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Ecological Research Series

Effects of Temperature on Diseases of Salmonid Fishes

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EFFECTS OF TEMPERATURE ON DISEASES OF SALMONID FISHES

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ABSTRACT

The effect of water temperature on infections of salmonid fish was investigated. Chondrococcus columnaris infection was studied in rainbow trout, coho and spring chinook salmon; Aeromonas salmonicida infection in coho and spring chinook salmon; and Aeromonas liquefaciens infection in steelhead trout. In all cases mortality rates were high at 64° to 69°F; moderate at 54° to 59°F; and low or zero at 39° to 49°F. Progress of the infections was accelerated at higher temperatures, and progressively retarded at decreasing temperature levels.

In infection of coho with Ceratomyxa shasta, mortality was high at 69°F, low at 49° to 54°, and zero at 39° to 44°F. This infection in rainbow trout resulted in high mortality at all temperatures except 39°. In both cases the course of the disease was most rapid at higher temperatures, and became progressively slower as the temperature decreased.

For infection of kokanee salmon fingerlings with sockeye salmon virus, the temperature range of 54° to 59°F was optimal. In this range mortality rates were high, and the course of the disease most rapid. At higher temperatures mortality rates were lower, and at 39° to 44°F, progress of the disease was retarded, though total mortality was often high.

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SECTION I

CONCLUSIONS

1. Water temperatures of 59°F and above produce high mortality rates in juvenile coho salmon injected with Aeromonas salmonicida. Even at 49° and 54° losses may exceed 40 percent.

2. Mortality rates of coho salmon injected with A. salmonicida are very low at temperatures of 39° and 44°F.

3. The mean time to death of coho salmon injected with A. salmonicida is estimated to be 3.5 days at 69°F, and this increases steadily as water temperature decreases, to a maximum of 31 days at 39°F.

4. The effect of temperature on the growth rate of A. salmonicida in vitro appears to be similar to its effect on the rate of progress of the infection in fish.

5. Among spring chinook salmon injected with A. salmonicida, the mean time to death is estimated to be 2.9 days at 74°F, and this increases progressively as water temperature decreases, to a maximum of 18.4 days at 39°F.

6. The percentage of fatal infections among steelhead trout injected with Aeromonas liquefaciens, is high at temperatures of 64°F and above,

moderate at 54° and 59°F, and zero at 49°F and below.

7. When coho and spring chinook salmon, and rainbow trout are infected with Chondrococcus columnaris by water contact, the percentage of fatal infections is high at temperatures of 64°F and above, moderate at 59°F and approaches zero at 49°F and below.

8. A temperature of 54°F is close to the threshold for development of fatal infection of salmonids by Chondrococcus columnaris.

9. The percentage of fatal infections in rainbow trout infected with Ceratomyxa shasta is high at water temperatures between 74° and 44°F.

10. The mean time to death of rainbow trout infected with C. shasta is approximately 14 days at 74°F, increasing to approximately 155 days at 44°F. Fish continually held at 39°F are not believed to develop fatal infection.

11. The percentage of fatal infections among coho salmon infected with C. shasta, is high at 64°F and above, moderate at 54° to 59°, and approaches zero at 49° and below.

12. The effect of temperature on the progress of infection by C. shasta appears similar in rainbow trout and coho salmon, as indicated by the mean time to death. There is a distinct difference in the effect of

temperature on susceptibility of these two species. Coho are not equally susceptible at all temperatures, while rainbow trout are.

13. Among kokanee salmon fingerlings exposed to the sockeye salmon virus mortality rates are high at water temperatures from 39° to 59°F, and significantly lower at 64° and above.

14. While fatal infections due to the virus may be high at 39°F, the mean time to death is much longer than at higher temperatures.

SECTION II

RECOMMENDATIONS

Water temperatures in many rivers of the Pacific Northwest from May through October are in a range favorable for the progress of the important infectious diseases of salmonids. During this period threshold temperatures for these diseases are reached and a maximum of 70°F is not uncommon. Temperatures favorable to the host generally occur from November through April. It is therefore recommended that no additional sources of heat should be allowed to enter these rivers. Added heat during the period from May through October could only serve to further enhance the severity of these diseases. Increasing water temperatures from November through April would shorten the period when conditions are most favorable for the host.

Data collected in this laboratory over the past 5 years as part of another study indicate that the threshold temperature for initiation of infection by Ceratomyxa shasta is approximately 50°F. Results in these studies revealed that once animals are infected with this organism fatal disease develops over a wide range of temperatures. Therefore it appears that when temperatures exceed 50°F in waters where this agent occurs, disease and deaths can be expected.

Water temperature should be considered before trout or salmon are released into streams or lakes. Releases should not be made when the

temperature exceeds 50°to 53°F.

Evidence gathered during this investigation indicates that infection with the sockeye salmon virus occurs over a wide range of temperatures. As a result no practical recommendation could be made pertaining to changes of temperature in waters containing this infectious agent. It is conceivable that temperatures above 64°F could be used to control progress of the disease in fish rearing facilities equipped with water treatment or reuse systems which could eliminate other pathogens.

SECTION III

INTRODUCTION

The chief objective of the work described in this report has been to determine the effect of water temperature upon the course of and mortality from the more important infectious diseases of the salmonid fish native to rivers of the Pacific Northwest.

The diseases which have been studied have included those caused by Ceratomyxa shasta, Chondrococcus columnaris, Aeromonas salmonicida, Aeromonas liquefaciens, and the Oregon sockeye salmon virus. Fish species which have been used in these studies were juvenile coho and chinook salmon and steelhead trout. Fingerling kokanee salmon were used in experiments with the virus.

The general experimental plan which has been followed with each disease agent has been to infect groups of susceptible fish of a given species by the most appropriate method, and to hold these groups in tanks of flowing water, controlled at one of several temperature levels. Eight temperatures, from 39°F to 74°F, with 5° increments, have been provided. For each experimental temperature, groups of 50 or more infected fish have been employed, distributed equally between 2 tanks. Parallel groups of normal uninfected fish have been held under identical conditions.

All experimental fish have been observed daily for appearance of symp-

toms, lesions, or fatal infections. Dead fish were removed immediately, and were autopsied and the appropriate organs examined culturally for the presence of the specific pathogen. Observations were continued until no further deaths occurred.

The effect of the various water temperatures upon each type of infection has been judged by the fraction of the group of fish held at each temperature that developed fatal infection caused by the specific pathogen, and by the mean death time for those that succumbed in each group.

SECTION IV

EQUIPMENT DESIGN AND FABRICATION PHASE

Before experiments dealing with the effect of water temperature on infectious diseases of fish could be undertaken, special equipment for holding experimental animals at various temperatures had to be designed and fabricated. This phase of the project was submitted to engineering firms for bids, and was ultimately carried out by the Corvallis firm of Cornell, Howland, Hayes and Merryfield.

The equipment provided for holding fish consists of 64 covered fiberglass tanks or aquaria of about 21 gallon capacity. Sixteen of these were new and 48 were already installed in the fish disease laboratory. Water is supplied to the laboratory from a well, at a constant temperature of 54°F. Eight of the 64 tanks are supplied with flowing water at that temperature. Eight tanks are supplied with water heated to one of the following temperatures: 59°, 64°, 69° and 74°F; and 8 tanks receive water chilled to 49°, 44° and 39°F. The rate of flow of these various streams of heated and chilled water is variable, with a maximum of 1.0 gallon per minute per tank. The temperature of each stream is automatically controlled by a recorder-controller and mixing valve within a range of ± 0.5°F. An alarm system gives warning of any failure that might develop in temperature control.

The heated water is supplied by a gas fired boiler capable of supplying

each of the 4 heated water streams at a rate of 8 gallons per minute. The refrigerated water is produced by a stainless steel chiller of special design and custom built, with a capacity adequate to supply each of the 3 refrigerated streams at 8 gallons per minute. All equipment, piping and valves that come in contact with the water supply are of stainless steel or polyvinyl chloride, to eliminate possible toxicity to fish. A small frame building, 16 x 10 ft. was constructed to house the boiler, chiller and air compressor.

In order to provide protection against possible failure of the well water supply to the laboratory, and to permit the periodic overhaul of the pump in the laboratory well, it was necessary to make an alternate source of water available. Piping was installed to connect the laboratory to an existing well located at a distance of about 150 yards. A new pump with a capacity of 300 gallons per minute was required to deliver the required volume of water from this second well.

SECTION V

EFFECT OF WATER TEMPERATURE ON INFECTION OF SALMONIDS BY *AEROMONAS SALMONICIDA* AND *AEROMONAS LIQUEFACIENS*

Materials and Methods

Two strains of *Aeromonas salmonicida* were used in the work reported here. Strain 5-G was isolated from the kidney of a coho salmon during an outbreak of furunculosis at the Siletz Hatchery in Oregon. Stock cultures were maintained by cultivating the organism in peptone-beef extract-glucose broth, centrifuging, resuspending the cells in sterile skim milk, and lyophilizing. The second strain, SS-70 was isolated from the kidney of a chinook salmon at the South Santiam Hatchery in Oregon. It was passed through a series of 13 transfers in juvenile coho salmon by intraperitoneal inoculation of a suspension of kidney tissue from the fish infected in the preceding transfer. Kidney tissue from the last fish in the series was then macerated, suspended in skim milk and lyophilized.

Aeromonas liquefaciens, strain K-1, was isolated from the kidney of shad during an epizootic in Coos Bay, Oregon. Stock cultures were maintained on peptone-beef extract-glucose agar covered with a layer of neutral mineral oil. This medium contains 10 gm of peptone (Difco) 5 gm of glucose, 10 gm of beef extract, 5 gm of sodium chloride, and 15 gm of agar, per liter.

Experimental fish employed in these experiments were juvenile coho, or spring chinook salmon, or juvenile steelhead trout. Their average weight ranged from 10 to 30 grams in different experiments. They were generously donated for this project in relatively large numbers by the Oregon Game Commission and the Fish Commission of Oregon.

Experimental infections in fish were produced by the intramuscular or intraperitoneal injection of 0.05 ml of a 48 hour culture of the organism grown in brain heart infusion broth (Difco Labs) or peptone-beef extract-glucose broth (PBG) and resuspended in frog Ringer saline at pH 6.9-7.0. The composition of the PBG medium with agar is described subsequently in this report. The broth is prepared in the same manner, but without the agar. The bacterial concentration was adjusted to represent from 0.5 to 2.0 LD_{50} doses, based on an earlier titration of the same organism in the same fish species held at $54^{o}F$. It would have been distinctly preferable to use a more natural method for establishing infection, but preliminary experiments and previous experience indicated that exposure of fish to high concentrations of these aeromonas species in their water supply, or the presence of infected fish in the tank with normal susceptible ones, could not be relied upon to produce fatal infections in a large percentage of those exposed. These organisms while pathogenic for fish, do not always possess highly invasive properties.

The method used to temper fish to the various experimental water temperatures was as follows: When first received from the hatchery,

the fish were placed in holding tanks supplied with well water at 54°F. At the beginning of an experiment, the number of fish to be used in one experimental group were transferred to an 18 gallon tank, also supplied with 54°F water. Water at the next temperature increment, either 49° or 59°F, from one of the controlled streams, was then introduced at the rate of about one half gallon per minute. Within 1 to 1½ hours, the water in the tank had reached the 49° or 59° level. The fish were then held at the new temperature for 48 hours. Water at the next temperature increment, either 44° or 64° was then introduced into the tank at the same rate as before, and the new temperature maintained for 48 hours. This process was repeated until groups of fish had been adjusted to each of the eight experimental temperature levels covering the range from 39° to 74°F at 5 degree intervals.

For the cultural examination of experimental fish at necropsy small fragments of kidney tissue were streaked on plates of Furunculosis Agar, Difco, modified by the addition of 1 gram of skim milk solids per liter of medium. Plates were incubated at room temperature (about 22°C) for 48 hours. Colonies producing zones of clearing on this medium were inoculated on two plates of regular Furunculosis Agar, tubes of Oxidative-Fermentative Medium, Difco, and Arginine Decarboxylase Medium, Difco. One of the plates was incubated at 37°C to inhibit A. salmonicida, the other at room temperature to permit growth. The latter plate was then used for determining morphology, the Gram reaction, motility, catalase and cytochrome oxidase reactions. A. salmonicida is a Gram negative,

non-motile rod that produces clearing on the Furunculosis Agar with casein, fails to grow at 37°C, and forms catalase and cytochrome oxidase. A. liquefaciens differs by growing at 37°C and in being motile.

The experimental design adopted in this work required the use of sixteen 18 gallon aquaria for each experiment. Thus 2 tanks were provided for each of the 8 water temperatures. Eight tanks, one at each temperature, were assigned to groups of fish to be infected with the pathogen being studied, while the remaining eight were assigned to groups of uninfected control fish that received sham injections. The number of fish per tank was at least 25, and in some experiments was increased to 35. Two complete and identical experiments were conducted concurrently, each one consisting of 8 groups of infected fish and 8 control groups. The purpose of this plan was to provide information concerning the degree of variation to be expected between groups of fish receiving, insofar as possible, exactly the same treatment.

The terms rainbow trout and steelhead trout are used in the text of this report. It should be understood that both terms refer to a single species, i.e. Salmo gairdneri, but steelhead are anadramous while rainbow trout do not migrate to the ocean.

Experimental Phase

Effect of Temperature on Infection with *Aeromonas salmonicida*

The effect of water temperature on experimental infection of juvenile coho salmon (*Oncorhynchus kisutch*) with *Aeromonas salmonicida*, strain 5-G was studied in two experiments. In each of these, 400 fish averaging 28 grams in weight, were distributed at random among 16 tanks, 25 fish per tank. Each tank contained 18 gallons of well water, flowing at a rate of 0.5 gallons per minute. Eight tanks contained fish to be infected, and eight contained fish to be used as uninfected controls. One tank in each group of eight received flowing water at 74°F, another pair received water at 69°F, a third pair received water at 64°F, and so on, so that the range of temperatures from 74°F to 39°F was covered, with groups of fish maintained at each 5 degree increment of temperature. The two experiments, involving 800 experimental fish, were carried out concurrently.

After tempering of the fish to the various temperature levels, those to be infected received an intramuscular injection of 2 LD_{50} of a 48 hour broth culture of *A. salmonicida*, strain 5-G diluted in frog Ringer saline. An LD_{50} was the approximate number of bacteria causing death in 50% of a group of 20 to 30 gram coho salmon injected with the organisms intramuscularly and held at 59°F for 5 days after the last death occurred. Control fish received a sham injection of 0.05 ml of a sterile

filtrate from a similar culture diluted to the same extent. Dead fish were collected daily, each was autopsied, and kidney tissue samples were cultured. This bacterium has been found to be recoverable from the kidney of about 74% of fish succumbing from this infection. All experimental groups were observed over a 55 day period.

Results of the two experiments are shown in Table 1. It is apparent that among the infected groups, the per cent mortality decreased in a stepwise manner from 100% at 69°F to 12% and 14% at 44° and 39° respectively. In three instances a 5 degree reduction in water temperature did not significantly influence the per cent mortality; this is evident at 69° and 64°, at 54° and 49°, and at 44° and 39°. However mortality was significantly lower at 59° than at 64°, at 54° than at 59°, and at 44° than at 49° (Appendix, page 95). The data indicate that the development of fatal disease in juvenile coho due to this organism was suppressed at water temperatures of 39° to 44°, and was enhanced progressively at temperatures of 49°, 59° and 64°.

The results of culturing kidney tissue from the infected groups of fish are recorded in Table 2. Aeromonas salmonicida was recovered from the majority of the individuals in each temperature group that succumbed to the infection. These cultural recoveries strengthen the evidence that death was due to the aeromonas infection. It may be presumed that the remaining fish in the groups at 59° and below from which the organism was not recovered also died from the infection, since control fish that

Table 1. Effect of water temperature on Aeromonas salmonicida infection in juvenile coho salmon.

Water temperature	Fraction of each group that died				Per cent mortality; 2 expts. combined.	
	Experiment 1		Experiment 2			
	Infected	Controls	Infected	Controls	Infected	Controls
74°F	25/25	13/25	25/25	20/25	100.0	66.0
69°F	25/25	2/25	25/25	0/25	100.0	4.0
64°F	24/25	3/25	23/25	5/25	94.0	16.0
59°F	16/25	0/25	19/25	0/25	70.0	0
54°F	9/25	0/25	12/25	0/25	42.0	0
49°F	11/25	0/25	12/25	0/25	46.0	0
44°F	2/25	0/25	4/25	0/25	12.0	0
39°F	4/25	0/25	3/25	0/25	14.0	0

1. Average weight of experimental fish was approximately 28 grams.
2. Fish were infected by an intramuscular injection of 2 LD_{50} (about 110 organisms) of a 48 hour broth culture of a virulent strain of A. salmonicida. Control fish received a sham injection of a sterile filtrate from a similar culture of the same organism.
3. All groups of fish were held at the indicated temperatures for 55 days. Dead fish were collected daily, autopsied, and cultures made from kidney tissue.
4. The deaths of control fish at 74°F are presumably due to the fact that this temperature is slightly above the upper limit of tolerance for juvenile coho. Both unfavorable physiological effects and activation of some resident microorganisms with potential pathogenic properties apparently contributed. A. liquefaciens was isolated from the kidney in some cases, and abundant external growth of a fungus was observed on many fish at this temperature.
5. The least significant difference between percent mortality values was determined to be 14.1% at the 0.05 probability level (Appendix, page 95).

Table 2. Recovery of Aeromonas salmonicida by culture of kidney tissue of juvenile coho salmon.

Water temperature	Proportion of experimentally infected fish yielding positive cultures at autopsy		
	Fatally infected fish		Surviving fish
	No. positive / No. tested	Per cent positive	No. positive / No. tested
74°F	43/50	86.0	0/7
69°F	41/50	82.0	0/9
64°F	31/47	66.0	0/3
59°F	25/35	71.4	0/15
54°F	11/21	52.4	0/29
49°F	14/23	60.8	0/27
44°F	6/6	100.0	n.t.
39°F	6/7	85.7	n.t.

1. Twenty five control fish from the 39° and 44° groups, and all fifty controls in each of the other 6 temperature groups were examined by culturing kidney tissue on furunculosis agar. A. salmonicida was not recovered from any of these fish.
2. n.t. indicates not tested.

had received a sham injection and were held under the same conditions, all remained healthy. Aeromonas salmonicida was not found in the kidney of any of the infected fish that survived to the end of the experiment. This may be seen in the data of Table 2. Presumably the bacteria injected had been disposed of by body defense mechanisms in these individuals. In order to provide further evidence on this question, these surviving fish were transferred to tanks supplied with water at 64°F, a temperature favorable to development of this infection, and were held at this temperature for a 10 day period. No deaths occurred, and when these fish were sacrificed and autopsied, A. salmonicida was not recovered from the kidney of any of them.

A linear relationship between the log of the number of days to death and water temperature was observed and confirmed by regression analysis (Fig. 1). A correlation coefficient of -0.8850 was calculated and found to be highly significant (Appendix, page 108). This relationship indicates that progress of the fatal infection was accelerated at the higher temperatures of 69° and 64°, retarded at the intermediate temperatures, and still further retarded at the low temperatures of 39° and 44°.

