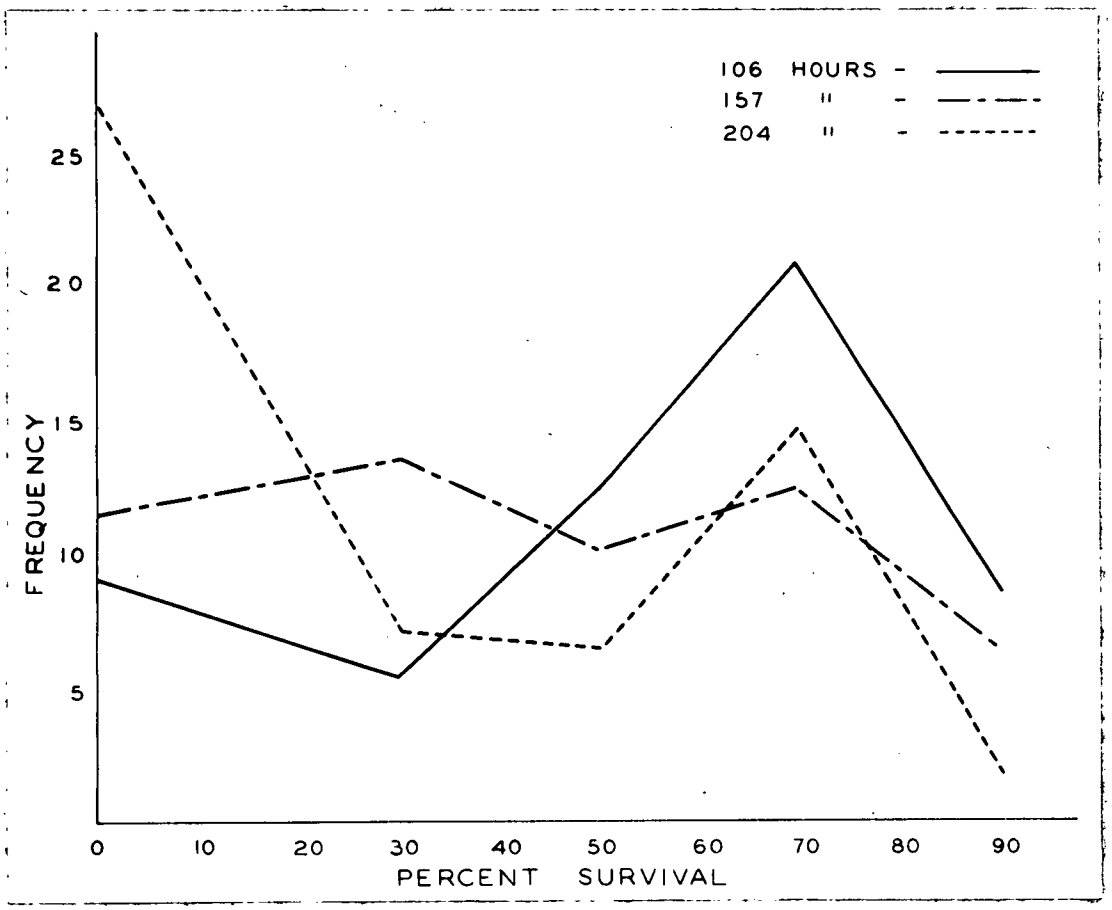


Figure 8. Graph showing the frequency of occurrence of percentage survival groups of eggs (further description in text).

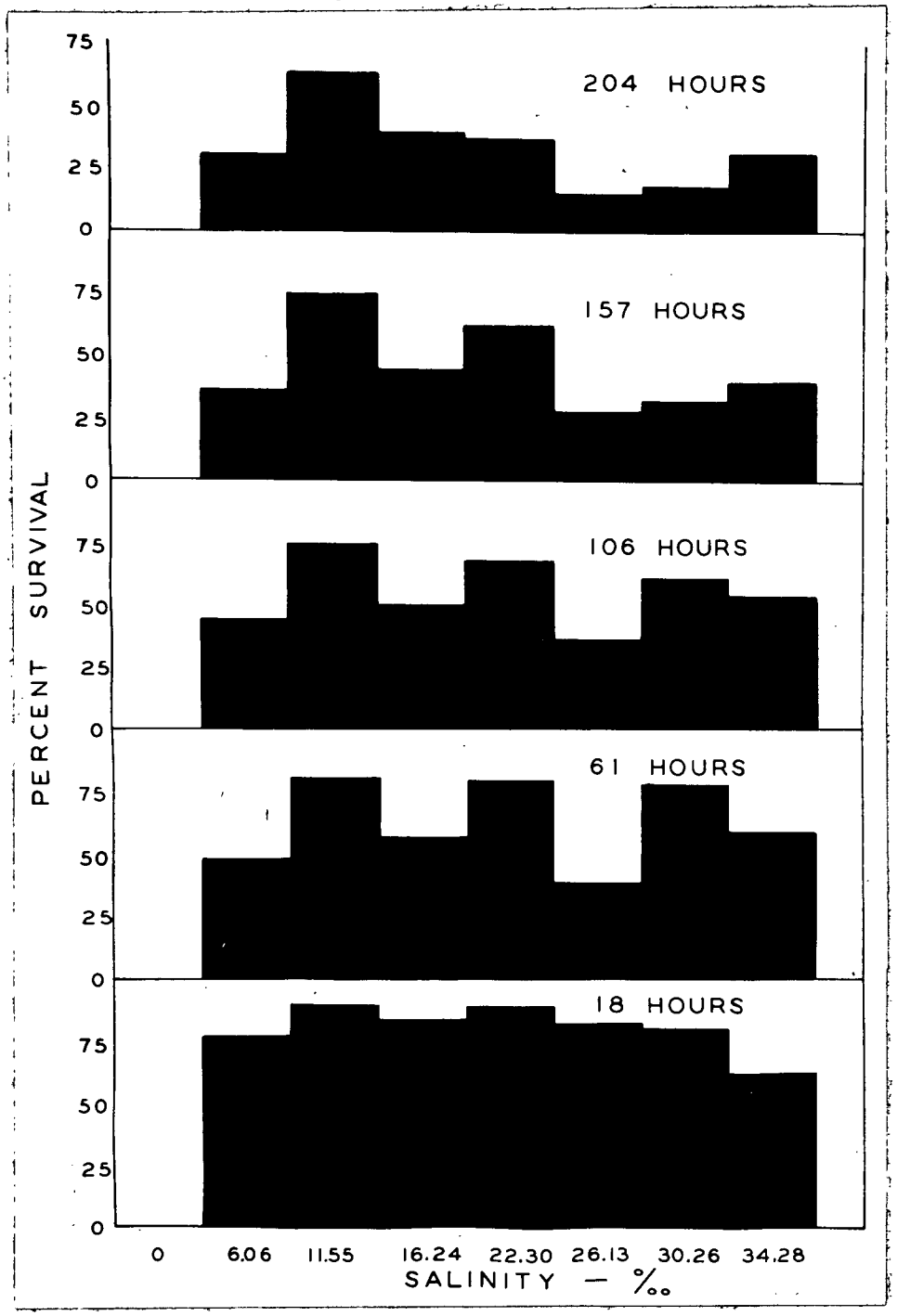


Figure 9. Percentage survivals (of original numbers of eggs) at different ages in various salinities.

result from the application of various salinities.

Figure 9 illustrates the percentage survival in the various salinities at the indicated ages. There is no survival in the 0°/oo solution, and although there is a significant difference in survival in the other salinities it is apparent that there is a wide range of tolerance extending between 6.06°/oo and 34.28°/oo salinities. As far as general trends are concerned there is obviously a marked irregularity during the first 61 hours of incubation, but subsequently there seems to be a higher survival in the 11.55°/oo salinity. The general trend seems to be that of rapid increase in egg survival from 0°/oo to 11.55°/oo. A more or less gradual decrease in survival to 30.26°/oo follows at which point there is marked irregularity in that the next higher salinity shows an increase in egg survival.