This effect of water temperature upon the average time from infection until death could be an expression of the combined influence of temperature on growth of the bacterium and upon the defense mechanisms of the host. In order to shed some light on this question the growth rates of the above strain of A. salmonicida were determined at each of the tem-

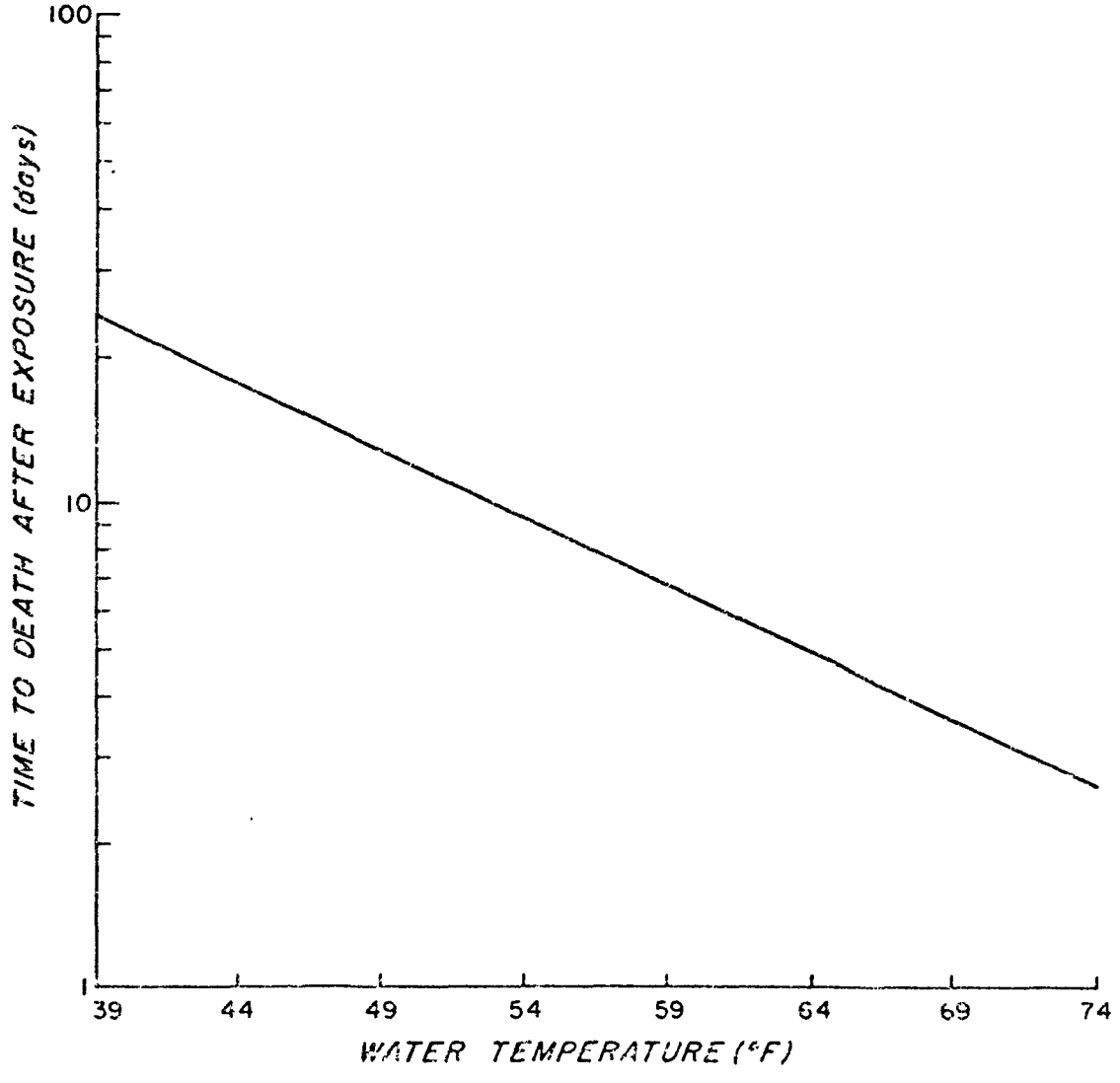

Fig. 1. Relationship between water temperature and log of time to death after infection of juvenile coho salmon with Aeromonas salmonicida.

peratures used in the fish experiment.

A flask containing 200 ml of peptone-beef extract-glucose broth was inoculated with the organism to give a concentration of 1.9×10^5 cells per ml. The inoculated medium was then distributed in 2 ml aliquots in screw cap culture tubes. A group of these tubes was then incubated at each of the 8 temperatures.

Growth was measured by determining optical density at 650 mμ at various intervals during an 80 hour incubation period. The growth rates observed are shown in Fig. 2.

A. salmonicida grew very slowly at 39° and 44°, and the rate of growth increased progressively with each 5 degree increase in temperature, reaching a maximum at 69°. Thus the effect of temperature on the growth rate of the organism in vitro appears to follow a pattern closely similar to its effect on mortality among infected fish. In other words high temperatures resulting in the most rapid growth of A. salmonicida in vitro also resulted in the shortest mean time to death among infected fish, while low temperatures resulting in very slow growth rates in vitro were associated with the longest mean times to death. No information is available concerning the possible influence of temperature on host defense mechanisms, but in any case the data of Fig. 2 indicate that the effect of temperature controls the growth of the bacterium which in turn has a major controlling influence on mortality in infected

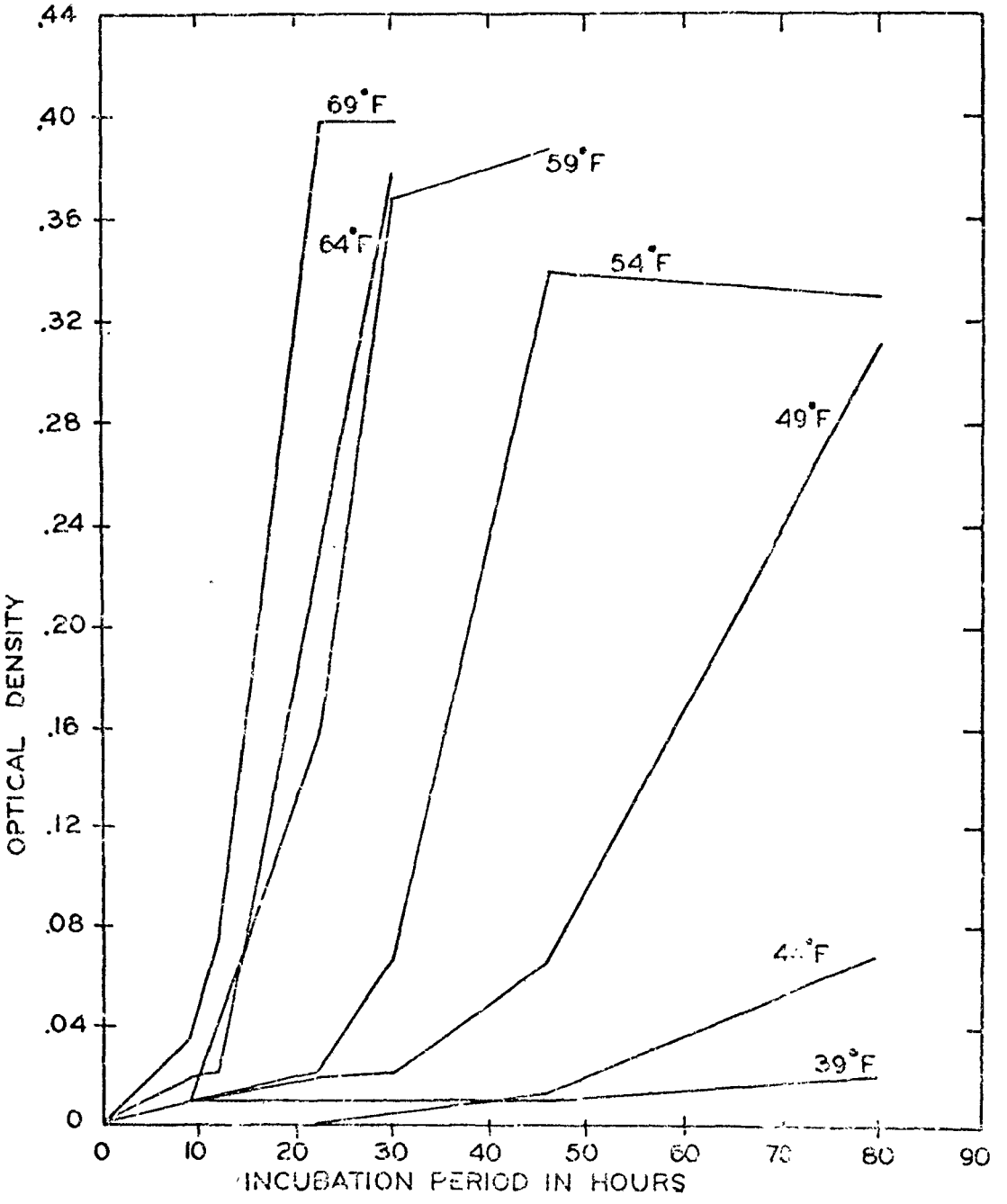

Fig. 2. Effect of temperature on growth rate of Aeromonas salmonicida in peptone-beaf-extract-glucose broth.

juvenile coho salmon.

The effect of temperature on infection of juvenile spring chinook salmon (Oncorhynchus tshawytscha) by Aeromonas salmonicida was studied in two experiments. As in the work with the coho salmon, 400 fish averaging 10 gm in weight, were used in each experiment. They were distributed at random among the 16 tanks, 25 fish per tank and tempered to the various temperatures in the manner previously described. The groups of fish to be infected received an intraperitoneal injection of 1.4 LD_{50} (about 425 organisms) doses of a 48 hour broth culture of A. salmonicida (strain SS-70). Control fish received a sham injection of 0.05 ml of sterile physiological saline. As in the coho experiments, dead fish were collected daily, autopsied, and cultures made from kidney tissue. All experimental groups were observed over a period of 35 days.

The results of these two experiments are presented in Table 3. It is apparent that there was variation in the percent mortality observed at the different temperatures, and some of the differences were statistically significant (Appendix, page 96), but the consistent reduction in mortality with decreasing temperature which was found in the coho experiments, was not observed with the spring chinook. The results obtained in 6 of the 8 temperature groups would have been compatible with such a trend, but the mortality noted at 59^{o}, and at 39^{o} were both higher than would have been expected, and in each case was significantly different from the values for the adjacent temperature groups. Reasons

Table 3. Effect of water temperature on Aeromonas salmonicida infection in juvenile spring chinook salmon.

Water temperature	Fraction of each group that died				Per cent mortality; 2 expts. combined.	
	Experiment 1		Experiment 2			
	Infected	Controls	Infected	Controls	Infected	Controls
74°F	22/25	0/25	20/25	0/25	84.0	0
69°F	18/25	0/25	22/25	1/25	80.0	2.0
64°F	19/25	1/25	17/25	0/25	72.0	2.0
59°F	23/25	0/25	25/25	0/25	96.0	0
54°F	16/25	2/25	19/25	1/25	70.0	6.0
49°F	16/25	0/25	14/25	0/25	60.0	0
44°F	6/25	1/25	13/25	2/25	38.0	6.0
39°F	18/25	0/25	16/25	1/25	68.0	2.0

1. Average weight of experimental fish was approximately 10 gm.
2. Fish were infected by an intraperitoneal injection of 1.4 LD_{50} (about 425 organisms) doses of a 48 hour broth culture of a virulent strain of A. salmonicida (strain SS-70). Control fish received a sham injection of 0.05 ml of sterile physiological saline.
3. All groups of fish were held at the indicated temperatures for 35 days. Dead fish were collected daily, autopsied, and cultures made from kidney tissue.
4. The least significant difference between percent mortality values was determined to be 14.9% at the 0.05 probability level (Appendix, page 96).

for these irregular results are not apparent, though it appears that some variable other than temperature has influenced them. The experimental method differed in two details from the coho experiments. A. salmonicida strain SS-70 was used because the stock cultures of strain 5-G, used with the coho, became non-viable for unknown reasons. The route of inoculation was intraperitoneal instead of intramuscular. However there seems to be no reason to assume that either of these differences could account for the irregularities. The experiments will be repeated when juvenile spring chinook salmon are again available.

The results of culturing kidney tissue from the fatally infected fish are shown in Table 4. Aeromonas salmonicida was recovered from the majority of these fish in each temperature group. Inoculated fish that survived were not cultured in this case.

The average interval between infection and death was determined for the infected chinook salmon by combining data from the two experiments. It was found to be 2.9 days at 74°, and to increase progressively as water temperature decreased, reaching a maximum of 18.4 days at 39°. When the log of the interval was plotted against temperature a linear relationship was revealed, exactly as in the coho salmon experiments. Confirmation of this relationship was again obtained by regression analysis (Fig. 3). A correlation coefficient at -0.8229 was calculated and found to be highly significant (Appendix, page 109). This figure show that in the chinook salmon also, the time to death was retarded at the lowest

Table 4. Recovery of Aeromonas salmonicida by culture of kidney tissue of juvenile spring chinook salmon.

Water temperature	Proportion of fatally infected fish yielding positive cultures at autopsy	
	No. positive / No. tested	Percent positive
74°F	35/42	83.2
69°F	34/40	85.0
64°F	31/36	86.1
59°F	33/48	68.8
54°F	33/35	94.3
49°F	20/25[1]	80.0
44°F	12/19	63.2
39°F	29/34	85.3

1. Only 25 of 30 dead fish in this group were cultured, as the tissues of the remaining 5 had undergone some decomposition.

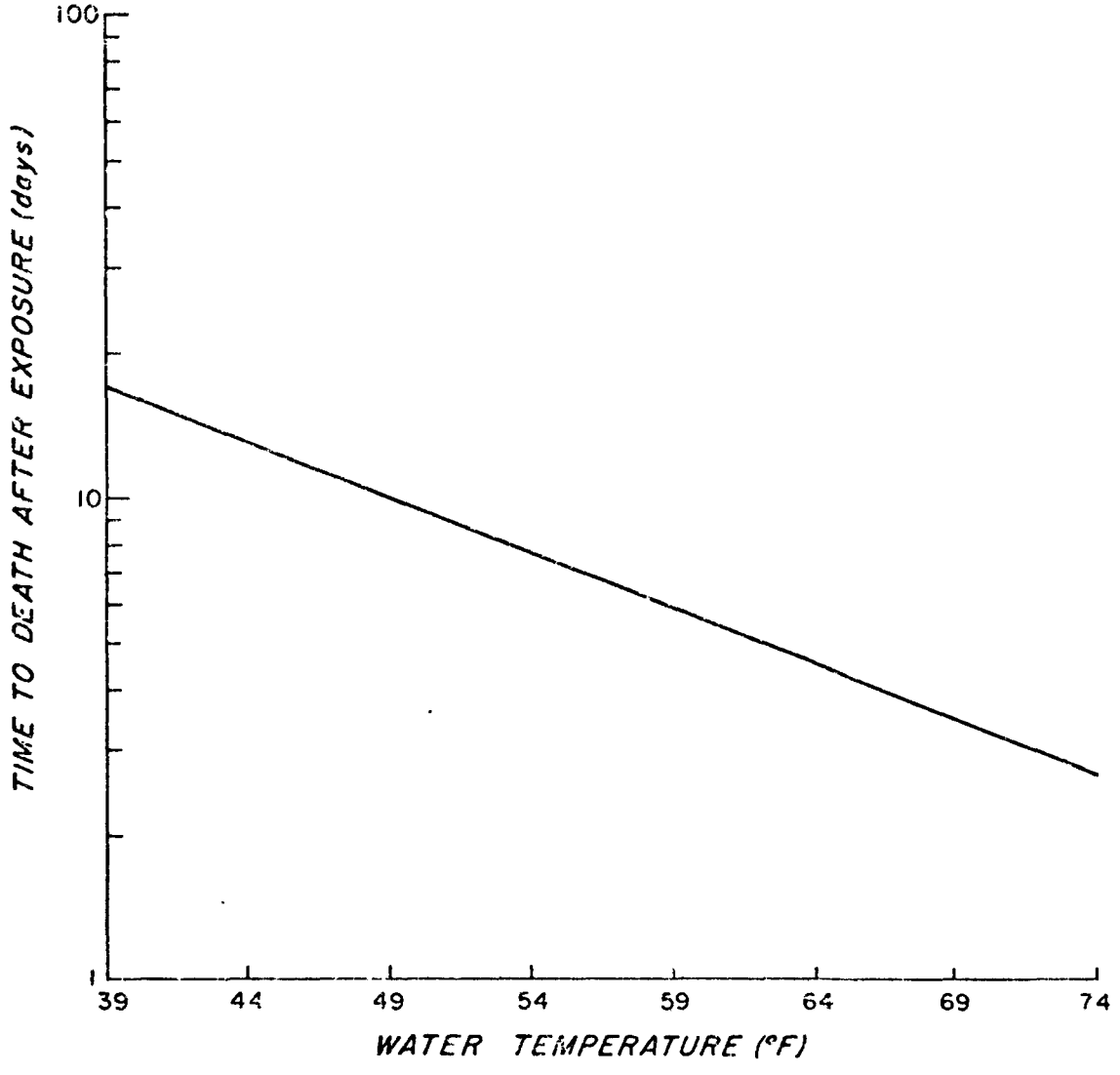

Fig. 3. Relationship between water temperature and log of time to death after infection of juvenile chinook salmon with Aeromonas salmonicida.

temperature levels, accelerated at the intermediate temperatures, and still further accelerated at the highest temperatures. At 39^{o} and 44^{o}, the chinook succumbed to the infection nearly twice as rapidly as did the coho, though at 54^{o} and above, the average time till death was closely similar for the two species.

Effect of Temperature on Infection with Aeromonas liquefaciens

Two experiments were carried out to determine the influence of water temperature on experimental infection of juvenile steelhead trout (Salmo gairdneri) with Aeromonas liquefaciens, strain K-1. In each of these, 560 fish averaging 25 grams in weight, were distributed at random among 16 tanks, 35 fish per tank. They were tempered to the various water temperatures as described previously. Groups of fish to be infected received an intramuscular injection of 0.5 LD_{50} doses (about 2.2×10^7 organisms) of a 24 hour culture of A. liquefaciens in peptone-beef extract-glucose broth. Control fish were injected with 0.05 ml of a sterile filtrate of the same culture, diluted to the same extent. All groups were held at their respective temperatures for 27 days. Dead fish were collected daily, autopsied, and cultures made from kidney tissue. Infected fish surviving at the end of this period were sacrificed and examined in the same manner. Ten control fish from each of the following temperature groups were also examined by kidney culture at the end of the experiment: 74°, 69°, 64°, 59°, and 54°F.

The mortality data from these experiments are presented in Table 5. The highest percent mortality occurred among the infected fish at 74°F, while all of the uninfected controls survived this high temperature. Mortality was significantly reduced at 69° and was essentially the same at 64°. A further significant reduction is evident at 59° and 54°, where 38.6 and 40.1 per cent of the fish died, respectively. Progress of the

infection was apparently halted at 49°, 44° and 39°, as no deaths occurred at these temperatures. Thus temperatures from 54° to 74° were favorable for the development of this infection in steelhead, and increased mortality was correlated with increased temperature in this range.

The mean time to death appeared to be about twice as long at 54° as at 74°, as indicated by the data in Table 5. However the influence of temperature on the mean time to death was less striking than in A. salmonicida infections, possibly due largely to the fact that no deaths occurred at 49° or lower.

The results of culturing kidney tissue from the infected groups of fish are presented in Table 6. It may be noted that among the infected fish that died, 68 to 93 percent in the various temperature groups, yielded cultures of A. liquefaciens. These data provide supporting evidence that death of these fish was caused by infection with this organism. It may be presumed that the remaining fish in these groups, from which the organism was not recovered, also died from the infection, since of the control fish at the same temperatures that had received a sham injection, all but one remained healthy. It is also of interest that among the surviving but infected groups of fish, some still harboured the organism in the kidney; 23 per cent of the survivors at 74° yielded positive cultures, and recoveries decreased to 17 per cent at 64°, 7 per cent at 59° and 2 per cent at 54°. None of those at 49° yielded the organism. Because of the small numbers of fish from which

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Table 5. Effect of water temperature on Aeromonas liquefaciens infection in juvenile steelhead trout.

Water temperature	Fraction of each group that died				Per cent mortality; 2 expts. combined.		Mean time from infection to death in days
	Experiment 1		Experiment 2				
	Infected	Controls	Infected	Controls	Infected	Controls	
74°F	29/35	0/35	28/35	0/35	81.5	0	1.7
69°F	18/35	0/35	27/35	0/35	64.3	0	1.7
64°F	26/35	0/35	21/35	0/35	67.2	0	1.8
59°F	9/35	1/35	18/35	0/35	38.6	1.4	2.1
54°F	11/35	0/35	17/35	0/35	40.1	0	3.3
49°F	0/35	0/35	0/35	0/35	0	0	-
44°F	0/35	0/35	0/35	0/35	0	0	-
39°F	0/35	0/35	0/35	0/35	0	0	-

1. Average weight of experimental fish was approximately 25 grams.
2. Fish were infected by an intramuscular injection of 0.5 LD_{50} (2.17×10^7 organisms) of a 24 hour culture of A. liquefaciens strain K-1 in trypticase-soy broth. Control fish received a sham injection of 0.05 ml of frog Ringer saline solution.
3. All groups of fish were held at the indicated temperatures for 27 days. Dead fish were collected daily, autopsied, and cultures made from kidney tissue. A. liquefaciens was recovered from 78 per cent of infected fish that died, and from 12% of infected fish that survived. Fifty of the uninfected controls were also examined by kidney culture and one fish yielded a culture of A. liquefaciens.
4. The least significant difference between percent mortality values was determined to be 16.1% at the 0.05 probability level (Appendix, page 97).

Table 6. Recovery of Aeromonas liquefaciens by culture of kidney tissue of juvenile steelhead trout.

Water temperature	Proportion of experimentally infected fish yielding positive cultures at autopsy			
	Fatally infected fish		Surviving fish	
	No. positive / No. tested	Per cent positive	No. positive / No. tested	Per cent positive
74°F	41/57	72.0	3/13	23.1
69°F	30/44	68.2	7/25	28.0
64°F	38/47	80.7	4/23	17.4
59°F	26/28	92.8	3/43	7.0
54°F	21/27	77.7	1/42	2.4
49°F	0	0	0/70	0
44°F	0	0	n.t.	-
39°F	0	0	n.t.	-

1. Ten uninfected control fish from each of the following temperature groups were tested for the possible presence of A. liquefaciens: 74°, 69°, 64°, 59°, and 54°F. Among these fifty fish, only one yielded a culture of the organism.
2. No deaths occurred in the 49°, 44°, and 39° groups of fish. All 70 of the survivors at 49° were cultured for the organism, but all were negative. The survivors at 44° and 39° were not cultured.

the organism was recovered, the differences in percent positive cultures between any two temperature levels differing by a 5^{o} increment are not statistically significant. However, the 28.0% value at 69^{o} differs significantly from the 7.0 and 2.4% and 0 values at 59^{o}, 54^{o} and 49^{o}, respectively. Also the 17.4% value at 64^{o} differs significantly from the 2.4% value at 54^{o} (Appendix, page 107).

These data suggest that within the temperature range studied, the higher temperatures favor survival of the pathogens in the tissues of the host, while the lower temperatures enhance the mechanisms that clear the microorganisms from these tissues.

Presumably the survivors were individuals possessing greater resistance to this bacterium than those that succumbed; however, they might serve as a reservoir of pathogens for later outbreaks when conditions are favorable.

Among 50 control fish that were examined by culture, 49 were negative and A. liquefaciens was isolated from one.

At the end of the 27 day experimental period, the infected fish that had been held at 39^{o}, 44^{o}, and 49^{o}, and among which no deaths had occurred, were transferred to tanks supplied with water at higher temperatures. The 39^{o} groups were transferred to 59^{o} water, the 44^{o} groups to 64^{o} water, and the 49^{o} groups to 69^{o} water. This was done to deter-

mine whether it was possible that small numbers of A. liquefaciens cells were surviving in some organ or tissue, and might be activated at the elevated temperatures and produce a fatal infection. These fish were observed for a period of 5 days at the higher temperatures, but no deaths occurred and all appeared healthy at the end of this period. This suggested that the organisms originally injected in these fish held at the 3 lower temperature levels had died out and been disposed of by the defense mechanisms of the animals.

Discussion

The work originally contemplated on the effect of water temperature on aeromonas infections is incomplete. It was planned to study A. salmonicida and A. liquefaciens infection in coho and chinook salmon and in steelhead trout. Progress has been slower than anticipated for several reasons. These have included the appearance of natural infections in populations of experimental fish, difficulties with temperature control equipment, and the sudden occurrence of high concentrations of dissolved nitrogen in the well water supply.

However the data reported indicate that fatal infection in coho salmon due to A. salmonicida was suppressed at 39° to 44°F, and mortality was progressively higher at temperatures from 49° to 64°. This was evident from the mortality rates and from the average intervals from infection until death, which were longest at the low temperatures, and decreased progressively with increasing temperature. In the case of juvenile spring chinook infected with this organism, the effect of temperature on mortality rates was irregular, suggesting the influence of some uncontrolled variable in the experiment; however it was again observed that the infection progressed slowly at the low temperatures and at progressively higher rates as the temperature increased. Fatal infection of steelhead trout with A. liquefaciens was prevented in the range of 39° to 49°; temperatures from 54° to 74° were favorable for development of this infection, and the mortality rate increased with temperature in this

range. Hence the limited data available thus far are consistent with the view that water temperatures above the range of 44° to 49° may cause increasing mortality from aeromonas infections in some salmonid species.

Development of a Differential Plating Medium for *Aeromonas Species* in Water Samples

During the course of this work the need arose for a bacterial culture medium that would permit the counting of aeromonas species in water specimens in the presence of other common bacterial flora. A medium was desired that would produce counts comparable to those obtained with the best plating media, while at the same time inhibiting growth of some other organisms found in water, and exhibiting differential colony reactions that would permit the recognition of aeromonas colonies. A relatively large number of formulations were compared with respect to the above properties and the following one was ultimately selected:

Peptone-Beef Extract-Glycogen (PBG) Agar

Bacto Peptone	10 grams liter
Beef extract	10 " "
Glycogen	4 " "
NaCl	5 " "
Sodium lauryl sulfate	0.1 " "
Brom thymol blue	0.1 " "
Agar	15 " "

final pH 6.9-7.1

Sodium lauryl sulfate was included in the medium as a selective agent for inhibition of some Gram positive bacteria. Brom thymol blue serves as both an indicator of pH change as well as adding to the inhibitory effect of sodium lauryl sulfate. Glycogen was included as the only carbohydrate because the aeromonas species are among the relatively few bacteria reported to be capable of fermenting this polysaccharide.

For use in plating a water sample, 1 ml of the sample in the desired dilution is added to a sterile Petri dish and mixed with 15 ml of the sterile PBG agar at 45-50^{o}C. After the agar has gelled and the surface has dried, it is overlaid with about 20 ml of 2.0% agar in distilled water. If Aeromonas salmonicida is to be isolated or counted, plates must be incubated at 25^{o}C for 3 days. Under these conditions it has been found that in addition to the above organism, a number of other bacteria also produce yellow colonies on this medium. These include some species of Citrobacter, Arizona, Edwardsiella, Enterobacter, and Serratia. However, all of these organisms, as well as Aeromonas hydrophila (liquefaciens) form large bright yellow colonies, 1 mm or more in diameter.

Colonies of A. salmonicida, Pleisomonas shigelloides, Vibrio anguillarum, and Vibrio parahaemolyticus, developing at this temperature, are pin point in size, and with a little experience can be readily distinguished from the former group. Organisms from natural sources producing these very small colonies can be presumptively identified as one of these

four species. Pleisomonas shigelloides can be distinguished by failure to produce gelatinase. The two Vibrio species produce lysine decarboxylase, but not arginine dihydrolase, reactions which would differentiate them from A. salmonicida and P. shigelloides. Production of a brown pigment and lack of motility will serve to differentiate A. salmonicida.

In addition, some colonies of this organism will produce small bubbles of gas in the agar layer, which has not been observed with any of the other bacteria mentioned above.

If the medium is to be used for isolation or counting of the Aeromonas hydrophila-liquefaciens complex, plates should be incubated at 37°C for 24 hours. Under these conditions colonies of these organisms are 0.3 to 0.5 mm in diameter and bright yellow in color. Those of Arizona, Citrobacter, Edwardsiella, Enterobacter and Serratia species are smaller, about 0.1 to 0.2 mm in diameter, and possess more of an orange color. The aeromonad colonies are often surrounded by a yellow halo in the green medium, a characteristic not observed with the other genera mentioned above. If there are as many as 200 to 300 A. hydrophila colonies per plate, the whole plate develops a yellow color, while comparable plates of the other organisms are greenish in color. Furthermore, some of the subsurface colonies of A. hydrophila produce small bubbles of gas in the agar layer, which is another differential characteristic. The Vibrio and Pleisomonas species do not grow at 37°C, and thus do not

require differentiation.

Over 75 species of bacteria have been examined on this medium. Many Gram positive organisms failed to grow, though Bacillus subtilis and other members of the genus grew sparsely. All Gram negative organisms grew, but only those genera listed above produced yellow colonies.

Although the medium has obvious limitations, it has been found to be useful in monitoring the numbers of both A. salmonicida and A. hydrophila in hatchery water, measuring their growth rates, and recovering cultures from viscera of experimentally infected fish. Thus far it appears to be superior to other available media for these purposes, but further experience will be required to completely define its usefulness and reliability.

The medium has been useful not only in counting aeromonas organisms in water, but for isolation of these organisms from fish, and measuring growth rates at various temperatures.

SECTION VI

EFFECTS OF WATER TEMPERATURE ON INFECTION OF SALMONIDS BY CHONDROCOCCUS COLUMNARIS

Materials and Methods

To determine the effect of water temperature on losses caused by the myxobacterium Chondrococcus columnaris, coho salmon (Oncorhynchus kisutch), spring chinook salmons (Oncorhynchus tshawytscha) and rainbow trout (Salmo gairdneri) were exposed to a virulent isolate of this bacterium. Groups of 25 or 35 fish of the species being tested were tempered to water temperatures ranging from 39 to 74°F at 5°F intervals. Two control and two experimental groups were held at each temperature. These animals were tempered in a manner previously described in the experiments with the aeromonads.

The C. columnaris isolate used in this study was obtained from a lesion on the gill of an adult spring chinook salmon at the Fish Commission of Oregon, Dexter Dam Holding Pond, Willamette River. This isolate was passed in coho salmon fry seven times to increase its virulence. After the seventh passage the culture was lyophilized. Immediately prior to each experiment the cells were removed from lyophilization, grown in cytophoga broth and passed once in the salmonid species being tested. Several isolates from the final fish passage were collected and pooled for use in the temperature experiment.

To prepare the exposure inoculum the cells were grown in tryptone yeast infusion broth containing 0.4% tryptone and 3.0% yeast infusion. After approximately 20 hours at 24°C the optical density was adjusted to 0.1 at 525 mμ with a Bausch and Lomb Spectronic 20 (1). The fish were then exposed in the experimental tanks to a 1:20 dilution of the adjusted broth culture for a 10 minute period. Normal water flow through the tanks was resumed after the exposure period. This dilution was determined by plate count to represent approximately 3 to 6 x 10^6 C. columnaris cells per ml. Dead fish were collected two times each day and bacteriological cultures were made from the gills and or kidney of each fish.

Experimental Phase

Table 7 shows the percent mortality and the incidence of C. columnaris in rainbow fry (average weight 2.9 g each) exposed to this bacterium at different water temperatures. Temperatures of 44 and 39°F were not included in this experiment. Fish infected with C. columnaris were observed at temperatures of 74°F down to 54°F with the larger number of losses occurring at the higher water temperatures. The greatest difference in loss occurred between 59 and 64°F. Control fish at 74°F had some mortality which was not due to C. columnaris.

Not only percent mortality but also the time to death was greatly influenced by water temperature. This is illustrated in Fig. 4, which shows the regression line relating water temperature and the log of the number of days from exposure to death. The equation and the data used in computing it are shown in Appendix, page 110. A correlation coefficient of -0.8573 was calculated and found to be highly significant. Thus the linear relationship between these two variables is demonstrated.

The results of the experiment with coho salmon (average weight 33 g each) are nearly identical to those observed with rainbow trout (Table 8). Within three days after exposure, all fish held at 69 and 74°F were dead. After one month at 64°F a loss of 99% had occurred, as compared to a 51% loss at 59°F. At 54°F only 4% of the test animals had died. No deaths due to C. columnaris occurred at 49°F or below. Among the

Table 7. Effect of water temperature on Chondrococcus columnaris infection in juvenile rainbow trout.

Water temperature	Fraction of each group that died[a]				Per cent mortality 2 expts. combined[c]		Per cent mortalities infected with C. columnaris[d]	
	Experiment 1		Experiment 2					
	Infected	Controls	Infected	Controls	Infected	Controls	Infected	Controls
74°F	25/25	8/25	25/25	5/25	100	26	100	0
69°F	24/24[b]	0/25	25/25	1/25	100	2	100	0
64°F	20/24[b]	0/25	25/25	1/25	92	2	100	0
59°F	7/25	0/25	13/25	0/25	40	0	100	0
54°F	4/25	2/25	0/25	1/25	8	6	100	0
49°F	0/25	0/25	0/25	0/25	0	0	0	0

a. At 18 days after exposure to C. columnaris.
b. One fish unaccounted for.
c. The least significant difference between mean percent mortality values for Exp. 1 and Exp. 2 was determined to be 16.1% at the 0.05 probability level (Appendix, page 98).
d. Based on recovery of the organism at autopsy.

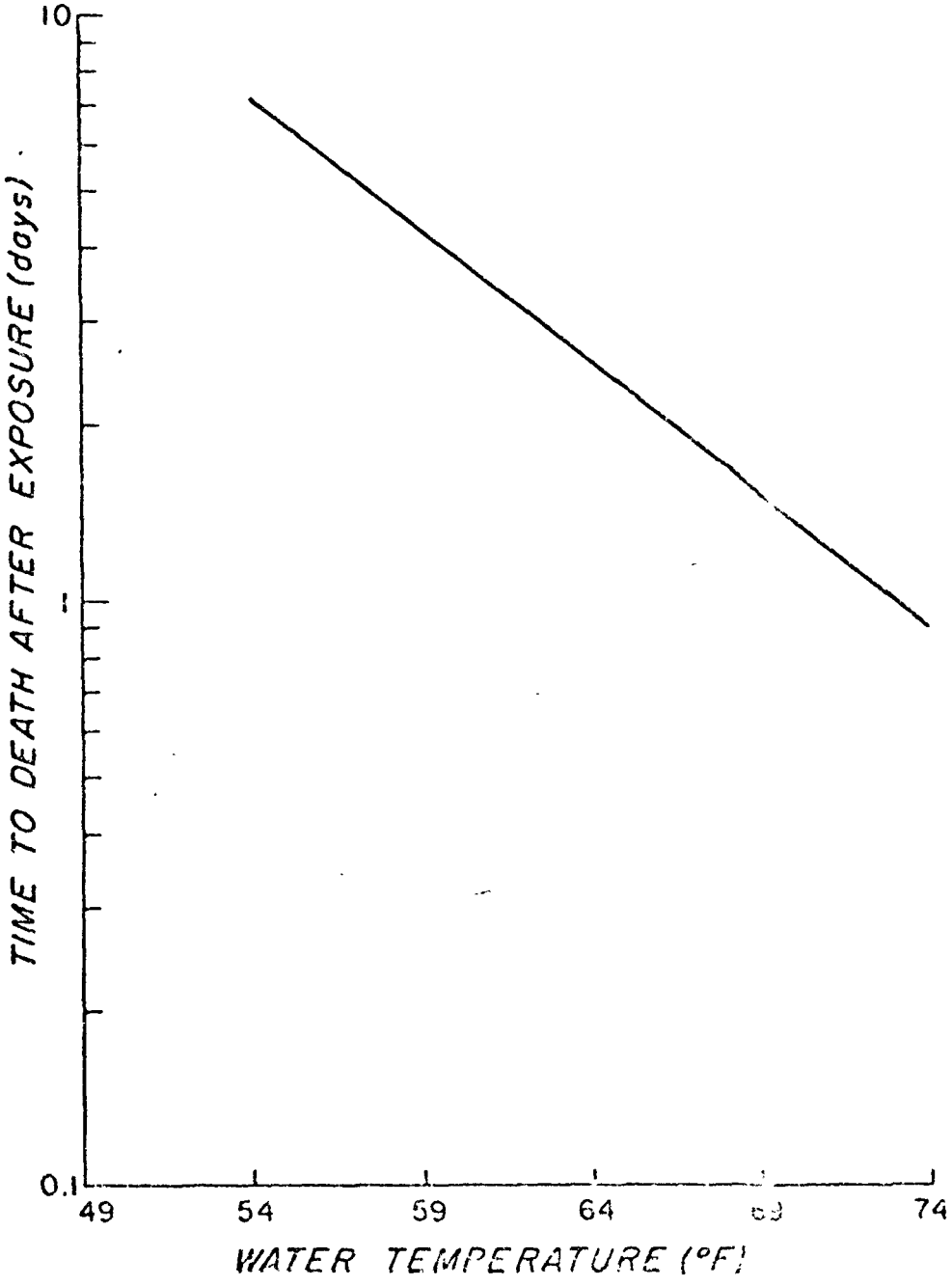

Fig. 4. Relationship between water temperature and log of time to death after exposure of juvenile rainbow trout to Chondrococcus columnaris.

Table 8. Effect of water temperature on Chondrococcus columnaris infection in juvenile coho salmon.

Water temperature	Fraction of each group that died[a]				Per cent mortality 2 expts. combined		Per cent mortalities infected with C. columnaris	
	Experiment 1		Experiment 2					
	Infected	Controls	Infected	Controls	Infected	Controls	Infected	Controls
74°F	35/35	1/35	34/34	0/35	100	1	100	0
69°F	35/35	1/35	35/35	0/35	100	1	100	0
64°F	40/40	3/35	36/37	0/35	99	4	98	0
59°F	16/35	0/35	20/35	2/35	51	3	97	0
54°F	1/35	0/34	2/35	1/35	4	1	75	0
49°F	0/35	0/35	0/35	0/35	0	0	0	0
44°F	0/35	0/35	0/35	0/35	0	0	0	0
39°F	0/35	1/35	0/35	0/35	0	1	0	0

a. At 25 days after exposure to C. columnaris.
b. The least significant difference between mean percent mortality values for Exp. 1 and Exp. 2 was determined to be 6.3% at the 0.05 probability level (Appendix, page 99).

control groups, only a few deaths occurred at the higher temperatures, and C. columnaris was never isolated from these fish.

Again, a linear relationship between water temperature and the log of the number of days from exposure to death was observed (Fig. 5). A correlation coefficient of -0.7699 was calculated, and as with the rainbow trout experiments, was highly significant (Appendix, page 111).

Fifteen days after the last death occurred fish surviving at 59° were transferred to 69° water and those surviving at 49° and 54° were transferred to 64° water. Subsequently, losses due to C. columnaris occurred among those groups originally held at 59° and 54°, but not among those held at 49°. Thus, some of the survivors, when moved to higher water temperatures, developed the disease.

Results of the spring chinook experiment (average weight, 10.2 g each) were similar to those observed previously with rainbow trout and coho salmon, although the percent mortality at temperatures of 59° and above was lower than in the other experiments (Table 9). Chondrococcus columnaris was isolated in gill or kidney cultures from approximately 88% of the deaths in fish exposed at 59° or higher; from 31% of those dying at 54°, and was not recovered from any of those held at 49° or below. Among the control groups only a few deaths occurred, and the columnaris bacterium was not isolated from these fish.