If, now, the percent mortality occurring between two successive observations is plotted for each salinity against age (time of each observation) the data given in Figure 9 are presented in a different manner and as a result another feature becomes evident. This feature is illustrated in Figure 10 which was plotted from the data in Table VII. The graph, with the exception of the 34.28°/oo line, indicates once again that lower salinities produce lower mortalities and in addition it seems to show

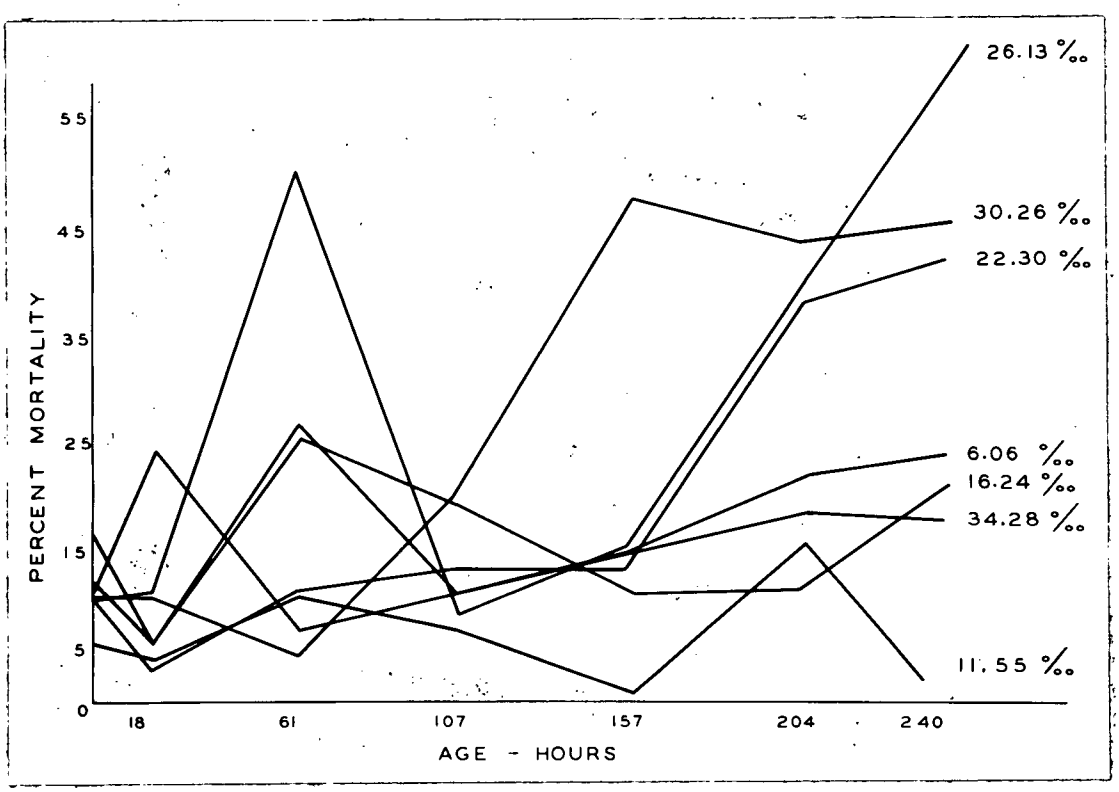


Figure 10. Percent mortality increments between successive observations at various ages in different salinities.

that the mortality curve for any one salinity is not smooth throughout the developmental period; each curve is characterized by irregularities. The irregularities tend to group about two ages in each case, the 61 and 204 hour observations. At both periods mortality increased markedly. As observations were made only on alternate days it is difficult to isolate the time at which the mortalities increased abruptly. However, the two periods do appear to fall within the critical stages of development indicated in the 1950 experiments. The first one occurs before 61 hours of incubation, probably at blastopore closure, and the second around 204 hours which would correspond to the pre-hatching critical period noted in 1950.

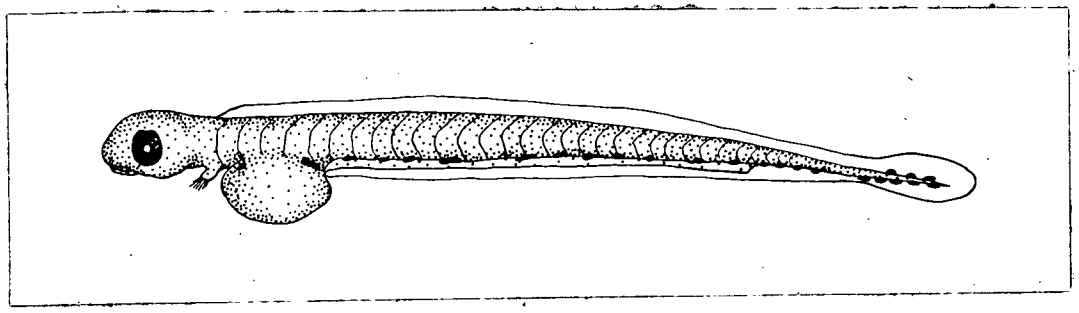
Development and Hatching in the various Salinities:- Observations indicated that the rate of development is the same in all salinities between 6.06°/oo and 34.28°/oo. Optic lobes were apparent at 46 hours and initial movement occurred at 118 hours in all of the salinities. Hatching commenced in all of the solutions on the tenth day and was completed by the fifteenth day. Close observations were made on the hatching process in 1951 and it was noted that the normal mode (the mode of hatching which produced the greatest survival) of hatching, which occurred over 80% of the time, took place in all but the 6.06°/oo solution. This method of hatching proved to be "head first", not

"tail first" as indicated in the 1950 preliminary experiments. The mechanism of hatching appears to start with a noticeable softening of the egg membrane; recently dead eggs or eggs which are not yet ready to hatch, have an egg membrane which is comparatively difficult to break, even with a dissecting needle. However, an egg about to hatch has an egg membrane which is easily broken, even by jarring. The dorsal part of the embryo's head is then seen to push against the egg membrane thus forming a bulge on one side of the egg. The bulge is then forced farther and farther out until it eventually breaks. The head of the emerging embryo is now free of the egg and by a series of violent threshings and wrigglings the yolk sac is forced out. The process of hatching is completed when the larva frees its tail by merely swimming out of the ruptured egg membrane. The entire process, observed in several cases, took approximately one hour and fifteen minutes. Although a few embryos started to hatch tail first these were never observed to emerge completely from the egg; any swimming movements of the tail seemed only to force the head farther into the egg shell. This was particularly apparent in the case of the 6.06°/oo salinity. In this salinity, six days after hatching had commenced, there were only a half dozen live larvae swimming about the four beakers; when the slides were examined perhaps 200 dead larvae were noticed

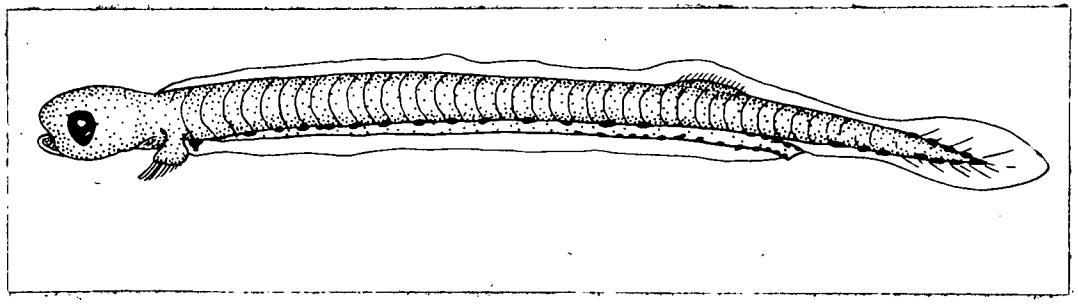
with only their tails protruding from the ruptured shells. Table VIII shows the number of larvae remaining alive in each salinity at the end of the hatching period. The percentage of the original number of eggs producing live larvae was very low, perhaps misleadingly low, as large numbers of the eggs did hatch but the mortality during and immediately following hatching was extremely high. Table VIII indicates that the 11.55°/oo, 16.24°/oo and 34.28°/oo salinity solutions produce the largest percent survival of larvae 6 days after hatching had commenced. These same salinities produced the highest survival of eggs. Figure 11-a shows the appearance of a recently hatched larva, and Figure 11-b a larva of about two weeks old.