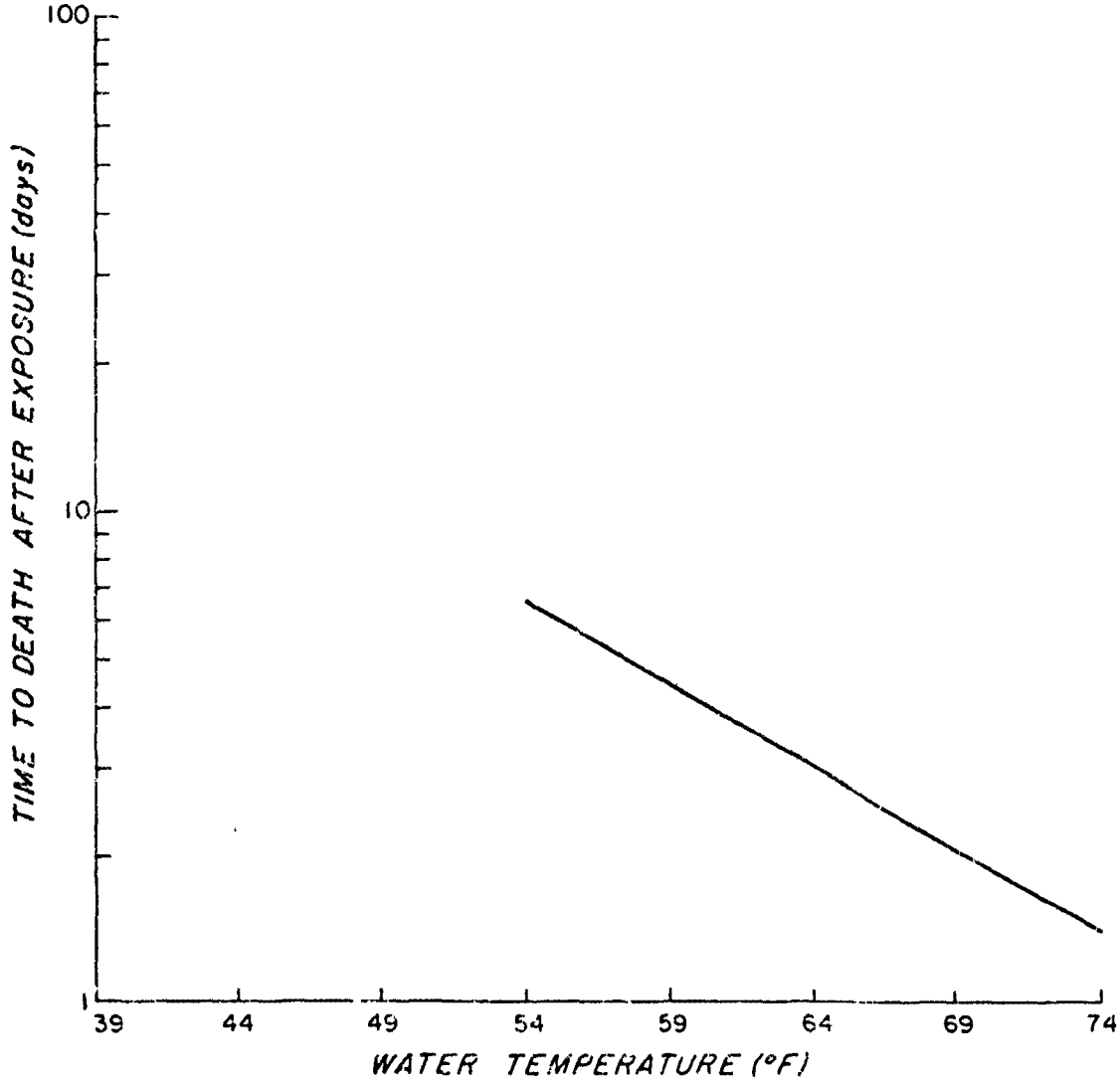

Fig. 5. Relationship between water temperature and log of time to death after exposure of juvenile coho salmon to Chondrococcus columnaris.

Table 9. Effect of water temperature on Chondrococcus columnaris infection in juvenile spring chinook salmon.

Water temperature	Fraction of each group that died[a]				Per cent mortality 2 expts. combined		Per cent mortalities infected with C. columnaris	
	Experiment 1		Experiment 2					
	Infected	Controls	Infected	Controls	Infected	Controls	Infected	Controls
74°F	24/25	0/25	22/25	0/25	92	0	87	0
69°F	19/25	1/25	16/25	0/25	70	2	83	0
64°F	16/25	1/25	10/25	0/25	52	2	95	0
59°F	7/24[b]	1/25	8/25	0/25	31	2	87	0
54°F	2/25	2/25	8/25	1/25	20	6	31	0
49°F	0/25	4/25	3/25	0/25	6	8	0	0
44°F	1/25	3/25	2/25	3/25	6	12	0	0
39°F	1/25	0/25	0/25	1/25	2	2	0	0

a. At 35 days after exposure to C. columnaris.
b. One fish unaccounted for.
c. The least significant difference between mean percent mortality values for Exp. 1 and Exp. 2 was determined to be 13.5% at the 0.05 probability level (Appendix, page 100).

The linear relationship between water temperature and the log of the number of days from exposure to death reported in the experiments with rainbow trout and coho salmon was again observed (Fig. 6). A correlation coefficient of 0.7192 was calculated and found highly significant (Appendix, page 112).

At the end of the 30 day observation period fish surviving at 54^{o} were transferred to 64^{o} water and survivors in the 49^{o} group were transferred to 59^{o} water. Fifteen of the 40 fish (38%) moved from 54 to 64^{o}F water and 4 of the 42 fish (10%) moved from 49 to 59^{o}F water became infected with C. columnaris.

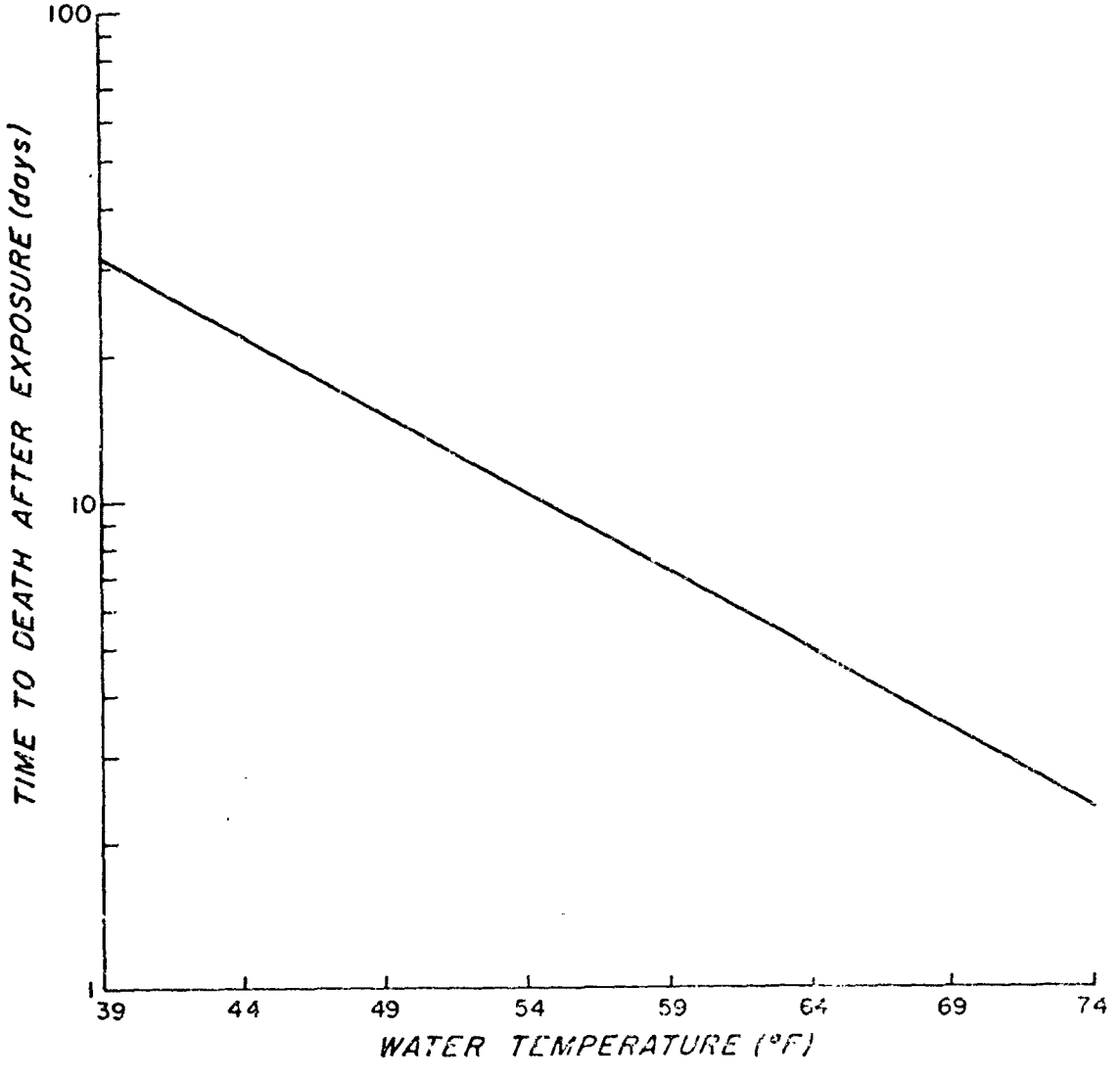

Fig. 6. Relationship between water temperature and log of time to death after exposure of juvenile chinook salmon to Chondrococcus columnaris.

Discussion

Fish infected with C. columnaris were observed at temperatures from 74°F down to 54°F with the greater number of losses occurring at the higher water temperatures. Results of the coho salmon and rainbow trout experiments are nearly identical. With spring chinook the percent mortality at 59°F and above was lower than in the other species. A temperature of 54°F appeared to be the threshold for infection by C. columnaris. At each temperature increment tested above 54°F exposure to the columnaris bacterium resulted in a greater number of infections and deaths.

The time to death was also greatly influenced by water temperature. For example, in the rainbow trout experiment all fish were dead at one and four days at 74 and 69°F, respectively, after exposure. At each lower temperature greater numbers of fish survived the exposure.

Chondrococcus columnaris was isolated from the gills or kidneys of most experimental animals in each group. The organism was not isolated from control groups. The symptoms and pathology observed in the test animals were similar to those described during epizootics of columnaris disease. In these experiments the fish were exposed to large numbers of the columnaris bacterium in the water; consequently isolation from the kidney may be a more accurate indication of infection.

No deaths caused by C. columnaris were found in fish held at 49°F or below.

However, as previously described, exposed fish held at water temperatures unfavorable for the progress of infection often developed fatal disease when transferred to water at higher temperatures (64^{o} or 69^{o}).

SECTION VII

EFFECTS OF WATER TEMPERATURE ON INFECTION OF SALMONIDS BY THE PARASITIC PROTOZOAN CERATOMYXA SHASTA

Materials and Methods

Three species of salmonids were examined in this study; rainbow trout (Salmo gairdneri) were obtained from the 1969 brood at Roaring River Hatchery (Oregon State Game Commission). Coho salmon (Oncorhynchus kisutch) were from the 1970 brood at Fall Creek Salmon Hatchery and spring chinook salmon (Oncorhynchus tshawytscha) were from the 1970 brood at Marion Forks Hatchery (both hatcheries operated by the Fish Commission of Oregon). Rainbow trout used as a positive control in the coho-spring chinook experiment were from the 1970 brood at Leaberg Trout Hatchery (OSGC).

The only practical method of initiating infection of C. shasta in fish is by exposure to water known to contain the infectious agent (2). During the summer and fall months, the Willamette River (below Corvallis) is a very effective location for producing C. shasta infections in several species of salmonids. The proximity of the Willamette River to the laboratory, made this site ideal for exposing fish to this protozoan in these experiments. The object of the exposure was to initiate at least a 50 percent infection attributable to C. shasta. From previous experiments conducted by this laboratory (3) it was estimated that a

48 to 72 hour exposure, to water in which the infectious agent was present, would suffice for our purposes. All experimental groups were held in a 96 cu ft live-box situated in the Willamette River current at the Albany site. Replacement time of water in the live-box was approximately 10 sec.

The rainbow trout were exposed to the infectious agent for 48 hours, between September 5 and September 7, 1970. The mean water temperature for this period was 60.7°F (S.D. = 0.45). The coho and spring chinook salmon were exposed for 72 hours, between September 17 and September 20, 1971. The mean water temperature was 59.4°F (S.D. = 0.62) for the interval.

The procedure used to temper fish to laboratory water temperatures after exposure to C. shasta was necessarily different from that used in the bacterial experiments. Previous information had suggested that the rate at which the C. shasta infection proceeds is temperature dependent. During the exposure period, therefore, the rate of the infectious process should be dependent on the river water temperature. Following this reasoning it was deemed necessary to temper the fish after exposure as rapidly as possible to the experimental temperatures without causing severe stress. The rates of change which filled these requirements were determined by preliminary experiments. A rate of 10°F per hour was used for rainbow trout and a rate of 6.6°F per hour was used for coho and spring chinook salmon. These rates of change were achieved by manual control of the temperature regulating equipment. All fish, both experi-

mental and control, were placed into their respective tanks, all of which contained 54°F water. The control equipment was also initially set at this temperature. This control equipment was then adjusted by either increasing or decreasing the temperature of the water coming into each tank to achieve the desired rate of change of temperature. When the eight experimental temperatures (74, 69, 64, 59, 54, 49, 44 and 39°F) were reached, the regulating instruments were calibrated and set for the remainder of the experiment.

It was anticipated that with a natural exposure in the Willamette River the fish would also become infected with bacterial diseases. Aeromonas liquefaciens and Chondrococcus columnaris were known to be prevalent in the river. For this reason prophylactic measures were taken to prevent their interference in the experiments. The antibiotic of choice in control of these diseases was Terramycin as TM_{50}. This compound was given to the fish incorporated in Oregon Moist Pellet (OMP) diet. Terramycin was chosen also because of its lack of activity against C. shasta. In each experiment all fish including controls were fed TM_{50} at a level of 25 g TM_{50}/100 lb of fish/day starting ten days prior to exposure. A level of 20 g TM_{50}/100 lb of fish/day was fed post exposure for three days in the rainbow trout experiment. The treatment was discontinued for four days, resumed for five additional days at which time the level was reduced to a dose of 5 g TM_{50}/100 lb of fish/day. To protect coho and spring chinook salmon a 30 min bath in water containing 5 µg/ml of soluble terramycin was given to the exposed fish before placing them

in the laboratory tanks. They were fed at the 20 g TM_{50}/100 lb of fish/day level for the interim of the experiment.

Dead fish were collected at least once daily and either examined while fresh or were frozen for later autopsy. Examination of dead animals consisted of microscopic observation (400 x, bright field) of wet mounted samples of intestinal scrapings. Slides containing two or more of the spore stage of C. shasta were considered as positive diagnoses.

The experimental design used for the rainbow trout experiment was the same as that used for the bacterial investigations described in this report. In order to conserve laboratory facilities during these very long-term experiments with C. shasta, the design was modified for the coho-spring chinook experiment. The experimental design was altered according to recommendations from the project's statistician. This modified design consists of conducting two concurrent experiments in each tank using two species of fish distinguished by removal of opposing pectoral fins. The second modification eliminated the use of two control groups for two experimental groups at each temperature, and substituted one control group. In the design used with rainbow trout 25 fish averaging 11.5 g were placed in each tank. With the modified design 25 coho salmon averaging 14 g and 25 spring chinook salmon averaging 8.7 g were placed in each tank. Rainbow trout used as an exposure control in the latter experiment averaged 15 g; 35 of these fish were exposed and 35 used as unexposed controls.

Experimental Phase

In these experiments with C. shasta two types of information were obtained. Quantal mortality data, expressed both as percent infection and quantitative response data expressed as mean time to death in days have been gathered. From this data some qualitative inferences have been drawn regarding effects of temperature changes in the host-parasite relationship.

Table 10 summarizes the mortality data acquired from two concurrent replications of an experiment utilizing rainbow trout. The data is arranged to show the effect of temperature on the C. shasta infection. The number of fish infected with C. shasta from experimental group 2 of both the 74°F and 69°F temperatures are lower than expected. This is due to a fatal C. columnaris infection in several fish prior to the time that they would have died of C. shasta. It can be seen, however, from all other experimental groups in the 74°F to 44°F range that temperature has little or no effect on the percent mortality due to C. shasta. This data suggests that rainbow trout may have no means to combat this parasite in this temperature range. At 39°F no mortality due to C. shasta was observed even after 237 days post exposure. However, when these same fish were tempered thereafter from 39°F to 64°F over a two week period and held for an additional four week period at 64°F, six percent succumbed to C. shasta infections.

Table 10. Incidence of Ceratomyxa shasta and mean time to death post-exposure of juvenile rainbow trout exposed to water containing the infective stage of the organism and then placed in temperature regulated disease free water.

Temperature[a]	Group No.	Total Deaths / Number Examined	% Mortality	No. Infected with C. shasta	% Infected[b] with C. shasta	Mean Time to Death of C. shasta Infected Fish[c]
74°	Exp. 1	25/25	100	17	68	15
	Control 1	0/25	0	0	0	—
	Exp. 2	25/25	100	9	36[d]	14
	Control 2	0/25	0	0	0	—
69°	Exp. 1	25/25	100	22	88	19
	Control 1	0/25	0	0	0	—
	Exp. 2	24/25	96	14	56[d]	19
	Control 2	0/25	0	0	0	—
59°	Exp. 1	23/25	92	22	88	45
	Control 1	2/25	8	0	0	—
	Exp. 2	23/25	92	21	84	40
	Control 2	0/25	0	0	0	—
54°	Exp. 1	21/22	96	15	68	56
	Control 1	0/25	0	0	0	—
	Exp. 2	21/22	96	18	82	57
	Control 2	0/25	0	0	0	—
49°	Exp. 1	21/25	84	21	84	83
	Control 1	0/25	0	0	0	—
	Exp. 2	19/25	76	19	76	92
	Control 2	0/25	0	0	0	—
44°	Exp. 1	18/24	75	17	71	157
	Control 1	0/25	0	0	0	—
	Exp. 2	20/25	80	20	80	154
	Control 2	0/25	0	0	0	—
39°	Exp. 1	0/25	0	0	0	—
	Control 1	0/25	0	0	0	—
	Exp. 2	0/25	0	0	0	—
	Control 2	0/25	0	0	0	—

[a] A 64°F temperature was included but due to mechanical failure of the temperature regulating device it was discontinued.

[b] The least significant difference between mean values for percent infected in Exp. 1 and Exp. 2 was determined to be 19.8% at the 0.05 probability level. (Appendix page 101).

[c] In days post-exposure.

[d] Losses were incurred due to Chondrococcus columnaris prior to onset of deaths caused by C. shasta.

Another parameter has been analyzed to determine the effect of temperature on the infection process. This second parameter is the dependence of the mean time to death (of dead specimens diagnosed positive for C. shasta) on temperature. When the log of the mean time to death in days is plotted against temperature a straight line should be obtained when the host shows either no defense against or a logarithmic interaction with the infectious organism. In this experiment with rainbow trout such a straight line function was obtained. This further supports the idea that rainbow trout are not able to overcome a C. shasta infection between 74°F and 44°F. If this assumption is correct, the temperature dependence of the mean time to death reflects the effects of temperature on the growth rate of the parasite.

At 39°F the host parasite relation is markedly altered since no mortality occurred. C. shasta may lie dormant in the fish which could explain the onset of the disease after the fish were elevated in temperature.

A linear relationship between water temperature and the log of the number of days from exposure to death was confirmed by regression analysis (Fig. 7). A correlation coefficient of -0.9830 was calculated and found highly significant (Appendix, page 113).

This experiment was terminated at each temperature when it was reasonable to assume no additional deaths would occur due to C. shasta. Fish remaining at termination were examined for the presence of C. shasta spores.

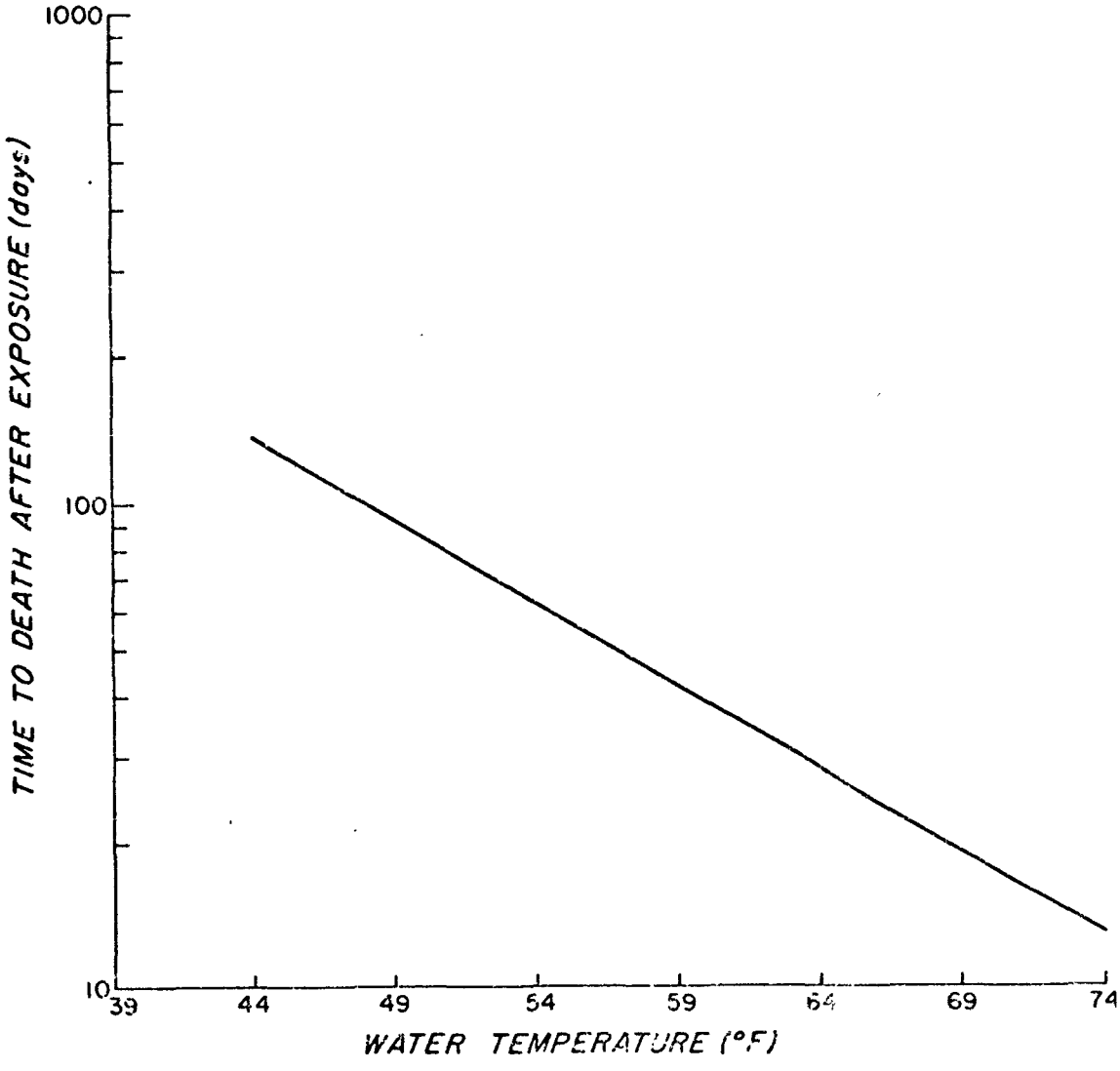

Fig. 7. Relationship between water temperature and log of time to death after exposure of juvenile rainbow trout to Ceratomyxa shasta.

Only two positive fish (at 44°F) were found. These fish appeared to have been recovering from the infection.

A second pair of experiments with C. shasta utilized the modified design explained previously.

Coho salmon and spring chinook salmon were the host animals in this study. The spring chinook portion of this experiment was terminated after the third week. When the coho salmon died at 74°F and 69°F (prior to the death of any spring chinook), the bacterial load, mostly of Aeromonas liquefaciens overcame the drug treatment. This bacterium killed all experimental spring chinook at 74° and 69°. The spring chinook were left in the tanks at the lower temperatures so as not to alter the results of the remaining coho experiment.

Mortality data from the coho salmon portion of the experiment is reported in Table 11. The results are noticeably different from that of the experiment with rainbow trout. In this study, in contrast to the rainbow trout experiment, the percent mortality attributable to C. shasta does not remain constant with decreasing temperature. It is instead reduced at 64°F and below with no mortality below 49°F. The previous experimental results (with rainbow trout) indicate that C. shasta can multiply in infected fish at temperatures down to 44°F when uninhibited. Coho salmon, therefore, may well be able to somewhat inhibit C. shasta development at 64°F and below.

Table 11. Incidence of Ceratomyxa shasta and mean time to death post-exposure of juvenile coho salmon exposed to water containing the infective stage of the organism and then placed in temperature regulated disease free water.

Temperature	Group No.	Total Deaths / Number Examined	% Mortality	No. Infected with C. shasta	% Infected[a] with C. shasta	Mean Time to Death of C. shasta Infected Fish[b]
74°	Exp. 1	25/25	100	13	52	12
	Exp. 2	23/25	92	17	68	13
	Control	0/23	0	0	0	—
69°	Exp. 1	23/25	92	20	80	22
	Exp. 2	23/25	92	22	88	23
	Control	2/25	8	0	0	—
64°	Exp. 1	13/22	59	12	55	34
	Exp. 2	13/23	57	12	52	43
	Control	0/25	0	0	0	—
59°	Exp. 1	3/24	13	3	13	39
	Exp. 2	7/23	31	7	30	41
	Control	0/25	0	0	0	—
54°	Exp. 1	5/25	20	5	20	87
	Exp. 2	7/25	28	6	24	87
	Control	0/25	0	0	0	—
49°	Exp. 1	0/25	0	0	0	—
	Exp. 2	1/25	4	1	4	146
	Control	0/25	0	0	0	—
44°	Exp. 1	0/25	0	0	0	—
	Exp. 2	0/25	0	0	0	—
	Control	0/25	0	0	0	—
39°	Exp. 1	0/25	0	0	0	—
	Exp. 2	0/25	0	0	0	—
	Control	0/25	0	0	0	—

[a] The least significant difference between mean values for percent infected in Exp. 1 and Exp. 2 was determined to be 18.4% at the 0.05 probability level. (Appendix page 102).

[b] In days post-exposure.

Although not as reliable in this experiment as in the rainbow trout experiment due to lower numbers of deaths, the mean time to death analysis remains a valid parameter. A plot of the log of the mean time to death verses temperature again yielded a straight line. Confirmation of this linear relationship was obtained by regression analysis (Fig. 8). The correlation coefficient obtained was -0.9574, which was highly significant (Appendix, page 114).

It appears that at 74°F neither species of fish has the ability to retard the growth of C. shasta. At 64°F and below the portion of the coho population able to resist a fatal C. shasta infection increases with decreasing temperature (Table 11). The portion of the population unable to resist fatal infection may, however, interact with the parasite to protract the mean time to death.

Thirty-five rainbow trout were also exposed at the same time as the coho salmon and were then held at 54°F. These fish were used to determine whether the Willamette River exposure would give repeatable results from one year to the next. Sixty-eight percent of these fish were diagnosed positive for C. shasta and the mean day of death was 67.7 days. These results were very near those observed with rainbow trout the previous year (Fig. 7).

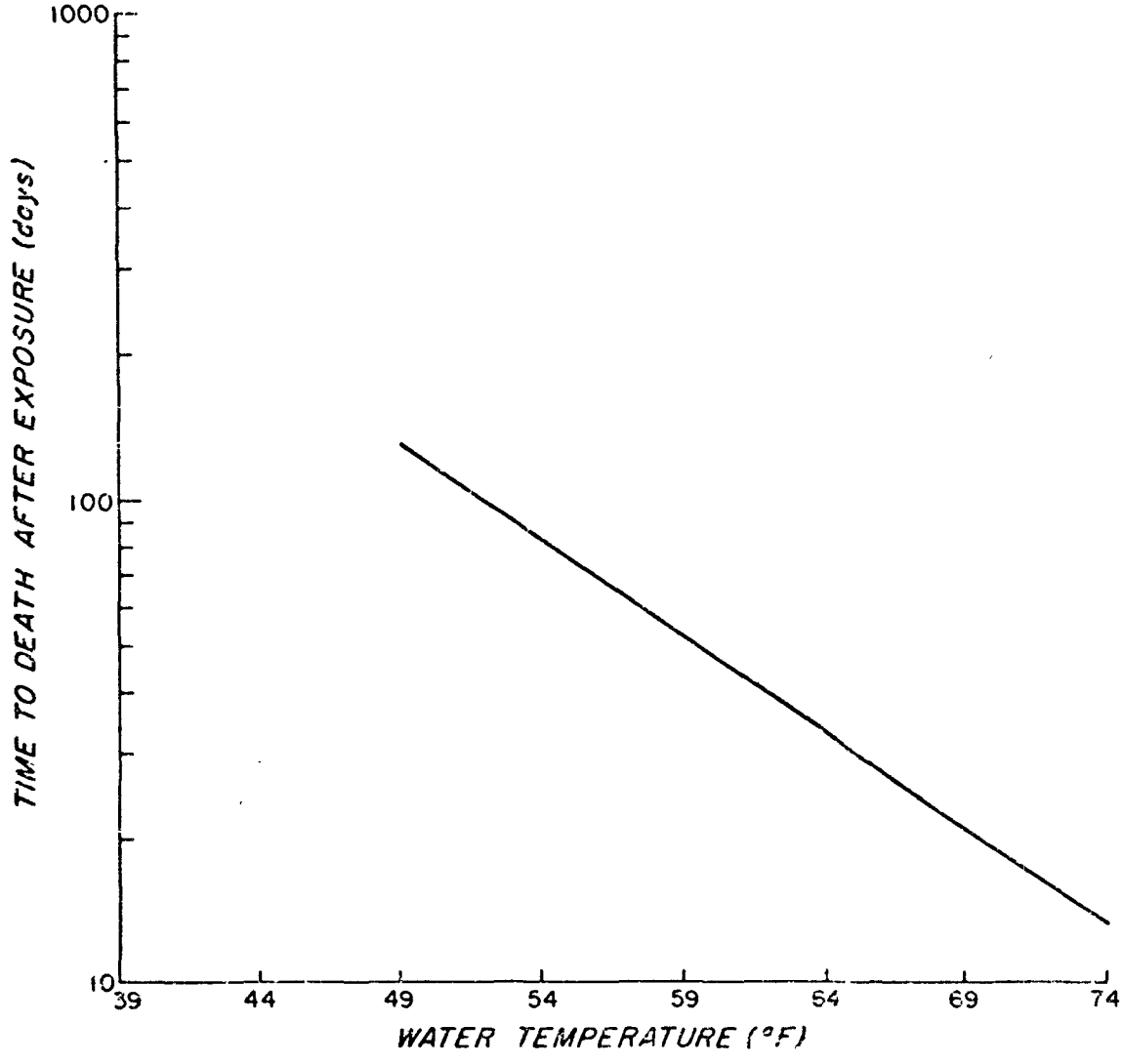

Fig. 8. Relationshop between water temperature and log of time to death after exposure of juvenile coho salmon to Ceratomyxa shasta.

Discussion

It is always the goal of this type of laboratory experiment to be able to apply the results to natural and management situations. It is felt that the results obtained in these experiments are applicable in these areas. The short period of exposure to infection used resulted in fish dying with identical pathology to that of fish held continuously in the Willamette River until death. The mortality may be somewhat higher in a natural situation since the fish are exposed to the infectious agent for a longer period of time.

Since the water temperature during the exposure was near 60°F nothing can be said about initiation of C. shasta infection at colder water temperatures. However, Keith Johnson working in this laboratory, has initiated an infection of C. shasta in Cutthroat trout (Salmo clarki) when the Willamette River had a maximum temperature of 48°F (exposure time, 3 days).

It has been shown in this study that rainbow trout held at water temperatures between 74°F and 44°F have little or no ability to overcome an infection of C. shasta once initiated. Coho salmon on the other hand do seem to be able to interact with the parasite. In this species the percent of the population which is susceptible to the disease diminishes with decreasing temperature. In both species, the mean time to death, of fish diagnosed positive for C. shasta, is inversely related to temperature.

SECTION VIII

EFFECT OF WATER TEMPERATURE ON INFECTION BY THE SOCKEYE SALMON VIRUS (IHN)

Materials and Methods

The virus used in the work reported here was isolated in this laboratory in 1958 from diseased fish collected at the Willamette River Salmon Hatchery in Oregon during an epizootic associated with a high mortality rate. Stocks of this agent have been maintained since that time by propagation in cultures of a salmonid cell line. Properties of the virus have been reported (4). Fish used in these experiments have all been fingerling kokanee salmon (Oncorhynchus nerka) generously supplied without charge by the Oregon Game Commission.

Because of the limited number of 21 gallon experimental tanks available at the main fish disease laboratory, it has not been possible to carry on experiments with all 5 fish pathogens concurrently in those facilities. Hence, in order to expedite the acquisition of data, work with the sockeye salmon virus has been done with the aid of one gallon glass aquaria, held in individual incubators at one of several temperatures. This equipment was practical to use because of the small size of the experimental fish. The water supply for these vessels was derived from the Corvallis Municipal supply, and had been processed to remove chlorine and fluoride by the Fisheries and Wildlife Department at Oregon State University. The water in all of these small aquaria was changed

at 48 hour intervals. The fish were fed Rangen's Salmon Mash twice a day in amounts slightly in excess of their usual consumption.

When fish were first received from a hatchery, they were distributed among a number of aquaria at a water temperature very close to that of the hatchery water. In the case of fry averaging less than 1 gm in weight, 30 fish or less were placed in each 1 gallon aquarium. For those averaging 1 to 3 gm, the maximum number in each aquarium was 15. The aquaria were then placed in incubators at one of the desired experimental temperatures and allowed to gradually adjust to the new temperature over a 24 hour period. It was realized that from a physiological viewpoint this was not an adequate acclimation procedure, but in actual practice it did not appear to create any difficulties.

Fish were exposed to the virus infection by adding measured amounts of standardized virus suspension to the water of their aquaria. Exposure periods of either 24 or 48 hours were allowed, after which the virus was removed and replaced by fresh water. Virus assays were carried out by the plaque method, using monolayer cultures of salmonid cell line CHSE 214, as described elsewhere (5).

Exposed fish that died 5 days or more after exposure were considered to be virus infected if they showed symptoms characteristic of the disease, and if 90% or more of unexposed controls held under the same conditions remained healthy. Typical symptoms included hemorrhages at

the base of pelvic and pectoral fins, extrusion of strings of milky fecal material, and development of a very dark body color. Control fish never showed any of these symptoms. The laboratory method for detection of the virus in the organs of dead fish, although used in a few instances, was considered to be impractical for application to large numbers of fish.

Experimental Phase

Preliminary Experiments

Before attempting to study the effect of water temperatures on infection with the sockeye salmon virus, it was necessary to determine an appropriate concentration of virus for initiating the experimental infection. In the first experiment bearing on this point, groups of 20 to 22 fingerling kokanee salmon, averaging 0.3 gm in weight were exposed to different concentrations of virus in their water for a 24 hour period. Concentrations varied from 2570 to 11 plaque forming units per ml, (pfu) at 3 fold intervals. All aquaria were held at 54°F and deaths recorded over a 24 day period. The results are shown in Table 12. A second experiment of the same type was carried out as soon as the first was completed. In this case the available kokanee fingerlings had increased to an average weight of 0.94 gm. The results are presented in Table 13.

In both preliminary experiments 90 to 100% of the fish exposed to 11 or more pfu of virus per ml, succumbed to the infection; hence the cumulative percent mortality did not increase with increasing virus concentration. Deaths were distributed over a much longer time period at the lower concentrations than was observed with the maximum virus level. This was probably because some of the fish exposed to the lower virus concentrations only became infected when exposed to virus being shed from others that had been infected during the initial exposure. Differences

in the distribution of deaths with time among the experimental groups are reflected in the values for the mean time to death in Tables 12 and 3. In both cases this value is minimal where the virus concentration was maximal, and vice versa. In comparing Tables 12 and 13 it may be noted that in the case of the 0.3 gm fish the mean time to death for all virus concentrations was consistently shorter than the comparable figure for the 0.94 gm fish.

The results of these two experiments indicated that any of several virus concentrations within the range which was studied could be used successfully for exposure of fish in temperature experiments. In order to allow as much latitude as possible for observing effects of water temperature it appeared desirable to use a lower concentration (e.g. 32 pfu/ml) for fish in the same weight range, i.e. 0.3 to 1.0 gm.

Effects of Water Temperature on Virus Infection

In the first experiment dealing with the effect of water temperature on the virus infection, the available kokanee fingerlings had an average weight of 1.1 gm. Incubator facilities for only 4 temperatures were available and those selected were 74^{o}, 64^{o}, 54^{o} and 39^{o}F. For each temperature level 20 fish were infected and another group of 20 held as controls. Two complete experiments were carried on concurrently. Dead fish were collected daily and deaths were presumed to be due to virus infection, unless they occurred in less than 5 days after exposure, or

Table 12. Mortality among fingerling kokanee salmon resulting from various concentrations of sockeye salmon virus in the aquarium water at 54°F.

Final virus concentration in water of aquaria; pfu/ml	Number of deaths occurring in each 5 day period after infection (5-9)	(10-14)	(15-19)	(20-24)	Fraction of each group that died	Mean time to death (days)
2570	20				20/20	6.4
857	14	5	1		20/20	8.9
286	19	2			21/21	7.7
95	14	5	1	1	21/21	9.7
32	14	7			21/21	9.5
11	11	8	2		21/21	10.7
heated virus control					0/17	
virus diluent control					0/22	

1. Average weight of experimental fish was approximately 0.30 gm.
2. Fish were exposed to the indicated virus concentration in their water for 48 hours.
3. The heated virus control group received virus equivalent to 2570 pfu/ml, which had been heated at 60°C for 30 minutes; this was added to the aquarium water.
4. The virus diluent control group received 4 ml of the virus diluent added to the aquarium water; the diluent was Eagle's MEM (tissue culture medium).

Table 13. Mortality among fingerling kokanee salmon resulting from various concentrations of sockeye salmon virus in the aquarium water at 54°F.

Final virus concentration in water of aquaria; pfu/ml	Number of deaths occurring in each 5 day period after infection					Fraction of each group that died	Mean time to death (days)
	(5-9)	(10-14)	(15-19)	(20-24)	(25-29)		
2570	20	2				22/22	7.3
857	6	9	6			21/22	12.0
286	9	11	1	1		22/22	10.4
95	3	9	3	1		16/22	13.1
32	5	7	6	2		20/22	13.8
11	1	9	5	4	2	21/22	15.8
4	1	7	2	2	4	16/22	17.9
1						0/22	--
0.4						0/21	--
0.1	1	3	12	3		19/22	16.3
virus diluent control						0/22	--

1. Average weight of experimental fish was 0.94 gm.
2. Fish were exposed to the indicated virus concentration in their water for 48 hours.
3. The virus diluent control group received 4 ml of the virus diluent added to the aquarium water; the diluent was Eagle's MEM (tissue culture medium).

unless some other factor, such as failure of aeration, was an obvious cause.

Results of these two experiments are shown in Table 14. The experimental groups held at 74°F were lost due to failure in the aeration apparatus. The percent mortality approached 100% among the fish at 39°, and was nearly 90% at 54°F. However at 64° it was only 41.5%, a definitely significant reduction (Appendix, page 103). This result suggested that the higher temperature had exerted a suppressive effect on the infection in some of the animals. Progress of the infection was apparently slower at 39°, as indicated by the longer average interval between exposure and death.

When a second pair of experiments was started, the available kokanee fingerlings were considerably larger, averaging 2.9 gm in weight. Because of their size it seemed advisable to expose them to a higher virus concentration than was used in the first experiments. Accordingly sufficient stock virus was added to the water to give a final concentration of 600 pfu/ml, and the exposure period was 24 hours. Equipment to provide 5 water temperatures was available at this point, and temperatures selected were 72°, 69°, 64°, 59° and 54°F. The number of fish in each infected and each control group was increased from 20 to 40, and two complete experiments were carried out in parallel, as before.

The results of these two experiments are presented in Table 15. In this

Table 14. Effect of water temperature on mortality of 1.1 gm kokanee salmon fingerlings exposed to sockeye salmon virus.

Water temperature	Fraction of each group that died				Per cent mortality; 2 expts. combined		Mean time to death (days)
	Experiment 1		Experiment 2				
	Exposed	Controls	Exposed	Controls	Exposed	Controls	
64°F	10/20	0/20	7/21	0/21	41.5	0	17.2
54°F	19/21	0/22	18/21	0/21	88.0	0	16.7
39°F	21/22	0/21	22/22	0/21	97.7	0	25.6

1. Average weight of experimental fish was approximately 1.1 gm.
2. Experimental fish held at 74°F were lost due to failure in aeration equipment.
3. Infected groups of fish were exposed to virus in the water of their aquaria at a concentration of 32 plaque forming units/ml for 24 hours.
4. All groups of fish were held at the indicated temperatures for a 45 day period.
5. The least significant difference between percent mortality values was determined to be 12.7% at the 0.05 probability level (Appendix, page 103).

Table 15. Effect of water temperature on mortality of 2.9 gm kokanee salmon fingerlings exposed to sockeye salmon virus.