RESULTS - VARYING SALINITY EXPERIMENTS

Egg Density and Survival:- The density of eggs on the slide sections used in the varying salinity experiment varied between 10 and 200 eggs per section. The possibility that egg density might have an effect on subsequent survival was tested for significance by application of the chi-square test (Fisher, 1930: 83-85). In this analysis a number of egg density groups were chosen and for each group two values were considered: (a) the total number of living eggs in each density group at the end of the salinity change (48 hours) and (b) the total number of eggs which



11 (a)



11 (b)

Figure 11. Lateral drawings of herring larva:- (a) recently hatched; (b) approximately 2 weeks old.

died in each density group during the 48 hours. The results of the analysis are given in Table IX. A chi-square value of 497 was obtained from the analysis indicating that egg survival is not independent of density (tabled value at 0.01 P and 7 degrees of freedom being 18.48). Figure 12 shows the average percentage survival for each egg density group plotted against the mid-point of each density group. There seems to be some evidence for the existence of an optimum survival around densities of 75 to 115 eggs per slide section. Quite obviously then this feature might lead to large experimental errors, especially when it is recalled that all survivals are weighted by being converted to percentage survival. However, during the varying salinity experiment, slide sections of various egg densities were randomly distributed over the different salinities, i.e., no selection was made as to whether high or low density egg slides were placed in certain salinities or not. Also, average survivals are used in the analysis, therefore experimental error resulting from differential egg survival caused by various densities would seem to be held to a minimum.

Survival Following Transfer to Different Salinities:- In Table X, the percentage survival of eggs transferred from each acclimation salinity to each one of the other salinities, from $0^{o}/oo$ to $34.28^{o}/oo$, are tabled for the

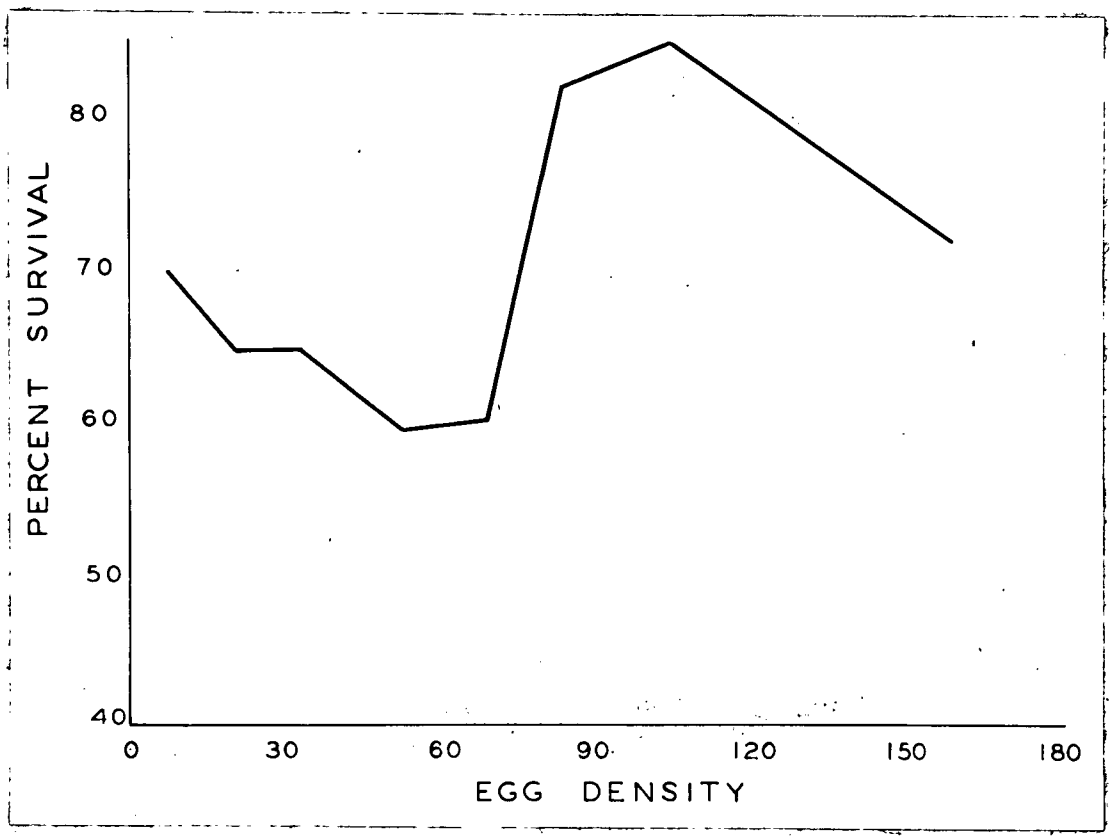


Figure 12. The survival of herring eggs on slide sections at various egg densities.

four observations. It can be seen that the survival of eggs on the control sections was not necessarily the highest. In the 27 control slide sections, survival on these sections as compared to the other changes, was higher in 7 cases, lower in 8 cases, and about average in the remaining 12 cases. On this basis alone, it appears that changing from one salinity to another produces no higher mortality than a change involving no salinity difference.

Conceivably, the magnitude of the salinity change could be a factor in egg survival in the secondary salinities. Table XI shows the percentage survival of eggs transferred, either up or down the range of salinities, from changes involving $0^{o}/oo$ (the control) to + or - $30^{o}/oo$ or $34^{o}/oo$ salinity. It is apparent from the average value for the four experiments at each increment that there is no obvious correlation between survival and magnitude of salinity change within the range of salinities under consideration. With but two exceptions the results show that the percent survival of eggs in salinity changes ranging from + $30^{o}/oo$ to - $34^{o}/oo$ varies only between survivals of 60% and 73%. The two exceptions indicate survivals of 51% and 45%.

If magnitude of salinity change, then, has no apparent relation to survival the average survival in the

various secondary salinities, regardless of the change which placed the eggs there, can be considered. Table XII is a summary of the data given in Table X. The average percentage survival is shown for eggs taken at various ages from each of the acclimation salinities and placed for approximately 48 hours in the indicated secondary salinities. Figure 13 shows graphically the results listed in Table XII. A number of features are indicated. In the first place, survivals at all ages are lowest in the $0^{o}/oo$ and $30.26^{o}/oo$ solutions. Secondly, there is an abrupt increase in survival in all cases in the $34.28^{o}/oo$ salinity. Also, it appears that during the initial changes at 36 hours, there is a considerable narrowing of the tolerance range, with survivals grouped about an optimum of $16.24^{o}/oo$. During the changes at 36 hours there was no survival of the eggs placed in pond water ($0^{o}/oo$). The figure indicates that during later development, the embryo is apparently capable of tolerating a wider range of salinities - even to the extent of tolerating, at least for 48 hours, fresh water. Finally, it seems significant that there is a lower survival during the initial (36 - 86 hour) change and in the final (190 - 237 hour) change. These are periods of development which would correspond to blastopore closure, and the pre-hatching periods. Perhaps this is another illustration of increased mortality during the