Water temperature	Fraction of each group that died				Per cent mortality; 2 expts. combined	
	Experiment 1		Experiment 2			
	Exposed	Controls	Exposed	Controls	Exposed	Controls
72°F	10/40	8/26	5/40	4/40	18.8	18.2
69°F	6/40	0/39	8/26	2/39	21.2	2.6
64°F	6/39	0/40	9/40	0/40	18.9	0
59°F	18/38	0/39	18/40	0/41	46.2	0
54°F	23/41	0/40	26/39	1/40	61.2	1.3

1. Average weight of experimental fish was approximately 2.9 grams.
2. Infected groups of fish had been exposed to the virus in the water of their aquaria for a 24 hour period; virus concentration was 600 plaque forming units/ml.
3. All groups of fish were held at the indicated temperatures for a 30 day period.
4. Deaths in the control groups at 72°F are presumably due to unfavorable physiological effects of the high temperature, and possibly the activation of some resident microorganisms with potential pathogenic properties.
5. The least significant difference between percent mortality values was determined to be 16.1% at the 0.05 probability level (Appendix, page 104).

case none of the experimental groups showed 100% mortality. The maximum average mortality for the two experiments was 61.2%, which occurred at 54°F. The mortality rate decreased at 59° and was still lower at 64° and 69°. The 72° temperature was at the threshold of tolerance for these fingerlings, as shown by a similar mortality rate in controls and infected groups. These experiments seem to indicate that within the temperature range covered, 54°F was the most favorable for the development of fatal infection in fish of this size, while at 64° and 69° the disease was partially suppressed and mortality was significantly lower. This result appears to confirm the findings in the first experiments. No consistent relationship between temperature and the mean time to death was observed, as may be seen in Table 16, although this period was longest among the 69° group.

The next pair of experiments was conducted during the following year when kokanee salmon fry were available again. At this time the fish were very small, and the average weight of those used in the experiments was 0.11 gm. Temperatures that were compared in these eperiments were 69°, 59°, 54°, 49°, and 39°F. For each temperature level in each experiment, 30 fish were exposed to virus and a similar group of 30 were held as controls. For these small fish, the virus concentration used to produce infection was 32 plaque forming units/ml of aquarium water. The exposure period was 24 hours.

Table 17 presents the results of this second pair of experiments. The

Table 16. Mean time to death for 2.9 gm kokanee salmon fingerlings exposed to sockeye salmon virus.

	Water temperatures at which experimental fish were held in °F				
	72°	69°	64°	59°	54°
Fraction of the exposed fish that died	15/80	14/66	15/79	36/78	49/80
Mean time to death (days)	--	17.4	10.1	12.8	13.0
Fraction of the control fish that died	12/66	2/78	0/80	0/80	1/80

1. Deaths in this control group presumably due to unfavorable physiological effects of the high temperature, and possibly the activation of some resident microorganisms with potential pathogenic properties.
2. Mean time to death is not included for the 72°F group because deaths were apparently due to a combination of factors mentioned above and virus infection.

Table 17. Effect of water temperature on mortality of 0.11 gm kokanee salmon fry exposed to sockeye salmon virus.

Water temperature	Fraction of each group that died				Per cent mortality; 2 expts. combined	
	Experiment 1		Experiment 2			
	Exposed	Controls	Exposed	Controls	Exposed	Controls
69°F	9/30	13/29	11/30	16/30	33.3	49.0
59°F	28/30	4/30	30/30	0/29	96.6	6.8
54°F	29/30	0/30	30/30	0/30	98.3	0
49°F	0/30	1/30	2/30	0/30	3.3	1.7
39°F	0/30	1/31	3/30	0/30	5.0	1.7

1. Average weight of experimental fish was approximately 0.11 grams.
2. Infected groups of fish had been exposed to the virus in the water of their aquaria for a 24 hour period; virus concentration was 32 plaque forming units/ml.
3. All groups of fish were held at the indicated temperatures for a 27 day period.
4. Deaths in the control groups at 69°F are presumably due to unfavorable physiological effects of the high temperature, and possibly the activation of some resident microorganisms with potential pathogenic properties.
5. The least significant difference between percent mortality values was determined to be 11.3% at the 0.05 probability level (Appendix, page 105).

percent mortality, as measured by the combined data of the two experiments was very low among the fish held at 39° and 49°F. It approached 100% however in the 54° and 59° groups, and declined again to a significant extent in the 69° groups. This was despite the fact that about half of the control fish at 69° succumbed to effects of this relatively high temperature. The data thus indicated that the temperature range of 54° to 59° was near optimal for development of fatal infection under these experimental conditions, and that infection was markedly retarded at 49° or lower, and to a lesser degree at 69°. The mean survival times for fish that died in each temperature group are shown in Table 18. The infection apparently progressed most rapidly at 54° and 59°, where mortality was also greatest. It ran a slower course at both lower and higher temperatures.

A third pair of experiments was next carried out with a population of kokanee salmon fry whose average weight was 0.95 gm. The experimental design was very similar to that in the previous experiments with the 0.1 gm fish, with the exception that only 20 fish were used in each small aquarium because of their larger size. In addition to the 5 experimental water temperatures used previously, groups of fish held at 64°F were included. The virus concentration in the water during the exposure period was 32 plaque forming units/ml.

Results of the third pair of experiments appear in Table 19. They are quite different from those obtained with the 2.9 gm and the 0.11 gm

Table 18. Mean time to death for 0.11 gm kokanee salmon fry exposed to sockeye salmon virus.

	Water temperatures at which experimental fish were held in °F				
	69°	59°	54°	49°	39°
Fraction of the exposed fish that died	20/60	58/60	59/60	2/60	3/60
Mean time to death (days)		12.8	14.7	17.0	20.0
Fraction of the control fish that died	29/59[1]	4/59	0/60	1/60	1/60

1. Deaths in this control group presumably due to unfavorable physiological effects of the high temperature on these tiny fish, and possibly the activation of some resident microorganisms with potential pathogenic properties.

Table 19. Effect of water temperature mortality of 0.95 gm kokanee salmon fry exposed to sockeye salmon virus.

Water temperature	Fraction of each group that died				Per cent mortality; 2 expts. combined	
	Experiment 1		Experiment 2			
	Exposed	Controls	Exposed	Controls	Exposed	Controls
69°F	12/18	9/19	--[1]	10/20	66.7	48.7
64°F	16/20	0/20	14/20	0/20	75.0	0
59°F	18/20	0/20	18/20	1/20	90.0	2.5
54°F	19/20	0/20	19/20	0/20	95.0	0
49°F	19/20	0/20	20/20	0/20	97.5	0
39°F	19/20	0/20	20/20	0/20	97.5	0

1. One group of 20 fish at 69°F was lost through failure of the air supply.
2. Average weight of experimental fish was 0.95 grams.
3. Infected groups of fish had been exposed to the virus in the water of their aquaria for a 24 hour period; virus concentration was 32 plaque forming units/ml.
4. All groups of fish were held at the indicated temperatures for a 29 day period.
5. Deaths in the control groups at 69°F are presumably due to unfavorable physiological effects of the high temperature, and possibly the activation of some resident microorganisms with potential pathogenic properties.
6. The least significant difference between percent mortality values was determined to be 9.33% at the 0.05 probability level (Appendix, page 106).

fish. In this case the final per cent mortality showed little relationship to water temperature. It was high at all temperatures, ranging from 90% to 97.5% at the four lower temperature levels. It declined slightly to 75% at 64^{o} (a value significantly different from 97.5% (Appendix, page 106). The distinct protective effective of the 39^{o} and 49^{o} temperatures, observed with the 0.11 gm fish was lacking in these experiments. There is however an indication of a retarding effect of the 39^{o} temperature on the course of the disease in Table 20, where the mean time to death at that temperature is about double the comparable value at the other temperatures. It is noteworthy that the 0.95 gm fish used in these experiments were obtained from the same population of kokanee salmon fry as the 0.11 gm fish. The data seem to indicate that these larger fish had become more susceptible to the virus infection; this is suggested by the high mortality at the low temperatures, and the fact that the mean survival times were distinctly shorter at comparable temperature levels.

Two further experiments with this virus in kokanee salmon populations were started, but had to be abandoned because of the development of natural bacterial infections, despite the presence of 2 ppm of oxytetracyclene in the water.

Table 20. Mean time to death for 0.95 gm kokanee salmon fry exposed to sockeye salmon virus.

	Water temperatures at which experimental fish were held in °F					
	69°	64°	59°	54°	49°	39°
Fraction of the exposed fish that died	12/18	30/40	36/40	38/40	39/40	39/40
Mean time to death (days)	---	9.9	9.0	10.8	10.6	20.1
Fraction of the control fish that died	19/39[1]	0/40	1/40	0/40	0/40	0/40

1. Deaths in this control group presumably due to unfavorable physiological effects of the high temperature, and possibly the activation of some resident microorganisms with potential pathogenic properties.

Discussion

It is in one sense unfortunate that all of the experiments reported with the sockeye salmon virus could not have been carried out with experimental fish of approximately the same size and from the same population. It appears that factors related to age or size, and perhaps to environmental or genetic background may have influenced some of the results obtained. However it was necessary to use kokanee fry or fingerlings at whatever time and from whatever source they became available.

One set of two experiments with 2.9 gm fish and one pair of experiments with 1.1 gm fish indicated that a water temperature of 54°F produced a higher percent mortality in infected fish than higher temperatures, up to 69°, and that the course of the disease was slower at the latter temperature. In another pair of experiments with 0.11 gm fish, temperatures of 54° and 59° both resulted in over 90% mortality, while very few fatal infections occurred at 39° and 49°. The course of the infection was also most rapid at 54° and 59°, as measured by the average time from infection until death. These results are not in conflict with experiments of Amend, (1970) who reported that mortality among fingerling sockeye salmon exposed to this virus was reduced to a low level if the fish were held at 68°F for 4 to 6 days after exposure (6). Work reported from this laboratory has indicated that this virus replicates abundantly in sockeye salmon cell cultures in the temperature range from 50°F to 68°F. Replication was retarded and virus yields were lower at 39°F, and

no replication occurred at 73.4°F (4). Thus the indications from experiments reported here that the temperature range from 54° to 59° is near optimal for development of fatal infection due to this virus in fingerling kokanee, is not out of line with other relevant data.

The experiments reported in Table 19 with fish of 0.95 gm average weight do not show the marked reduction in percent mortality in 64° water that was found in the experiments of Table 14 and 15. Presumably some unrecognized variable factor may be responsible for this difference. It is also of interest to note that only in the case of the very small fry, averaging 0.11 gm in weight, was the percent mortality reduced to a very low level (Table 17) by the low temperatures of 39° and 49°. This might suggest that these tiny fish were more resistant to the virus infection than larger fry from the same population (Table 19).

SECTION IX

ACKNOWLEDGMENTS

This project was supported by the Environmental Protection Agency over a three year period beginning April 1, 1969 and ending March 31, 1972. The total funds provided by this agency amounted to $182,355. The assistance provided by Dr. Donald A. Hilden and Dr. Gerald R. Bouck, who have served as Project Officers, is acknowledged with sincere thanks.

A major contribution to this project was made by the Fish Commission of Oregon and the Oregon Game Commission. In fact, without the support of these agencies none of the work described in this report would have been possible. They provided the large numbers of juvenile salmon and trout required for the experiments, without charge. The monetary value of these fish has been roughly estimated at about $3000 per year for each of the two years during which experimentation was carried on.

Another important contribution to the project was made by the Department of Microbiology at Oregon State University. All of the experimentation with sockeye salmon virus in fingerling kokanee salmon was carried on by Philip McAllister, a graduate research assistant in the above department. During 2 years of work on this phase of the project, he was supported entirely by an NDEA fellowship, so that all of his work was really donated to the project.

SECTION X

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**********************************************************************
*ANOVA12 - ONE/TWO FACTOR ANALYSIS OF VARIANCE.        OS-3  VER.3.5
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PROBLEM I-D! 2ND TRY

SOURCE	DF	SS	MS	F
EXP TYPE	1	1.89637813E 04	1.89637813E 04	428.2576
TEMP	7	2.00329687E 04	2.86185268E 03	64.6290
EXP TYPE X TEMP	7	6.22846875E 03	8.89781250E 02	→20.0939
ERROR	16	7.08500000E 02	4.42812500E 01	
TOTAL	31	4.59337188E 04		

SOURCE MEANS

EXP TYPE

(EXP)	(CON)
59.75000	11.06250

TEMP

(74)	(69)	(64)	(59)
83.00000	52.00000	56.25000	35.00000
(54)	(49)	(44)	(39)
21.00000	23.00000	6.00000	7.00000

EXP TYPE X TEMP

(EXP , 74)	(EXP , 69)	(EXP , 64)	(EXP , 59)
100.00000	100.00000	94.00000	70.00000
(EXP , 54)	(EXP , 49)	(EXP , 44)	(EXP , 39)
42.00000	46.00000	12.00000	14.00000
(CON , 74)	(CON , 69)	(CON , 64)	(CON , 59)
66.00000	4.00000	18.50000	0
(CON , 54)	(CON , 49)	(CON , 44)	(CON , 39)
0	0	0	0

Analysis of Final Percent Mortality Data in Text Table 3. A. Salmonicida in Coho Salmon.

r = 2; t = 2.12 for P = 0.05; t = 2.92 for P = 0.01; $\sqrt{44.28} = 6.6544$

Least significant difference 14.11 percent for P = 0.05
" " " " " 19.43 " " " P = 0.01

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*ANOVA12 - ONE/TWO FACTOR ANALYSIS OF VARIANCE.        OS-3  VER.3.5
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                        PROBLEM I-D: 2ND TRY

  SOURCE                DF          SS              MS              F

EXP TYPE                 1  3.78125000E 04   3.78125000E 04     763.8889

TEMP                     7  1.75950000E 03   2.51357143E 02       5.0779

EXP TYPE X TEMP          7  2.51950000E 03   3.59928571E 02       7.2713

ERROR                   16  7.92000000E 02   4.95000000E 01

TOTAL                   31  4.28835000E 04


SOURCE          MEANS

EXP TYPE
          (EXP )          (CON )
        71.00000         2.25000

TEMP
          ( 74)           ( 69)           ( 64)           ( 59)
        42.00000        41.00000        37.00000        48.00000
          ( 54)           ( 49)           ( 44)           ( 39)
        38.00000        30.00000        22.00000        35.00000

EXP TYPE X TEMP
       (EXP ,  74)     (EXP ,  69)     (EXP ,  64)     (EXP ,  59)
          84.00000        80.00000        72.00000        96.00000
       (EXP ,  54)     (EXP ,  49)     (EXP ,  44)     (EXP ,  39)
          70.00000        60.00000        38.00000        68.00000
       (CON ,  74)     (CON ,  69)     (CON ,  64)     (CON ,  59)
                 0         2.00000         2.00000                 0
       (CON ,  54)     (CON ,  49)     (CON ,  44)     (CON ,  39)
          6.00000                 0        6.00000         2.00000
```

Analysis of Final Percent Mortality Data in Text Table 3. A. Salmonicida in Spring Chinook Salmon.

$r = 2$; $t = 2.12$ for $P=0.05$; $t = 2.92$ for $P = 0.01$; $\sqrt{49.5} = 7.0356$

Least significant difference = 14.92 percent for $P=0.05$
" " " " " = 20.54 " " " $P=0.01$

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**********************************************************************
*ANOVA12 - ONE/TWO FACTOR ANALYSIS OF VARIANCE.        OS-3  VER.3.5
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                        PROBLEM I-D: 2ND TRY

  SOURCE                DF          SS              MS              F

EXP TYPE                 1  1.05088753E 04   1.05088753E 04     182.9490

TEMP                     7  7.75114719E 03   1.10730674E 03      19.2771

EXP TYPE X TEMP          7  7.73882219E 03   1.10554603E 03      19.2464

ERROR                   16  9.19065000E 02   5.74415625E 01

TOTAL                   31  2.69179097E 04


SOURCE          MEANS

EXP TYPE
        (EXP )          (CON )
      36.42500          .15125

TEMP
        ( 74)           ( 69)           ( 64)           ( 59)
      40.72500        32.12500        33.57500        20.00000
        ( 54)           ( 49)           ( 44)           ( 39)
      20.00000               0               0               0

EXP TYPE X TEMP
     (EXP ,  74)     (EXP ,  69)     (EXP ,  64)     (EXP ,  59)
      81.45000        64.25000        67.15000        38.55000
     (EXP ,  54)     (EXP ,  49)     (EXP ,  44)     (EXP ,  39)
      40.00000               0               0               0
     (CON ,  74)     (CON ,  69)     (CON ,  64)     (CON ,  59)
               0               0               0         1.45000
     (CON ,  54)     (CON ,  49)     (CON ,  44)     (CON ,  39)
               0               0               0               0
```

Analysis of Final Percent Mortality Data in Text Table 5. A. Liquefacions in Steelhead Trout.

r=2; t=2.12 for P=0.05; t=2.92 for P=0.01; $\sqrt{57.44} = 7.5790$

Least significant difference = 16.07 percent for P = 0.05
" " " " " = 22.13 " " " P = 0.01

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*ANOVA12 - ONE/TWO FACTOR ANALYSIS OF VARIANCE.        OS-3  VER.3.5
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                        PROBLEM I-D: GP3-PT1

  SOURCE                DF          SS                MS              F

EXP TYPE                 1  1.53688905E 04  1.53688905E 04     283.3435

TEMP                     5  1.33285267E 04  2.66570534E 03      49.1454

EXP TYPE X TEMP          5  9.35519069E 03  1.87103814E 03      34.4948

ERROR                   12  6.50894444E 02  5.42412037E 01

TOTAL                   23  3.67035024E 04


SOURCE          MEANS

EXP TYPE
          (EXP )          (CON )
        56.61108         6.00000

TEMP
          (   8)          (   7)          (   6)          (   5)
        63.00000        51.00000        46.63328        20.00000
          (   4)          (   3)
         7.00000               0

EXP TYPE X TEMP
        (EXP ,   8)     (EXP ,   7)     (EXP ,   6)     (EXP ,   5)
         100.00000       100.00000        91.66656        40.00000
        (EXP ,   4)     (EXP ,   3)
           8.00000               0
        (CON ,   8)     (CON ,   7)     (CON ,   6)     (CON ,   5)
          26.00000         2.00000         2.00000               0
        (CON ,   4)     (CON ,   3)
           6.00000               0
```

Analysis of Final Percent Mortality Data in Test Table 7, C. Columnaris in Rainbow Trout.

r=2; t=2.18 for P=0.05; t=3.06 for P=0.01 $\sqrt{54.2} = 7.3649$

Least significant difference 16.06 percent for P=0.05
" " " " " 22.54 " " " P=0.01

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ANOVA12 - ONE/TWO FACTOR ANALYSIS OF VARIANCE.        OS-3  VER.3.5
OREGON STATE UNIVERSITY COMPUTER CENTER               DATE - 12/05/72
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PROBLEM I-D: GP2-PT1

SOURCE	DF	SS	MS	F
EXP TYPE	1	1.45782935E 04	1.45782935E 04	1637.7096
TEMP	7	1.72962713E 04	2.47089589E 03	277.5777
EXP TYPE X TEMP	7	1.61281572E 04	2.30402245E 03	258.8314
ERROR	16	1.42426165E 02	8.90163529E 00	
TOTAL	31	4.81451481E 04		

SOURCE MEANS

EXP TYPE

(EXP)	(CON)
44.29531	1.60706

TEMP

(8)	(7)	(6)	(5)
50.71425	50.71425	51.46700	27.14275
(4)	(3)	(2)	(1)
2.85700	0	0	.71425

EXP TYPE X TEMP

(EXP , 8)	(EXP , 7)	(EXP , 6)	(EXP , 5)
100.00000	100.00000	98.64853	51.42850
(EXP , 4)	(EXP , 3)	(EXP , 2)	(EXP , 1)
4.28550	0	0	0
(CON , 8)	(CON , 7)	(CON , 6)	(CON , 5)
1.42850	1.42850	4.28550	2.85700
(CON , 4)	(CON , 3)	(CON , 2)	(CON , 1)
1.42850	0	0	1.42850

Analysis of Final Percent Mortality Data in Text Table 9. C. Columnaris in Coho Salmon.

r = 2; t = 2.12 for P = 0.05; t = 2.92 for P = 0.01. $\sqrt{8.9} = 2.9836$

Least significant difference 6.33 percent for P = 0.05
" " " " " 8.71 " " " P = 0.01

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*ANOVA12 - ONE/TWO FACTOR ANALYSIS OF VARIANCE.        OS-3  VER.3.5
OREGON STATE UNIVERSITY COMPUTER CENTER                DATE - 12/05/72
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PROBLEM I-D: GP4-PT1

SOURCE	DF	SS	MS	F
EXP TYPE	1	8.22938656E 03	8.22938656E 03	201.9441
TEMP	7	7.22271241E 03	1.03181606E 03	25.3201
EXP TYPE X TEMP	7	8.85962991E 03	1.26566142E 03	31.0586
ERROR	16	6.52012944E 02	4.07508090E 01	
TOTAL	31	2.49637418E 04		

SOURCE MEANS

EXP TYPE

(EXP)	(CON)
35.32294	3.25000

TEMP

(8)	(7)	(6)	(5)
46.00000	36.00000	29.00000	16.29175
(4)	(3)	(2)	(1)
13.00000	3.00000	9.00000	2.00000

EXP TYPE X TEMP

(EXP , 8)	(EXP , 7)	(EXP , 6)	(EXP , 5)
92.00000	70.00000	56.00000	30.58350
(EXP , 4)	(EXP , 3)	(EXP , 2)	(EXP , 1)
20.00000	6.00000	6.00000	2.00000
(CON , 8)	(CON , 7)	(CON , 6)	(CON , 5)
0	2.00000	2.00000	2.00000
(CON , 4)	(CON , 3)	(CON , 2)	(CON , 1)
6.00000	0	12.00000	2.00000

Analysis of Final Percent Mortality Data in Text Table 8, C. Columnaris in Spring Chinook Salmon

r=2; t=2.12 for P = 0.05; t = 2.92 for P = 0.01. $\sqrt{40.75}$ = 6.3836

Least significant difference 13.53 percent for P = 0.05
" " " " " 18.64 " " " P = 0.01

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*ANOVA12 - ONE/TWO FACTOR ANALYSIS OF VARIANCE.          OS-3  VER.3.5
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                        PROBLEM I-01 GP1-PT2

   SOURCE                DF          SS              MS              F

EXP TYPE                  1  2.77095276E 04  2.77095276E 04      323.5509

TEMP                      6  5.28712083E 03  8.81186806E 02       10.2892

EXP TYPE X TEMP           6  5.28712083E 03  8.81186806E 02       10.2892

ERROR                    14  1.19698719E 03  8.56419424E 01

TOTAL                    27  3.94827565E 04


SOURCE          MEANS

EXP TYPE
          (EXP )          (CON )
        62.91664               0

TEMP
          (   8)          (   7)          (   5)          (   4)
        26.00000        36.00000        43.00000        37.50000
          (   3)          (   2)          (   1)
        40.00000        37.70825               0

EXP TYPE X TEMP
       (EXP ,   8)     (EXP ,   7)     (EXP ,   5)     (EXP ,   4)
        52.00000        72.00000        86.00000        75.00000
       (EXP ,   3)     (EXP ,   2)     (EXP ,   1)
        80.00000        75.41650               0
       (CON ,   8)     (CON ,   7)     (CON ,   5)     (CON ,   4)
                0               0               0               0
       (CON ,   3)     (CON ,   2)     (CON ,   1)
                0               0               0
```