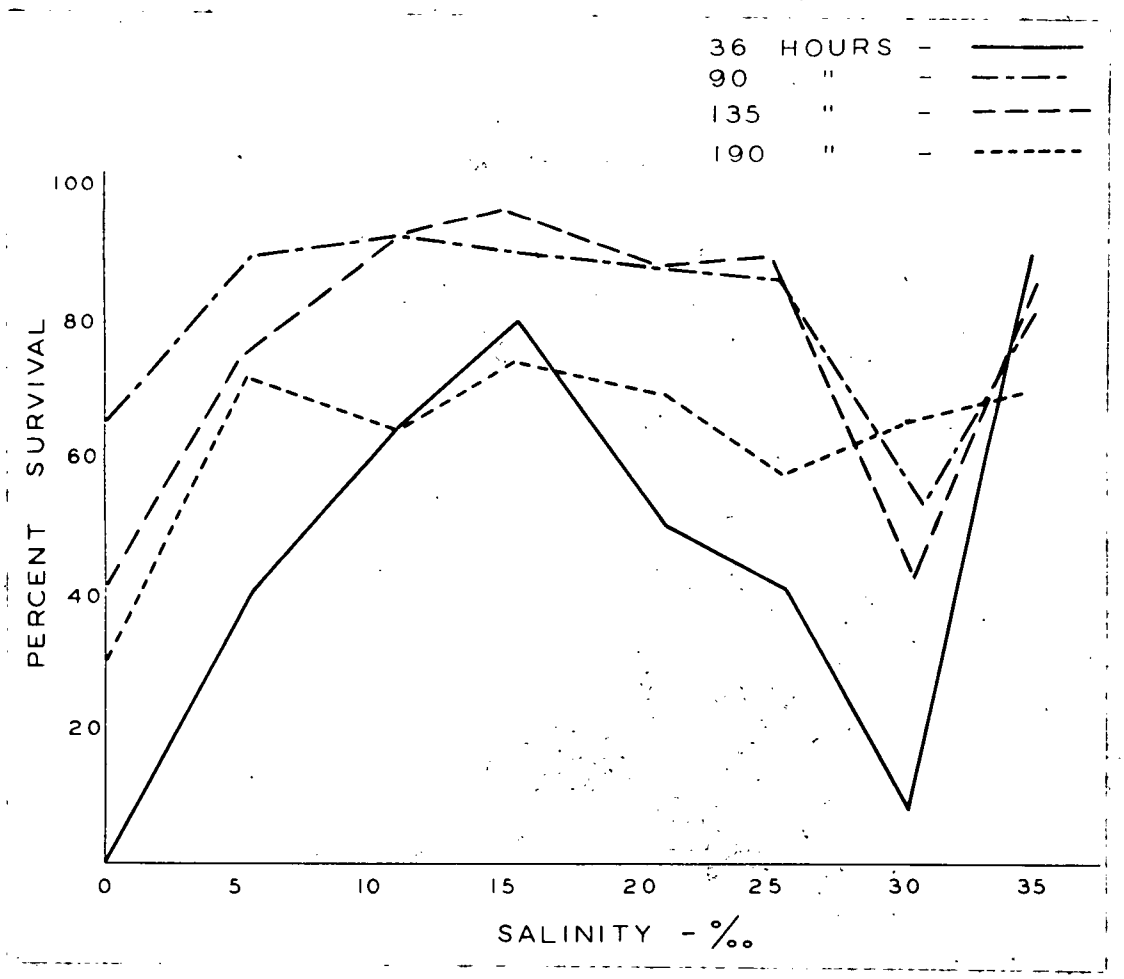


Figure 13. The survival of herring eggs transferred at the indicated ages into various salinities for a period of about 48 hours.

critical periods previously discussed.

Table XIII represents the survival of eggs, at two different ages, taken from the secondary salinities and transferred once again to either higher or lower salinities. The percentage survival in each change indicates once again a high tolerance. The controls show no consistently higher survival and the magnitude of the change indicates no apparent relationship with survival.

Development in the Secondary Salinities:- Development in salinities between 11.55°/oo and 34.28°/oo seemed to be identical. In 6.06°/oo activity prior to hatching was less than in the higher salinities, and in the 0°/oo salinity, although the embryos survived the salinity change, after 90 hours, there was little if any movement. During the stage of eye pigmentation, the embryos in this solution had little, if any, pigmentation, whereas at the same age in higher salinities the eyes were heavily pigmented (Figure 14 a & b).

RESULTS - REARING OF LARVAE

Using the procedures outlined previously no difficulties were encountered in rearing the majority of the herring larvae past yolk sac absorption. Table XIV represents the number of larvae surviving in rearing jars of the indicated salinities at time intervals of 3 days.

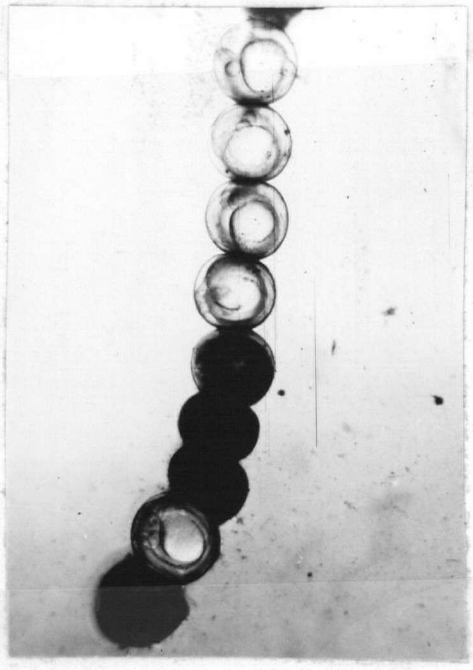


Figure 14a

Eggs at 180 hours in $0^{o}/oo$ salinity

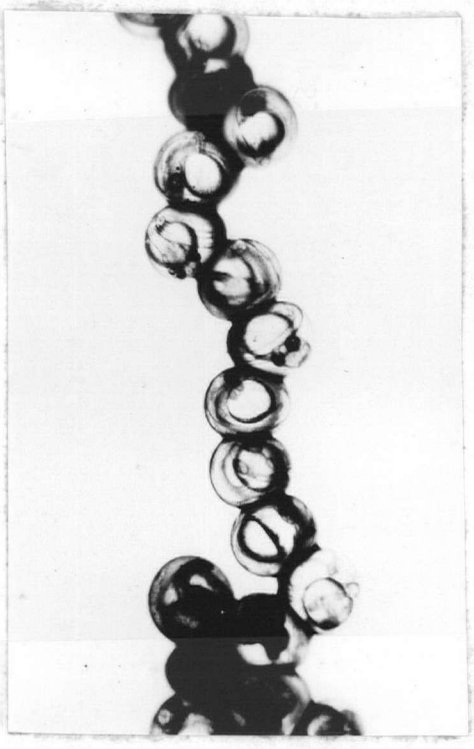


Figure 14b

Eggs at 180 hours in $34.28^{o}/oo$ salinity

Figure 14 a and b. Embryonic development at about 180 hours in eggs which have been transferred to $0^{o}/oo$ and $34.28^{o}/oo$ salinities. Note the lack of eye pigmentation in the $0^{o}/oo$ solution.

Since hatching extended over several days some of the larvae in each jar were 3 or 4 days old on day 1 in the table. By the end of the sixth day most of the larvae had completely absorbed their yolk sacs and many could be seen actively pursuing brine shrimp nauplii. At this time many of the herring larvae were noted to have numbers of nauplii within their intestines. No abnormalities were noted and growth and activity seemed to be the same in all of the salinities between 6.06°/oo and 34.28°/oo.

On March 25, 20 larvae were taken from the 34.28°/oo salinity and placed into a rearing jar of pond water (0°/oo salinity). Three hours later these larvae were all dead. From the rather limited observations on the few larvae available it would appear that the herring larva has a salinity tolerance range which extends, at least, between 6.06°/oo and 34.28°/oo. The determination of an optimum salinity range is obviously impossible with such small numbers. Further work along this line seems warranted as the mechanism of osmoregulation in the herring larvae would seem to be well developed for life in both brackish and salt water.

DISCUSSION

Salinity Tolerance of Fish:- Woodbury (1948) in a literature review states that "sudden variations in the

composition or concentration and even the gradual attainment of certain high or low salt concentration levels may reduce the vitality or even kill animals that live in or have to drink it." Apparently there is an optimum salinity both as to quality (composition) and quantity for a number of aquatic species (Ferguson, 1948; Loosonoff, 1945; and Leim, 1924). The range of conditions that may be considered optimum varies with different species and doubtless, also, the sensitivity of each species may differ at different stages of the life cycle.

When living in sub-optimal conditions, there must be a constant struggle on the part of the organism. The outcome of this struggle for existence depends in part on the hereditary potential of that organism. Fluctuating salinity would tend alternately to intensify or ease this struggle from time to time. Natural selection probably operates very effectively in the periods of intensified struggle, and probably the evolutionary process would proceed at a relatively rapid pace under such circumstances. Woodbury (1948) showed that there is a considerable variation among the members of a given species in their abilities to withstand fluctuations of salinity. No doubt then, those which reach their limit of tolerance first will perish before others with greater limits of tolerance and thus leave those that can withstand the sub-optimal conditions to

survive and propogate their hereditary survival characteristics.

In the relatively stable conditions of the open ocean many of the simpler forms have the cells of the body exposed to sea water and osmotic exchanges are made directly with it. With increased complexity of the more highly organized forms, there is generally an increasing separation of the internal environment from the external by enclosing the body within a more or less waterproof coat and relegating the osmotic exchanges to specialized cells. These are usually associated with the gills or kidneys of fish.

Baldwin (1949) describes three types of osmoregulation in fish:

1. Fresh water teleosts have an internal osmotic pressure greater than the external so that water enters from the outside tending to dilute the blood. This in turn results in production of hypotonic urine.

2. Marine teleosts living in a medium with a salinity of about $35^{o}/oo$ have an external osmotic pressure greater than the internal and consequently use a different physiological method of regulation. The fish drinks water absorbing both salts and water. The salt is eliminated by special cells in the gills, and the water then passes as an isotonic urine out of the kidney. Nash (1931) and Keys

(1933) found that glomerular development is generally much greater in fresh water and euryhaline teleosts than in marine teleosts. Regarding the two types of osmoregulation mentioned above, it is important to note that the fish kidney is capable of regulating only toward a high osmotic concentration of the blood, and the mechanism in the gills toward a lowered osmotic concentration. The latter mechanism is absent in fresh water forms.

3. Marine elasmobranchs have internal and external pressures about equal. The problem of excretion is solved by allowing about 2 percent urea to accumulate in the blood, which then brings water in through the gills and allows a slow stream of nearly isotonic urine to pass out the kidneys.

For many species osmoregulation is exercised only within narrow limits. The amount of such control varies considerably among different species. As the Pacific herring spawns in inshore waters which may vary from 8.21°/oo to 27.68°/oo salinity on some spawning grounds such as Queen Cove, Vancouver Island, B.C., it would seem that this particular fish must have a high salinity tolerance. Nash (1931) showed in the case of two marine clupeid fishes, Sardinella macrophythalmus and Sardinella anchovia, that the kidneys indicated a glomerular count approaching that of fresh water teleosts. It seems possible then that the

Pacific herring tolerates relatively fresh water because of a high glomerular count. As a matter of fact, Kyle (1926) states that the herring is one of the most primitive of fishes and has only been a marine form since the Cretaceous period.

Sumner (1905) suggests that one factor in the death of salt water fishes in fresh water is the extraction from their tissues of an amount of salts sufficient to reduce the percentage below a certain necessary minimum.

Salinity Tolerance of Eggs:- The mechanism of osmoregulation in the case of fish eggs has not been studied to the same extent as in fish. However, a few features have been brought out by various workers. Vernidub (1947) found that the reaction of developing fish eggs to exterior influences varies at different stages of development, and that at some stages the influencing agent does not produce effects on morphogenesis (division, gastrulation, growth of the developing embryo, pigmentation of the eyes, etc.) while at other stages, the same influence results in the disturbance and death of eggs. Battle (1929) showed in the case of the four-bearded rockling Enchelyopus cimbrius that changes in salinity will so alter the physiological processes concerned with growth and differentiation that the embryo develops abnormally. Ford (1929), working with eggs of the European herring, showed the

existence of a wide tolerance range over which development and hatching could take place. Apparently then eggs of different fish species react in different ways to changes in the environment.

Rollefson (1932) has shown that in cod eggs the active protoplasm is concentrated at the blastodisc but in addition it also spreads over the entire surface of the yolk as a thin film. During gastrulation the blastodisc flattens and spreads over the yolk, which is thus covered by embryonic tissue. This same sequence seems to take place in the herring egg. Rollefson then explains increased resistance of eggs on the basis of the covering up of the yolk by the embryonic tissue. In addition he proved that the cod membrane is always permeable and allows salts and dyes to enter the perivitelline fluid, but the protoplasmic layer was shown to be impermeable.

Discussion of Experimental Results:- Results of the salinity experiments have shown the existence of a wide salinity tolerance for eggs and larva of the Pacific herring. In conditions of different constant salinities, the range is from 6.06°/oo to 34.28°/oo. There seems to be a trend toward higher survivals in salinities of 11.55°/oo and 16.24°/oo, except in the case of eggs held in 34.28°/oo where there was also a high survival. Experiments in which eggs were moved from one salinity to another at various

ages also showed that there is a wide salinity tolerance range, a range which appears to increase after the first two days of development. Developing eggs of later ages will survive for at least two days even in pond water of $0^{o}/oo$ salinity.

Ford (1929), in his salinity experiments on the European herring Clupea harengus, made observations which are essentially in agreement with the results obtained for Clupea pallasii. He showed that a high egg survival was obtained over a wide range of salinities, $4.8^{o}/oo$ to $37.5^{o}/oo$. In the low salinities, $4.8^{o}/oo$ and $9.3^{o}/oo$, many larvae were unable to wriggle free of the egg envelope. In the present experiment this appeared to be the case in the $6.06^{o}/oo$ salinity, but hatching was normal in all other salinities. This inability to hatch beyond a mere protrusion of the tail from the egg capsule, was also noted by Battle (1929). In the case of the four-bearded rockling, this feature is explained as being correlated with an inadequate development of the tail musculature.

Regarding fertilization in the various salinities, eggs were successfully fertilized in all salinities from $6.06^{o}/oo$ to $34.28^{o}/oo$, and fertilization may have occurred even in the $0^{o}/oo$ solution. Ford indicated successful fertilization of European herring eggs in salinities as low as $4.8^{o}/oo$. These observations suggest that only a very

low salt concentration is necessary to produce successful fertilization and subsequent development.

With the exception of the 34.28°/oo salinity the present experiments indicate that it is the higher salinities which produce the highest mortalities. Leim (1924) found this to be the case with the eggs of the shad Alosa sapidissima, a clupeid living in the ocean but spawning in fresh and brackish waters.

Schechter (1943) showed that the first effects of low salinity on eggs of the snail, Thais floridana, is immobilization, a feature which was evident in herring eggs which were transferred to fresh water.

It was shown in the above experiments that the highest percentage of eggs hatching occurred in salinities of 11.55°/oo, 16.24°/oo and 34.28°/oo. Without the contradictory results produced in the highest salinity it would seem that an increase or decrease in salinity over the range of 11.55°/oo - 16.24°/oo produces lethal effects. In this respect Scott (1929) showed, for eggs of the winter flounder, Pseudopleuronectes americanus Walbaum, that the time of hatching is not affected by varying salinity, but that the average percentage hatch is lower for eggs in dilute sea water. He also noted that developing embryos held at low salinities were more often relatively inactive. These results are in agreement with

those obtained for herring eggs.