Analysis of Data in Text Table 10 for Percent of Rainbow Trout Infected with *C. Shasta*

$r = 2$; $t = 2.14$ for $P = 0.05$; $t = 2.98$ for $P = 0.01$; $\sqrt{85.64} = 9.2543$

Least significant difference = 19.80 percent for $P = 0.05$
" " " " " = 27.58 " " " $P = 0.01$

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**********************************************************************
*ANOVA12 - ONE/TWO FACTOR ANALYSIS OF VARIANCE.        OS-3  VER.3.5
OREGON STATE UNIVERSITY COMPUTER CENTER               DATE - 12/05/72
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                        PROBLEM I-D& GP1-PT1

  SOURCE              DF          SS              MS              F

EXP TYPE               1  4.91374599E 03  4.91374599E 03   115.7391

TEMP                   7  9.44196107E 03  1.34885158E 03    31.7711

EXP TYPE X TEMP        7  4.72098054E 03  6.74425791E 02    15.8855

ERROR                  8  3.39642933E 02  4.24553667E 01

TOTAL                 23  1.94163305E 04


SOURCE          MEANS

EXP TYPE
          (CON )          (EXP )
               0        30.35337

TEMP
          (   8)          (   7)          (   6)          (   5)
        40.00000        56.00000        35.57300        14.31167
          (   4)          (   3)          (   2)          (   1)
        14.66667         1.33333               0               0

EXP TYPE X TEMP
        (CON ,   8)     (CON ,   7)     (CON ,   6)     (CON ,   5)
                 0               0               0               0
        (CON ,   4)     (CON ,   3)     (CON ,   2)     (CON ,   1)
                 0               0               0               0.
        (EXP ,   8)     (EXP ,   7)     (EXP ,   6)     (EXP ,   5)
          60.00000        84.00000        53.35999        21.46750
        (EXP ,   4)     (EXP ,   3)     (EXP ,   2)     (EXP ,   1)
          22.00000         2.00000               0               0

END OF *ANOVA12 EXECUTION.
```

Analysis of Data in Text Table 11 for Percent of Coho Salmon Infected with C. Shasta

$r = 2$; $t = 2.31$ for $P = 0.05$; $t = 3.36$ for $P = 0.01$; $\sqrt{3/2 \times 42.54} = 7.9802$

Least significant difference = 18.43 percent for $P = 0.05$
" " " " " = 26.81 " " " $P = 0.01$

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**********************************************************************
*ANOVA12 - ONE/TWO FACTOR ANALYSIS OF VARIANCE.        OS-3  VER.3.5
OREGON STATE UNIVERSITY COMPUTER CENTER               DATE - 12/05/72
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PROBLEM I-01: GP4-PT1

SOURCE	DF	SS	MS	F
EXP TYPE	1	1.72504150E 04	1.72504150E 04	644.6292
TEMP	2	1.79707546E 03	8.98537732E 02	33.5774
EXP TYPE X TEMP	2	1.79707546E 03	8.98537732E 02	33.5774
ERROR	6	1.60561279E 02	2.67602132E 01	
TOTAL	11	2.10051272E 04		

SOURCE	MEANS		
EXP TYPE			
	(EXP)	(CON)	
	75.82967	0	
TEMP			
	(6)	(4)	(1)
	20.83325	44.04750	48.86375
EXP TYPE X TEMP			
	(EXP , 6)	(EXP , 4)	(EXP , 1)
	41.66650	88.09500	97.72750
	(CON , 6)	(CON , 4)	(CON , 1)
	0	0	0

Analysis of Final Percent Mortality Data in Text Table 14. Sockeye Salmon Virus in 1.1 Gram Kokanee Salmon

r = 2; t = 2.45 for P = 0.05; t = 3.71 for P = 0.01; $\sqrt{26.76}$ = 5.1730

Least significant difference 12.67 percent for P = 0.05
" " " " " 19.19 " " " P = 0.01

```
**********************************************************************
*ANOVA12 - ONE/TWO FACTOR ANALYSIS OF VARIANCE.        OS-3  VER.3.5
OREGON STATE UNIVERSITY COMPUTER CENTER               DATE - 12/13/72
**********************************************************************

                    PROBLEM I-D: GP2-PT1

  SOURCE              DF          SS              MS              F

EXP TYPE               1  4.14403260E 03  4.14403260E 03       79.9433

TEMP                   4  1.19465647E 03  2.98664117E 02        5.7616

EXP TYPE X TEMP        4  2.37925942E 03  5.94814856E 02       11.4747

ERROR                 10  5.18371758E 02  5.18371758E 01

TOTAL                 19  8.23632026E 03


SOURCE          MEANS

EXP TYPE
          (EXP )          (CON )
        33.62870         4.83970

TEMP
          (   8)          (   7)          (   6)          (   5)
        19.56725        12.72425         9.47125        23.09200
          (   4)
        31.31625

EXP TYPE X TEMP
      (EXP ,   8)     (EXP ,   7)     (EXP ,   6)     (EXP ,   5)
         18.75000        22.88450        18.94250        46.18400
      (EXP ,   4)
         61.38250
      (CON ,   8)     (CON ,   7)     (CON ,   6)     (CON ,   5)
         20.35450         2.56400               0               0
      (CON ,   4)
          1.25000
```

Analysis of Final Percent Mortality Data in Text Table 15. Sockeye Salmon Virus in 2.9 Gram Kokanee Salmon.

$r = 2$; $t = 2.23$ for $P = 0.05$; $t = 3.17$ for $P = 0.01$; $\sqrt{51.83} = 7.1998$

Least significant difference = 16.06 percent for $P = 0.05$
" " " " " 22.82 " " " $P = 0.01$

```
**********************************************************************
*ANOVA12 - ONE/TWO FACTOR ANALYSIS OF VARIANCE.        OS-3  VER.3.5
OREGON STATE UNIVERSITY COMPUTER CENTER              DATE - 12/05/72
**********************************************************************

                    PROBLEM I-01 GP1-PT1

  SOURCE              DF          SS              MS            F

EXP TYPE               1  6.31122945E 03  6.31122945E 03   244.5855

TEMP                   4  9.72105609E 03  2.43026402E 03    94.1825

EXP TYPE X TEMP        4  1.17204664E 04  2.93011661E 03   113.5538

ERROR                 10  2.58037717E 02  2.58037717E 01

TOTAL                 19  2.90107897E 04


SOURCE          MEANS

EXP TYPE
          (EXP )          (CON )
        47.33340        11.80530

TEMP
          (   7)          (   5)          (   4)          (   3)
        41.20700        51.66650        49.16675         2.50000
          (   1)
         3.30650

EXP TYPE X TEMP
      (EXP ,   7)     (EXP ,   5)     (EXP ,   4)     (EXP ,   3)
         33.33350        96.66650        98.33350         3.33350
      (EXP ,   1)
          5.00000
      (CON ,   7)     (CON ,   5)     (CON ,   4)     (CON ,   3)
         49.08350         6.66650               0         1.66650
      (CON ,   1)
          1.61300
```

Analysis of Final Percent Mortality Data in Text Table 27, Sockeye Salmon Virus in 0.11 Gram Kokanee Salmon.

r = 2; t = 2.23 for P = 0.05; t = 3.17 for P = 0.01; $\sqrt{25.80} = 5.0797$

Least significant difference 11.33 percent for P = 0.05
" " " " " 16.10 " " " P = 0.01

```
**********************************************************************
*ANOVA12 - ONE/TWO FACTOR ANALYSIS OF VARIANCE.        OS-3  VER.3.5
OREGON STATE UNIVERSITY COMPUTER CENTER               DATE - 12/05/72
**********************************************************************

                        PROBLEM I-D: GP3-PT1

  SOURCE                 DF          SS              MS            F

EXP TYPE                  1  3.88331201E 04  3.88331201E 04  4438.0710

TEMP                      5  5.88330104E 02  1.17666021E 02    13.4475

EXP TYPE X TEMP           5  2.67185564E 03  5.34371128E 02    61.0710

ERROR                    10  8.74999981E 01  8.74999981E 00

TOTAL                    21  4.21808058E 04


SOURCE          MEANS

EXP TYPE
          (EXP )          (CON )
        88.75791         4.76073

TEMP
          (   7)          (   6)          (   5)          (   4)
        57.01750        37.50000        46.25000        47.50000
          (   3)          (   1)
        48.75000        48.75000