Perhaps it is beyond the scope of this paper to attempt an explanation of some of the above results as it is evident that much more experimentation should be done, especially between the ranges of $0^{o}/oo$ and $11.55^{o}/oo$ salinities. However, as it is apparent that the eggs are capable of tolerating a wide range of salinities, one might deduce that the egg membrane is impermeable to water and thus no problems of osmoregulation exist. This hypothesis does not, however, explain all of the facts. Developing eggs at early stages die within a few hours when placed in pond water, but at a later age, eggs will survive for several days when placed in pond water. This might, then, indicate that the egg membrane is permeable to water and it is this very feature which causes death of the eggs. As Rollefson (1932) has shown for cod eggs, it may be the growth of the embryonic area (impermeable to water in cod eggs) over the yolk which explains why herring eggs are able to tolerate fresh water at a later stage of development. If this embryonic layer, in the case of herring eggs, is water impermeable, then it might be expected that as the egg developed the tolerance range would increase. The results indicate that following blastopore closure there is a considerable widening of the tolerance range.

Another trend, which appeared as a result of the salinity experiments, was a more or less well defined optimum salinity (11.55°/oo - 16.24°/oo) for development and hatching of the herring eggs. Why the optimum should occur at this range is easily explained if we consider that the herring egg is freely permeable to water. Sea water of normal salinity, around 35°/oo, is generally considered as having an osmotic pressure between two and three times as great as that normally found in the internal environment of marine organisms. Thus the salinity which would be isotonic as far as the herring egg is concerned would probably be within the range of 11.55°/oo to 16.24°/oo salinity. In sea water within this range then, eggs would have little or no osmoregulatory problems and therefore would probably be in a better position to survive. This may also provide an explanation as to why herring spawn in a locality such as Queen's Cove where salinities have been shown to vary from 8.2°/oo to 27.68°/oo.

In the 1950 preliminary experiments it was noted that egg slides, during the course of the experiment, became covered with a brown filamentous, granular layer. A sample of this growth was taken to the Biology Department for identification; unfortunately it could not be identified beyond the observation that it appeared to be some form of fungus growth. In 1951 the same growth was apparent in all

but the 34.28°/oo solution. In this salinity the egg slides remained clear throughout the experiment. On the basis of these observations, the obvious explanation for the high survival in the 34.28°/oo salinity seems to be that during development in the experiments, eggs are attacked by a fungus growth which may produce a considerable mortality in the salinities between 6.06°/oo and 30.26°/oo. However, in the case of the 34.28°/oo solution the salt concentration at this level acts as an antiseptic which apparently seems to inhibit growth of the fungus.

CONCLUSIONS

At the beginning of this paper it was suggested that salinity might be a limiting factor in the survival of the eggs and larvae of the Pacific herring, Clupea pallasii. Experimental studies have shown the existence of a wide salinity tolerance range in which normal development, hatching and survival of larvae take place. Thus a salinity change, in itself, does not seem to be a limiting factor as far as survival of eggs and larvae is concerned.

During the 1950 and 1951 experiments, the existence of critical stages during embryonic development were demonstrated. At these critical periods mortalities increased considerably, not only in eggs which were moved from one salinity to another, but in eggs which remained

in one constant salinity throughout their development. The inference is that at these two critical stages, any one of several environmental disturbances might readily produce heavy mortalities. Spawning of the Pacific herring takes place only on a few major spawning grounds where spawn is deposited from the intertidal zone to a depth of perhaps 10 fathoms. It seems quite possible then that the developing eggs might be extremely vulnerable to any abrupt change in the physical as well as the chemical environment, e.g. wave action, wind and rain. If some more or less abrupt change of the external environment were to take place during one of these critical periods a large percentage of the eggs might conceivably be killed off in one of the spawning areas. It is suggested that the observed fluctuations in the herring fishery might be the result of an unfavourable environmental factor, occurring during the early stages of development, rather than as the result of predation on eggs and larvae or the unfavourable dispersion of larvae from the spawning ground. Admittedly dispersal and predation may be important in producing minor fluctuations of abundance. It seems more likely, however, that a major fluctuation could result from some unfavourable environmental factor, occurring during a critical period of development, causing a catostrophic mortality of spawn.

In summary, the salinity experiments have produced

the following results:

1. The existence of a wide salinity tolerance range in the case of both eggs and larvae.

2. There is an optimum survival of eggs in the salinity range of 11.55°/oo to 16.24°/oo.

3. The tolerance range apparently increases with an increase in age of the developing egg. Eggs will survive in fresh water at a later stage of development whereas during early development the eggs die in 0°/oo.

4. Only in 0°/oo and 6.06°/oo salinity are there any apparent differences in development and mode of hatching.

5. Evidence seems to indicate that the egg membrane is permeable to water.

6. The existence of two critical stages in development (during which mortality increases markedly) is demonstrated. These critical periods correspond to blastopore closure, and the period immediately prior to and during hatching.

7. There is apparently an optimum density of eggs per slide which will yield the greatest survival, 75 - 125 eggs per slide.

8. Some evidence is presented for the occurrence of a "chain reaction" in which a slide of eggs, formerly having a high survival, is suddenly killed in a matter of

one or two days.

9. Larvae were successfully reared in all salinities between 6.06°/oo and 34.28°/oo for several weeks beyond yolk sac absorption.

10. The need for further investigation of many of the features noted is indicated.

- A P P E N D I X -

Table I

THE SURVIVAL OF HERRING EGGS

AGE DAYS	EXPERIMENT # 1		# 2		# 3	
	Number	Percent	Number	Percent	Number	Percent
0	6,558		1,254		2,656	
1	5,861	89.4	920	73.4	2,133	80.3
2	3,368	51.4	889	70.9	2,076	78.1
3	1,808	27.6	858	68.4	2,023	76.2
4	1,312	20.0	788	62.8	1,927	72.6
5	1,120	17.1	625	49.8	1,866	70.3
6	816	12.4	418	33.3	1,848	69.6
7	620	9.5	290	23.1	1,800	67.8
8	490	7.5	203	16.1	1,652	62.1
9	394	6.0	199	15.9	1,449	54.6
10	182	2.8	140	11.2	1,426	53.7
11	158	2.4	130	10.4	1,426	53.7
12	135	2.1	113	9.0		
13	123	1.9	111	8.9		

Table II

SIGNIFICANCE OF THE DIFFERENCE BETWEEN TWO REGRESSION COEFFICIENTS (EXPERIMENT 1 vs. 2 and EXPERIMENT 1 vs 3)

Experiment 1 vs. 2		Experiment 1 vs. 3	
# 1	# 2	# 1	# 3
N = 14	N = 14	N = 14	N = 12
σy = 0.564	y = 0.37	σy = 0.564	σy = 0.87
σx = 4.03	x = 4.03	σx = 4.03	σx = 3.45
b_{yx} = -0.142	b_{yx} = -0.091	b_{yx} = -0.142	b_{yx} = -0.024
r = -1	r = -0.99	r = -1	r = -0.96
σd_b = 0.055		σd_b = 0.048	
d_b = 0.051		d_b = 0.118	
Calc. t = 0.93		Calc. t = 2.46	
Tabled t = 2.0 at 0.05 P		Tabled t = 2.0 at 0.05 P	
Therefore no significance.		Therefore a significant difference between regression coefficients.	

Table III

DIET AND THE SURVIVAL OF HERRING LARVAE

DIET (a)		DIET (b)		DIET (c)	
Day	Number of Herring larvae	Day	Number of Herring larvae	Day	Number of Herring larvae
1	25	1	25	1	25
2	23	2	25	2	24
3	23	3	24	3	24
4	21	4	23	4	24
5	18	5	20	5	22
6	10	6	13	6	22
7	4	7	7	7	21
8	0	8	6	8	21
		9	3	9	20
		10	3	10	20
		11	3	11	19
		12	3	12	19
		13	2	13	19
		14	2	14	18
		15	2	15	18
				16	18
				17	17
				18	16
				19	16
				20	16

TABLE IV

GROWTH OF HERRING LARVAE ON DIET C

Lengths at 20 days, hatched at 6.00 mm.

DAY	LENGTH
1	12.1 mm.
2	10.8 "
3	11.3 "
4	12.2 "
5	12.1 "
6	9.6 "
7	11.0 "
8	10.4 "
9	12.3 "
10	10.7 "
11	9.0 "
12	11.1 "
13	7.8 "
14	10.9 "
15	12.5 "
16	11.2 "
Total	175.0
Mean Length	10.9 mm.

TABLE V

PERCENT SURVIVAL AT VARIOUS AGES OF EGGS FERTILIZED AND HELD IN CONSTANT SALINITIES

SALINITY	ORIGINAL NO.	AGE IN HOURS				
		18	61	106	157	204
0°/oo	5446	0	0	0	0	0
6.06°/oo	2783	77	48	44	37	30
11.55°/oo	876	88	80	75	74	63
16.24°/oo	894	84	56	50	45	40
22.30°/oo	1185	87	79	67	62	37
26.13°/oo	1065	82	38	36	28	16
30.26°/oo	1086	80	77	61	32	17
34.28°/oo	1258	62	58	52	40	36

TABLE VI

MULTIPLE CLASSIFICATION

SALINITY - SURVIVAL - AGE

SLIDES	SALINITY (°/oo) TREATMENT (% Survival)							SUM OF 56 SLIDES	YIELD/SLIDE
	6.06	11.55	16.24	22.30	26.13	30.26	34.28		
					18 HOURS				
1	72	100	74	100	8	95	100		
2	65	95	81	100	12	97	94		
3	88	89	94	100	54	42	91		
4	81	88	86	100	56	85	100		
5	32	82	69	78	100	89	95		
6	43	93	75	46	77	63	85		
7	74	82	60	88	49	96	96		
8	68	98	67	90	84	97	96		
SUM	523	727	606	702	440	664	753	4415	78.8
					61 HOURS				
1	67	100	74	93	8	55	100		
2	34	78	80	100	12	47	78		
3	76	89	88	91	48	42	83		
4	70	84	75	96	44	78	98		
5	32	82	30	37	89	89	93		
6	39	93	45	33	61	59	67		
7	67	82	54	74	49	96	92		
8	68	81	52	76	84	97	94		
SUM	453	689	498	600	395	553	705	3893	69.5

TABLE VI Continued

MULTIPLE CLASSIFICATION

SALINITY - SURVIVAL - AGE

SLIDES	SALINITY (°/oo) TREATMENT (% Survival)							SUM OF 56 SLIDES	YIELD/SLIDE
	6.06	11.55	16.24	22.30	26.13	30.26	34.28		
					106 HOURS				
1	60	99	70	65	5	8	63		
2	19	68	80	45	12	8	47		
3	70	89	87	78	48	29	58		
4	68	81	72	79	44	87	73		
5	14	74	24	27	63	59	58		
6	32	93	23	16	53	42	47		
7	62	76	41	74	35	61	92		
8	51	80	47	76	20	70	91		
SUM	376	660	444	460	280	314	529	3063	54.7
					157 HOURS				
1	58	86	44	22	0	4	63		
2	17	68	69	22	1	6	38		
3	50	75	85	83	38	1	48		
4	66	84	69	74	33	23	73		
5	12	51	19	13	35	37	58		
6	17	78	27	13	31	25	47		
7	59	76	40	50	24	36	74		
8	43	68	45	61	20	31	85		
SUM	322	586	398	338	182	163	486	2475	44.2

TABLE VI Continued

MULTIPLE CLASSIFICATION

SALINITY - SURVIVAL - AGE

SLIDES	SALINITY (°/oo) TREATMENT (% Survival)							SUM OF 56 SLIDES	YIELD/SLIDE
	6.06	11.55	16.24	22.30	26.13	30.26	34.28		
					204 HOURS				
1	39	74	41	10	0	1	63		
2	2	65	64	16	1	2	36		
3	37	62	78	61	2	0	6		
4	44	79	61	61	13	14	9		
5	1	42	26	7	14	0	43		
6	11	67	13	5	16	23	47		
7	47	74	35	8	12	16	74		
8	27	61	36	6	4	13	82		
SUM	208	524	354	174	62	69	360	1751	31.3
SUM OF 40 SLIDES	1882	3186	2300	2274	1359	1763	2833	15,597	
% SUR. PER SLIDE	47.5	79.6	57.5	51.7	34.0	44.1	70.8		

TABLE VI Continued

MULTIPLE CLASSIFICATION

1. Correction: $\frac{(15,597)^2}{280} = 868,809$

2. Total: $(72)^2 + (65)^2 \ldots\ldots (85)^2 = 249,950$

3. Subclasses: $\frac{(523)^2 + (453)^2 \ldots\ldots + (360)^2}{8} - 868,809$
$= 153,772$

4. Within Subclasses: $249,950 - 153,772 = 96,178$

5. Age Treatments: $\frac{(4415)^2 + (3893)^2 + \ldots. (1751)^2}{56}$
$- 868,809$
$= \frac{53,221,269}{56} - 868,809$
$= 81,571$

6. Salinity Treatments: $\frac{(1882)^2 + (3186)^2 + \ldots. (2833)^2}{40}$
$- 868,809$
$= \frac{37,134,535}{40} - 868,809$
$= 59,554$

TABLE VI Continued

MULTIPLE CLASSIFICATION

7. Subclass Discrepance: $153,772 - (81,571 + 59,554)$

$= 12,647$

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES (VARIANCE)
Age Treatments	4	81,571	20,398
Salinity Treatments	6	59,554	9,926
Interaction	24	12,647	527
Slides Treated Alike	245	96,178	393
TOTAL	279	249,950	

8. Analysis of Variance of Percent Egg Survival in Constant Salinity Experiment:

a. Interaction: $F = \frac{527}{393} = 1.34$

b. Salinity Treatments: $F = \frac{9926}{393} = 25.26$ **

c. Age Treatments: $F = \frac{20,398}{393} = 51.90$ **

TABLE VII

MORTALITY INCREMENTS (PERCENT)

CONSTANT SALINITY EXPERIMENT

AGE IN HOURS	SALINITY (°/oo)						
	6.06	11.55	16.24	22.30	26.13	30.26	34.28
0	17	6	12	9	9	10	9
18	6	4	6	3	11	10	23
61	27	10	26	10	52	4	7
106	10	7	19	15	8	19	9
157	14	1	10	15	14	48	14
204	22	16	11	38	40	44	16
240	23	4	21	42	64	46	14

TABLE VIII

PERCENTAGE SURVIVAL OF LARVAE HATCHED IN

VARIOUS SALINITIES, FOUR DAYS AFTER EMERGENCE

SALINITY °/oo	FERTILIZED EGGS	LIVE EGGS @ 204 HRS.	LIVE LARVAE AFTER 6 DAYS	% OF ORIGINAL EGGS	% OF EGGS ALIVE AT 204 HRS.
0	5446	0	0	0	0
6.06	2783	1005	5	0.2	0.5
11.55	876	587	73	8.3	12.4
16.24	894	409	84	9.4	20.5
22.30	1185	465	13	1.1	2.8
26.13	1065	195	8	0.8	4.1
30.26	1086	222	4	0.4	1.8
34.28	1258	467	148	11.8	31.7

TABLE IX

EGG DENSITY AND SURVIVAL,

CHI SQUARE ANALYSIS

DENSITY GROUP	$^{*}a$	$^{*}a'$	$\frac{1}{a + a'}(an' - a'n)^2$
0- 15	190	85	5×10^6
16- 30	390	206	188×10^6
31- 45	463	245	227×10^6
46- 60	835	629	3569×10^6
61- 72	885	651	3419×10^6
73- 90	1414	360	2291×10^6
91-125	1975	424	5030×10^6
126-250	2196	951	6×10^6
D.F. = 7	n = 8348	n' = 3551	$14{,}735 \times 10^6$

$^{*}a$:- Number of eggs surviving in each group after salinity treatment.