EXP TYPE X TEMP
     \(EXP ,   7)    (EXP ,   6)    (EXP ,   5)    (EXP ,   4)
         66.66700        75.00000        90.00000        95.00000
      (EXP ,   3)    (EXP ,   1)
         97.50000        97.50000
     \(CON ,   7)    (CON ,   6)    (CON ,   5)    (CON ,   4)
         47.36800               0        2.50000               0
      (CON ,   3)    (CON ,   1)
                0               0
```

Analysis of Final Percent Mortality Data in Text Table 19, Sockeye Salmon Virus in 0.95 Gram Kokanee Salmon.

$r = 2$; $t = 2.23$ for $P = 0.05$; $t = 3.17$ for $P = 0.01$; $\sqrt{2 \times 8.749} = 4.1833$.

Least significant difference 9.33 percent for $P = 0.05$
" " " " " 13.26 " " " $P = 0.01$

χ^2 Analysis of Percentages of Surviving Fish Yielding Cultures of A. liquefaciens. Text Table 6.

A. Comparison of Percent Positive at 69^oF (28%) and 59^oF (71%)

Water Temp.	Number Positive	Number Negative	Total
69^o	7	18	25
59^o	3	40	43
Total	10	58	68

$$\chi^2 \text{ (Yates Modification)} = \frac{(17.5 \times 2.5 - 40.5 \times 7.5)^2 \times 68}{25 \times 43 \times 10 \times 58}$$

$$= 7.38$$

From χ^2 Table for n = 1
p = less than 0.01 for this value of χ^2

Hence difference is highly significant.

B. Comparison of Percent Positive at 64^oF (17.4%) and 54^oF (2.4%)

Water Temp.	Number Positive	Number Negative	Total
69^o	4	19	23
54^o	1	41	42
Total	5	60	65

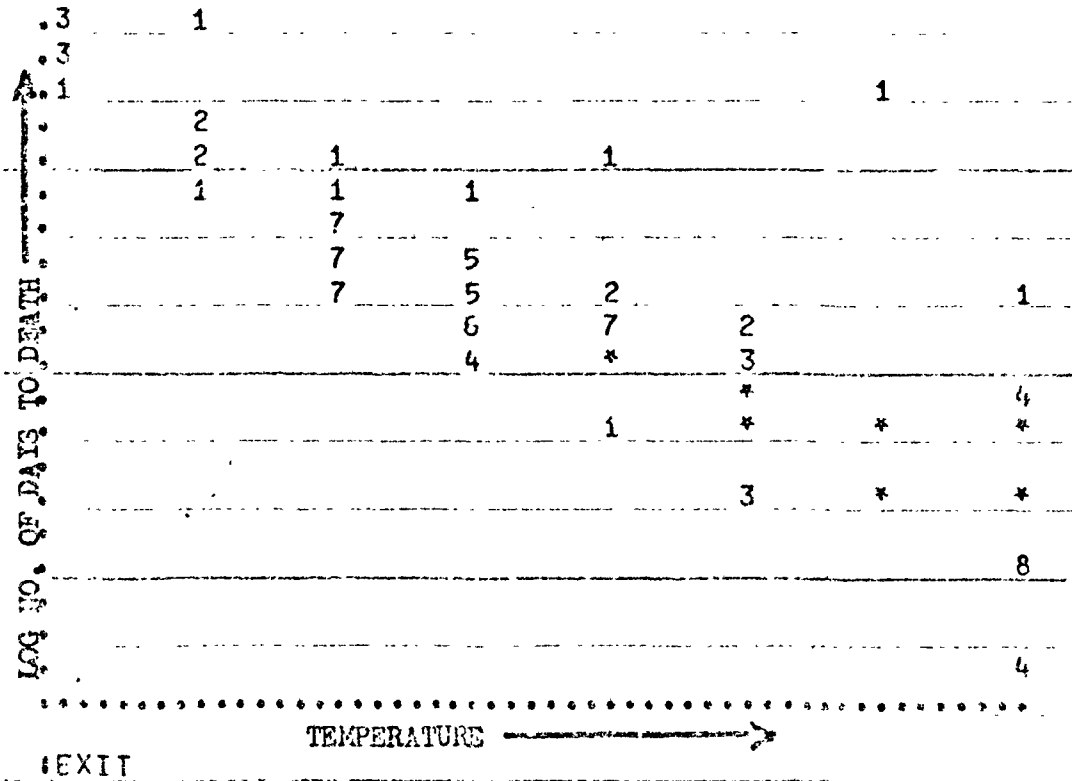
$$\chi^2 \text{ (Yates modification)} = \frac{(18.5 \times 0.5 - 4.5 \times 41.5)^2 \times 65}{23 \times 42 \times 60 \times 5}$$

$$= 7.09$$

From χ^2 Table for n = 1
p = less than 0.01 for this value of χ^2

Hence difference is highly significant.

REGRESSION ANALYSIS. AEROMONAS SALMONICIDA IN COHO SALMON. TEXT FIG. 1



RELATION BETWEEN WATER TEMPERATURE AND LOG NO. OF DAYS TO DEATH. A. SALMONICIDA INFECTION IN COHO SALM.

Y(2)= 2.4629315E 01 + -2.7651534E-02 X(1)

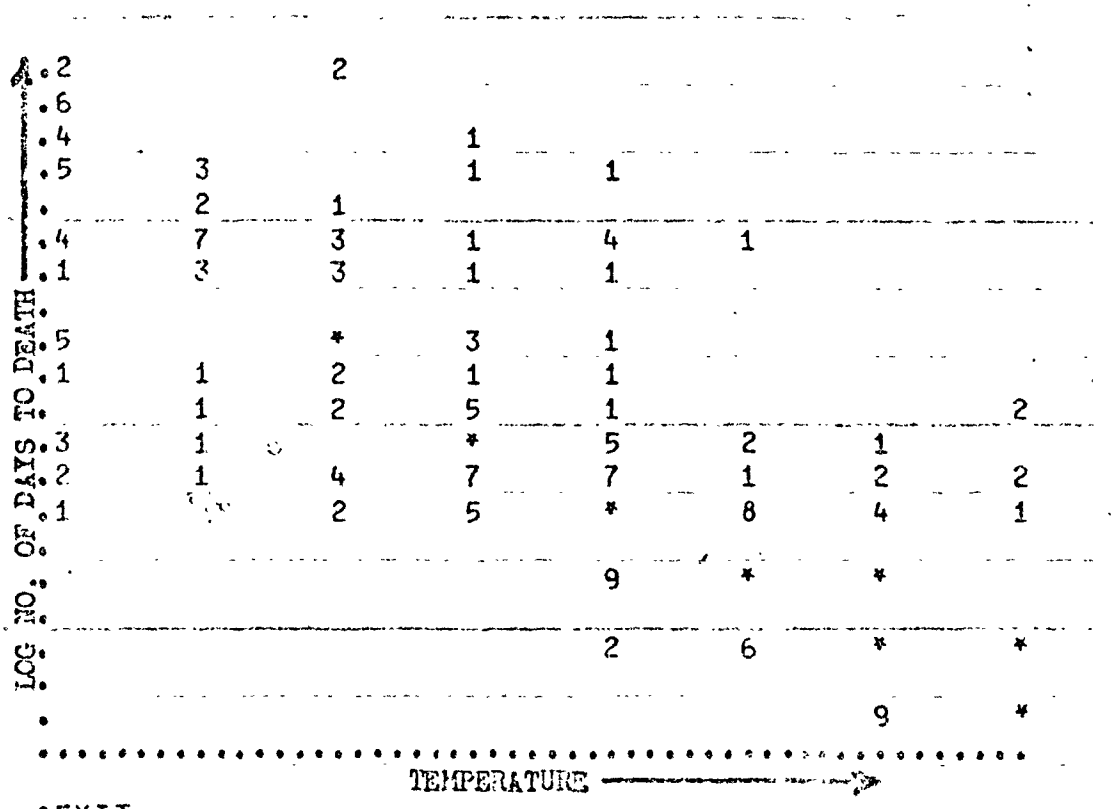
Y = LOG NO. DAYS TO DEATH (1)= TEMPERATURE

R SQUARED = .78319516 = % OF VARIATION ACCOUNTED FOR WITH LINE.

VAR	S.E. OF REGR. COEF.	T
0	6.800487282E-02	4.1015[illegible]73E 01
1	9.45055545E-04	-2.9259[illegible]27E 01

CORRELATION COEFFICIENT = -0.8849775 = LINEAR RELATIONSHIP BETWEEN TEMPERATURE AND LOG NO. OF DAYS TO DEATH

REGRESSION ANALYSIS. AEROMONAS SALMONICIDA
IN CHINOOK SALMON. TEXT FIG. 3



)EXIT

RELATION BETWEEN WATER TEMPERATURE AND LOG NO. OF DAYS TO DEATH. A. SALMONICIDA INFECTION IN CHINOOK SALMON.

Y(2)= 2.1215819E 01 + -2.2966.89E-02 X(1)

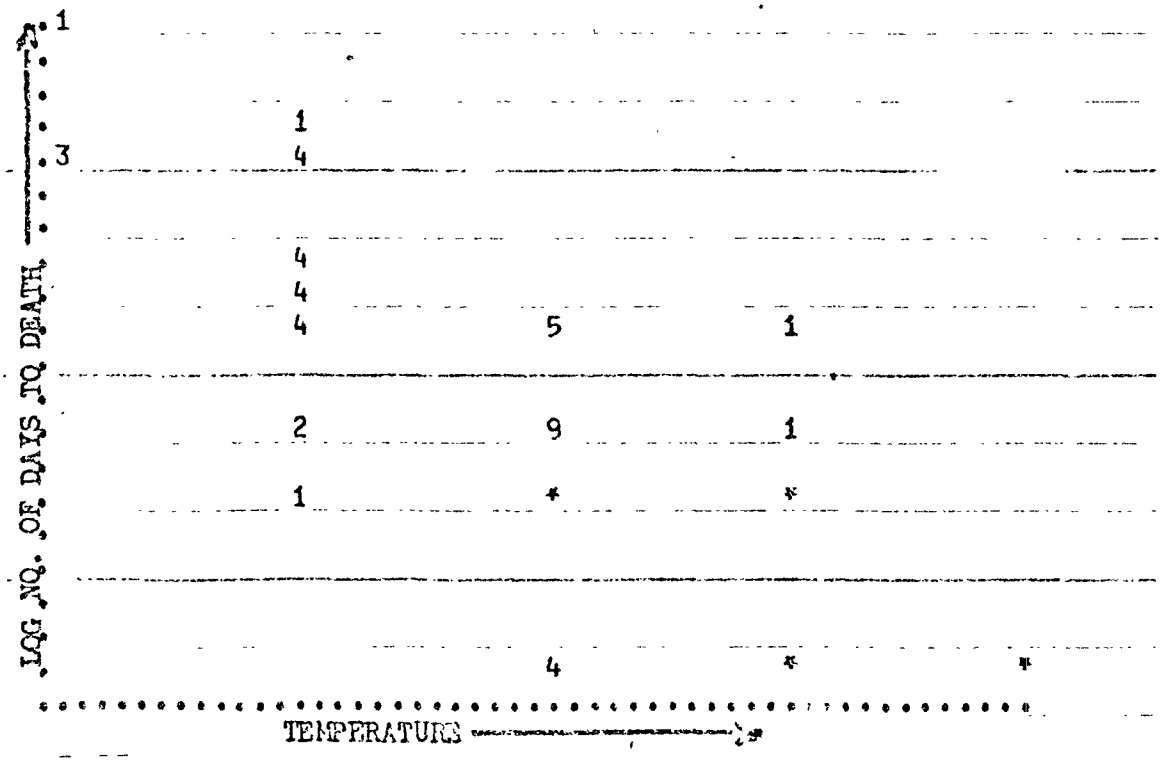
Y = LOG NO. DAYS TO DEATH (1) = TEMPERATURE

R SQUARED = .67720766 = % OF VARIATION ACCOUNTED FOR WITH LINE.

VAR	S.E. OF REGR. COEF.	T
0	5.59644448E-02	3.790[illegible]236E 01
1	9.44280422E-04	-2.432[illegible]771E 01

CORRELATION COEFFICIENT = -0.8229263 = LINEAR RELATIONSHIP BETWEEN TEMPERATURE AND LOG NO. OF DAYS TO DEATH

REGRESSION ANALYSIS. CHONDROCOCCUS COLUMNARIS
IN RAINBOW TROUT. TEXT FIG. 4



RELATION BETWEEN WATER TEMPERATURE AND LOG NO. OF DAYS TO DEATH. C. COLUMNARIS INFECTION IN RAINBOW TROUT.

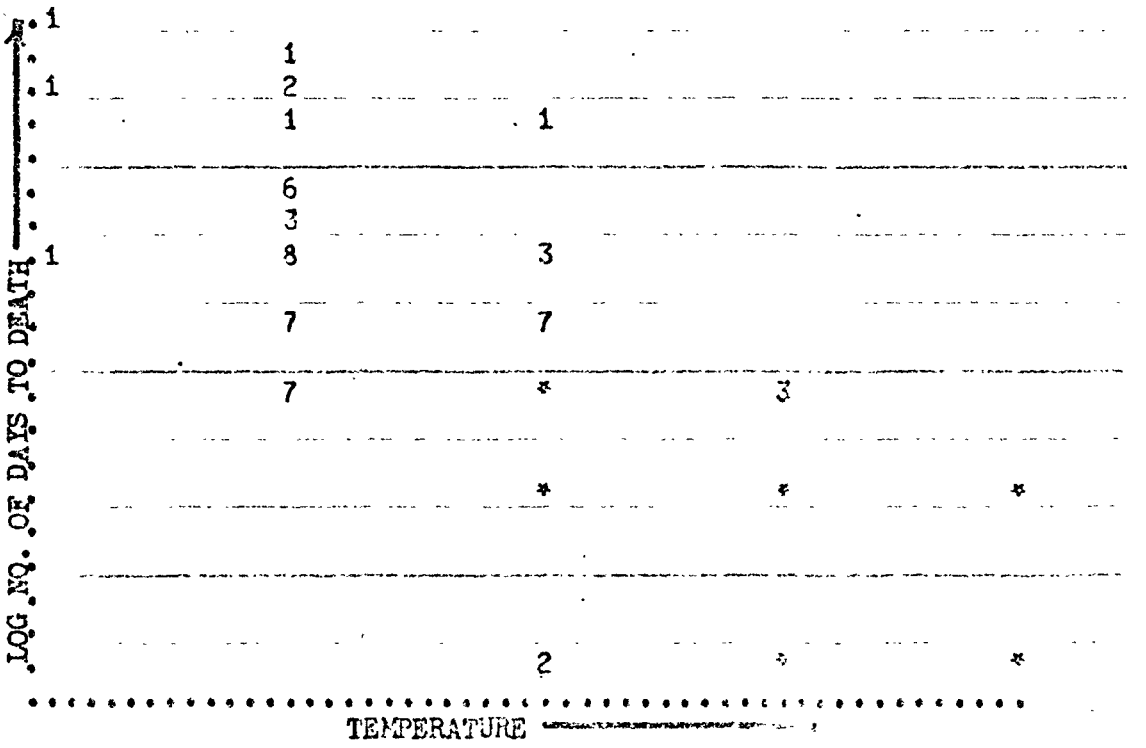
Y(2)= 3.2806148E 00 + -4.49 942E-02 X(1)
Y = LOG NO. DAYS TO DEATH ( ) = TEMPERATURE

R SQUARED = .73489354 = % OF VARIATION ACCOUNTED FOR WITH LINE.

VAR	S.E. OF REGR. COEF.	T
0	1.42169166E-01	2.3075[illegible]08E 01
1	2.09631512E-03	-2.14514[illegible]8E 01

CORRELATION COEFFICIENT = -0.8572593 = LINEAR RELATIONSHIP BETWEEN TEMPERATURE AND LOG NO. OF DAYS TO DEATH

REGRESSION ANALYSIS. CHONDROCOCCUS COLUMNARIS
IN COHO SALMON. TEXT FIG. 5



!EXIT

RELATION BETWEEN WATER TEMPERATURE AND LOG NO. OF DAYS TO DEATH. C. COLUMNARIS INFECTION IN COHO SALMON

Y(2)= 2.6270343E 00 + -3.352 30E-02 X(1)

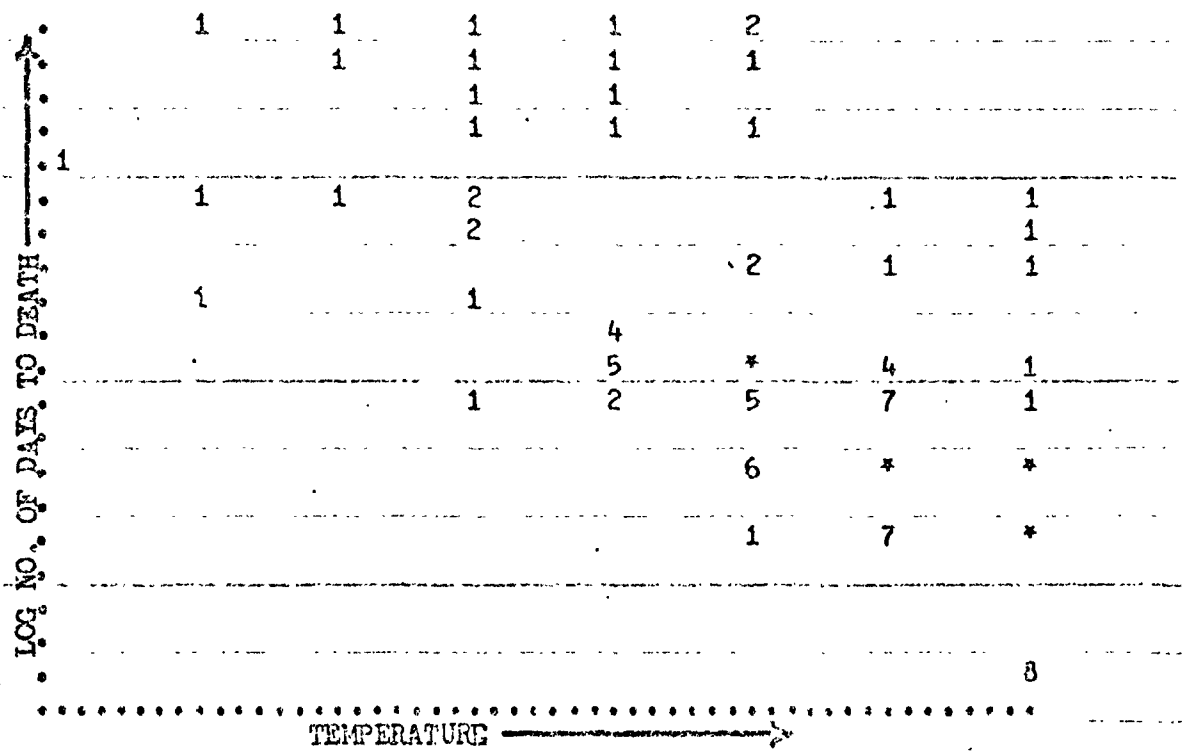
Y= LOG NO. DAYS TO DEATH (1)= TEMPERATURE

R SQUARED = .59286895 = % OF VARIATI ACCOUNTED FOR WITH LINE.

VAR	S.E. OF REGR. COEF.	
0	1.18372935E-01	2.213 630E 01
1	1.75347405E-03	-1.911 324E 01

CORRELATION COEFFICIENT = -0.7699790 = LINEAR RELATIONSHIP BETWEEN TEMPERATURE AND LOG NO. OF DAYS TO DEATH

REGRESSION ANALYSIS. CHONDROCOCCUS COLUMNARIS
IN CHINOOK SALMON. TEXT FIG. 6



!EXIT

RELATION BETWEEN WATER TEMPERATURE AND LOG NO. OF DAYS TO DEATH, C. COLUMNARIS INFECTION IN CHINOOK SALMON

Y(2)= 2.7559243E 00 + -3.2223786E-02 X(1)

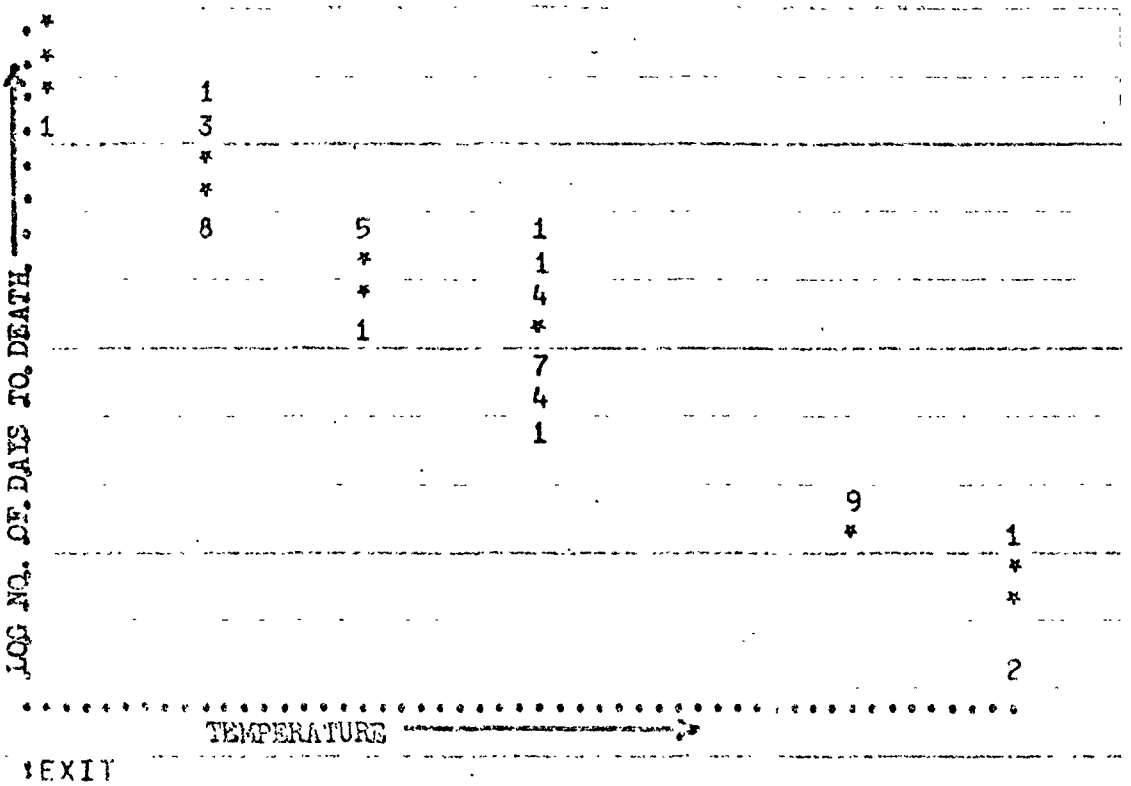
Y = LOG NO. DAYS TO DEATH (1) = TEMPERATURE

R SQUARED = .51730671 = % OF VARIATION ACCOUNTED FOR BY LINE.

VAR	S.E. OF REGR. COEF.	T
0	1.76388738E-01	1.5624 509E 01
1	2.64065457E-03	-1.2205 260E 01

CORRELATION COEFFICIENT = -0.7192404 = LINEAR RELATIONSHIP BETWEEN TEMPERATURE AND LOG NO. OF DAYS TO DEATH

REGRESSION ANALYSIS. CERATOMYXA SHASTA IN RAINBOW TROUT. TEXT FIG. 7



EXIT

RELATION BETWEEN WATER TEMPERATURE AND LOG NO. OF DAYS TO DEATH. C. SHASTA INFECTION IN RAINBOW TROUT

Y(2)= 3.6397320E 00 + -3.4173144E-02 X(1)

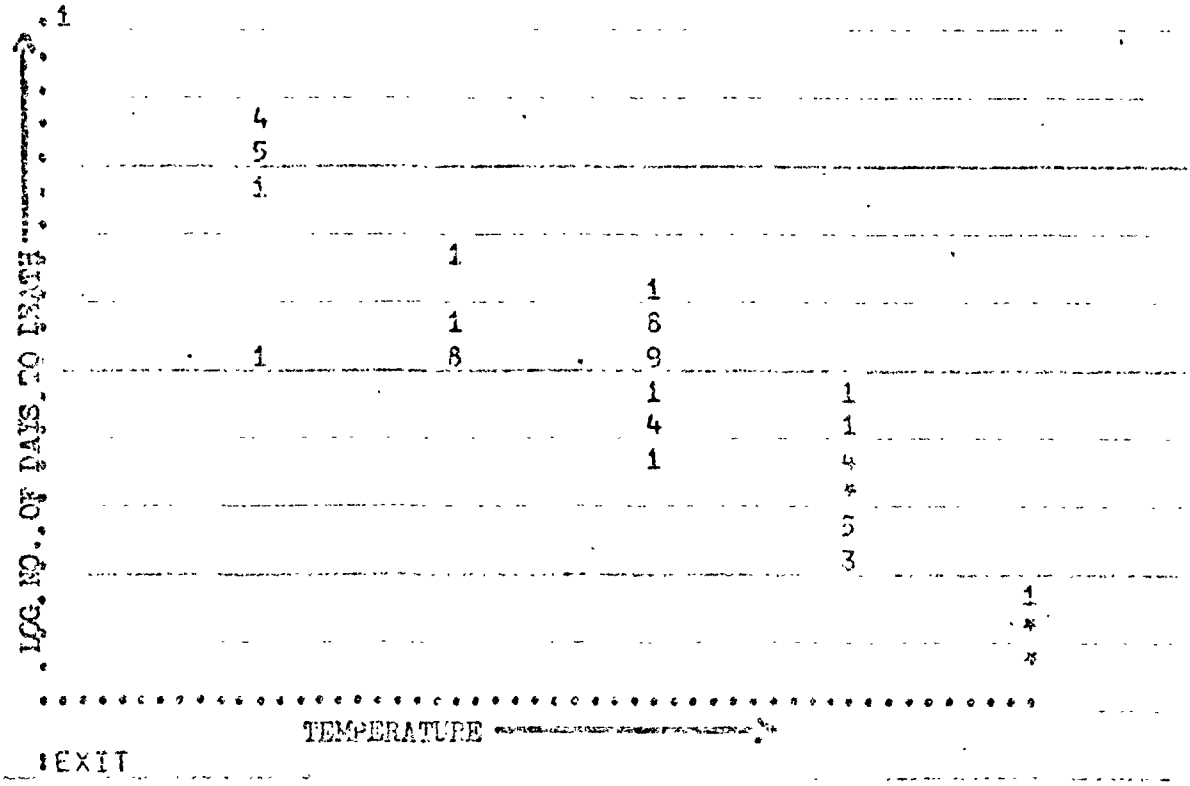
Y = LOG NO. DAYS TO DEATH (1) = TEMPERATURE

R SQUARED = .96647996 = % OF VARIATION ACCOUNTED FOR WITH LINE.

VAR	S.E. OF REGR. COEF.	T
0	2.51298796E-02	1.4483826E 02
1	4.33055056E-04	-7.8913391E 01

CORRELATION COEFFICIENT = -0.9830971= LINEAR RELATIONSHIP BETWEEN TEMPERATURE AND LOG NO. OF DAYS TO DEATH

REGRESSION ANALYSIS. CERATOMYXA SHASTA IN
COHO SALMON. TEXT FIG. 8



!EXIT

RELATION BETWEEN WATER TEMPERATURE AND LOG NO. OF DAYS TO DEATH. C. SHASTA INFECTION IN COHO SALMON

Y(2)= 4.8494299E 00 + -3.9553275E-02 X(1)
Y = LOG NO. DAYS TO DEATH (1) = TEMPERATURE

R SQUARED = .91679954 = % OF VARIATION ACCOUNTED FOR WITH LINE.

VAR	S.E. OF REGR. COEF.	
0	7.427023456-02	5.4922[illegible]181E 01
1	1.10628789E-03	-3.5752244GE 01

CORRELATION COEFFICIENT = -0.9574965 = LINEAR RELATIONSHIP BETWEEN TEMPERATURE AND LOG NO. OF DAYS TO DEATH

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SELECTED WATER RESOURCES ABSTRACTS

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W

Effects of Temperature On Diseases Of Salmonid Fishes

5 Report Date July, 1972

6

8 Performing Organization Report No.

J. L. FRYER AND K. S. PILCHER

Oregon State University
Department of Microbiology

18050 DIJ

13 Type of Report and Period Covered Final Report 4/1/69 - 3/31/72

12 Sponsoring Organization **Office of Research and Monitoring Environmental Protection Agency**

Environmental Protection Agency, report number, EPA-660/3-73-020, January 1974.

The effect of water temperature on infections of salmonid fish was investigated. Chondrococcus columnaris infection was studied in rainbow trout, coho and spring chinook salmon; Aeromonas salmonicida infection in coho and spring chinook salmon; and Aeromonas liquefaciens infection in steelhead trout. In all cases mortality rates were high at 64° to 69°F; moderate at 54° to 59°F; and low or zero at 39° to 49°F. Progress of the infections was accelerated at higher temperatures, and progressively retarded at decreasing temperature levels. In infection of coho with Ceratomyxa shasta, mortality was high at 69°F, low at 49° to 54°, and zero at 39° to 44°F. This infection in rainbow trout resulted in high mortality at all temperatures except 39°. In both cases the course of the disease was most rapid at higher temperatures, and became progressively slower as the temperature decreased. For infection of kokanee salmon fingerlings with sockeye salmon virus, the temperature range of 54° to 59°F was optimal. In this range mortality rates were high, and the course of the disease was most rapid. At higher temperatures mortality rates were lower, and at 39° to 44°F, progress of the disease was retarded, though total mortality was often high.

17 Descriptors Animal diseases, effluents, fish diseases, heated water, infection, microorganisms, pathogenic bacteria, pathology, thermal pollution, water pollution, water quality, water temperature

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