$^{*}a'$:- Number of eggs dying during salinity treatment.

n = Total a

n' = Total a'

$$\chi^2 = \frac{14{,}735 \times 10^6}{29 \times 10^6} = \frac{497}{}$$

$\therefore$ A significant difference.

TABLE X

PERCENT SURVIVAL OF EGGS TRANSFERRED

TO SECONDARY SALINITIES

@ 36 HOURS

	TO							
FROM	0°/oo	6.06	11.55	16.24	22.30	26.13	30.26	34.28
6.06°/oo	0	68	66	85	67	26	22	96
11.55	0	41	69	100	42	20	0	96
16.24	0	48	52	96	68	51	4	87
22.30	0	33	48	93	79	70	4	70
26.13	0	0	7	42	5	12	2	90
30.26	0	14	62	100	23	61	9	80
34.28	0	86	75	90	22	37	22	97
AVERAGE SURVIVALS	0	41	63	87	50	40	9	88

TABLE X Continued

TRANSFERRED @ 90 HOURS

FROM	TO							
	0°/oo	6.06	11.55	16.24	22.30	26.13	30.26	34.28
6.06°/oo	61	84	81	83	96	84	60	92
11.55	87	86	100	100	87	94	97	86
16.24	59	100	95	89	79	72	53	51
22.30	46	86	98	98	74	77	66	75
26.13	89	75	92	92	100	97	86	94
30.26	18	97	100	86	97	100	21	92
34.28	95	98	99	100	100	86	5	86
AVERAGE SURVIVALS	65	89	95	93	90	87	55	82

TABLE X Continued

TRANSFERRED @ 135 HOURS

FROM	TO							
	0°/oo	6.06	11.55	16.24	22.30	26.13	30.26	34.28
6.06°/oo	31	77	78	96	96	73	8	87
11.55	69	100	95	100	100	96	42	70
16.24	87	96	100	94	98	64	25	-
22.30	36	98	90	93	89	100	36	100
26.13	5	36	100	100	71	100	85	84
30.26	7	100	100	100	83	100	0	70
34.28	50	51	100	100	89	95	84	98
AVERAGE SURVIVALS	41	80	95	98	89	90	40	85

TABLE X Continued

TRANSFERRED @ 190 HOURS

FROM	TO							
	0°/oo	6.06	11.55	16.24	22.30	26.13	30.26	34.28
6.06°/oo	67	84	39	63	81	18	67	49
11.55	38	98	64	81	50	71	53	78
16.24	19	59	96	100	57	79	71	83
22.30	0	76	29	74	30	19	72	78
26.13	0	50	43	64	85	34	58	66
30.26	4	60	81	62	96	80	-	-
34.28	65	93	92	91	97	88	74	81
AVERAGE SURVIVALS	28	74	63	76	71	56	66	72

TABLE XI

MAGNITUDE OF SALINITY CHANGE AND PERCENT SURVIVAL

@ 36 AND 90 HOURS

	SALINITY CHANGE IN °/oo													
	-34	-30	-25	-20	-15	-10	-5	0	+5	+10	+15	+20	+25	+30
36 HOURS							0	68	66	85	67	26	22	96
						0	41	69	100	42	20	0	96	
					0	48	52	96	68	51	4	87		
				0	33	48	93	79	70	4	70			
			0	0	7	42	5	12	2	90				
		0	14	62	100	23	61	9	80					
	0	86	75	90	22	37	22	97						
90 HOURS							61	84	81	83	96	84	60	92
						87	86	100	100	87	94	97	86	
					59	100	95	89	79	72	53	51		
				46	86	98	98	74	77	66	75			
			89	75	92	92	100	97	86	94				
		18	97	100	86	97	100	21	92					
	95	98	99	100	89	95	84	86						

TABLE XI Continued

135 AND 190 HOURS

SALINITY CHANGE IN °/oo

	-34	-30	-25	-20	-15	-10	-5	0	+5	+10	+15	+20	+25	+30
135 HOURS							31	77	78	96	96	73	8	87
						69	100	95	100	100	96	42	70	
					87	96	100	94	98	64	25	-		
				36	98	90	93	89	100	36	100			
			5	36	100	100	71	100	85	84				
		7	100	100	100	83	100	0	70					
	50	51	100	100	89	95	84	98						
90 HOURS							67	84	39	63	81	18	67	49
						38	98	64	81	50	71	53	78	
					19	59	96	100	57	79	71	83		
				0	76	29	74	30	19	72	78			
			0	50	43	64	85	34	58	66				
		4	60	81	62	96	80	-	-					
	65	93	92	91	97	88	74	81						
AVERAGE	70	45	61	60	68	69	70	71	73	69	59	51	61	81

TABLE XII

SUMMARY OF EGG SURVIVALS IN SECONDARY SALINITIES (PERCENT)

AGE IN HOURS	SALINITY °/oo							
	0	6.06	11.55	16.24	22.30	26.13	30.26	34.28
36-86	0	41	63	87	50	40	9	88
90-132	65	89	95	93	90	87	55	82
135-188	41	80	95	98	89	90	40	85
190-237	28	74	63	76	71	56	66	72

TABLE XIII

PERCENTAGE SURVIVAL OF EGGS

THROUGH TWO SALINITY CHANGES

AGES - 37 - 87 - 132 HOURS

SALINITY CHANGES °/oo	PERCENT SURVIVAL
6.06 - 6.06 - 6.06	100 - 67 - 45
16.24 - 11.55 - 6.06	100 - 52 - 50
34.28 - 22.30 - 6.06	100 - 89 - 79
30.26 - 16.24 - 6.06	100 - 100 - 68
22.30 - 16.24 - 6.06	100 - 89 - 75
11.55 - 16.24 - 22.30	100 - 100 - 84
6.06 - 11.55 - 22.30	100 - 66 - 38
34.28 - 26.13 - 22.30	100 - 37 - 29
22.30 - 22.30 - 22.30	100 - 79 - 25
34.28 - 34.28 - 34.28	100 - 97 - 86
16.24 - 22.30 - 34.28	100 - 68 - 39
6.06 - 16.24 - 34.28	100 - 85 - 10

TABLE XIII Continued

AGES 135 - 188 - 205 HOURS

SALINITY CHANGES °/oo	PERCENT SURVIVAL
6.06 - 6.06 - 6.06	100 - 77 - 38
34.28 - 16.24 - 6.06	100 - 100 - 100
22.30 - 16.24 - 6.06	100 - 93 - 29
34.28 - 34.28 - 34.28	100 - 100 - 100
16.24 - 22.30 - 34.28	100 - 98 - 75
6.06 - 11.55 - 34.28	100 - 75 - 40

TABLE XIV

SURVIVAL OF HERRING LARVAE

RAISED IN DIFFERENT SALINITIES

SALINITY	AGE IN DAYS*									
°/oo	1	4	7	10	13	16	19	22	25	28
6.06	5	4	4	3	3	1	-	-	-	-
11.55	73	60	56	53	53	49	36	24	16	11
16.24	84	78	65	46	46	44	35	17	11	5
22.30	13	12	12	12	12	11	9	5	2	1
26.13	8	7	6	6	6	5	4	3	3	2
30.26	4	4	4	4	4	2	1	1	1	-
34.28	148	146	143	143	115‡	79	41	30	20	11

* Day 1 - Some of the larvae at this time are already 3 or 4 days old as explained in the text.

‡ At this point, 20 live larvae were removed - see text.

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