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Title: The Effect of Temperature and Sodium Chloride Concentration on the Survival of Vibrio parahaemolyticus in Trypticase Soy Broth and Fish Homogenate

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The effect of temperature and sodium chloride concentration on the survival of three strains of Vibrio parahaemolyticus was studied in Trypticase soy broth and in fish homogenate. The inoculum level for studies at 48 ± 1, 5 ± 1, -5 ± 1 and -18 ± 1°C was approximately 10,000 cells per ml. Samples at 48 ± 1°C were removed at 0.5, 5, 10, and 20 min. Low temperature sampling periods were 1, 9, 16, and 30 days.

Cells of Vibrio parahaemolyticus suspended in Trypticase soy broth without sodium chloride were quite unstable and readily killed. The presence of sodium chloride appeared to be protective to the cells at 48 ± 1°C, with the optimum concentration being strain dependent. Heating, however, reduced the numbers of Vibrio parahaemolyticus at all sodium chloride concentrations. Temperatures of 5 ± 1, -5 ± 1, and -18 ± 1°C reduced the number of organisms per

milliliter regardless of the sodium chloride concentration. However, in the presence of sodium chloride, viable cells were still detected at the end of the storage period.

Fish homogenate was protective since it stabilized the survival count even with no additional sodium chloride. The presence of sodium chloride in concentrations of 3, 6, and 9% at 48 ± 1°C further stabilized the viable cells. Storage of the samples at -5 ± 1 and -18 ± 1°C, even in the presence of sodium chloride, resulted in considerable decreases in viable counts of Vibrio parahaemolyticus after 30 days. Comparison of cell recovery in 3% sodium chloride Trypticase soy agar with Colwell's Vibrio maintenance medium at 48 ± 1°C showed significantly higher counts were obtained on 3% sodium chloride Trypticase soy agar for strains SB04-422 and 17802. The same comparison studies at -5 ± 1 and -18 ± 1°C showed no significant differences.

Strain 17802 appeared to be more temperature sensitive than the other strains used in this study. Optimum sodium chloride concentrations for survival were strain dependent.

Plating on 3% sodium chloride Trypticase soy agar for comparison of cell recovery from both 6% sodium chloride Trypticase soy broth and 6% fish homogenate gave significantly higher counts than plating on 6% sodium chloride Trypticase soy agar.

THE EFFECT OF TEMPERATURE AND SODIUM CHLORIDE CONCENTRATION ON THE SURVIVAL OF *VIBRIO PARAHAEMOLYTICUS* IN TRYPTICASE SOY BROTH AND FISH HOMOGENATE

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INTRODUCTION

The survival of Vibrio parahaemolyticus in foods is of concern since this organism is a major cause of gastro-enteritis in Japan and four outbreaks have been attributed to it in the United States. In addition, it has been isolated from the coastal waters and fish of the United States. Therefore, the potential exists for it to be a cause of a greater number of outbreaks here. Prior to 1969, no outbreaks of food poisoning had been directly attributed to Vibrio parahaemolyticus in the United States. In the 1969 surveillance reports of food-borne illness by the Communicable Disease Center (1970) two related outbreaks attributed to Vibrio parahaemolyticus were reported. They occurred in July and August and affected a total of 71 people. In both cases shellfish was the food involved. In 1970, two more outbreaks occurred: one affecting 161, and the other, five people (Center for Disease Control, 1970). Again shellfish was incriminated.

Vibrio parahaemolyticus is a bacterium estimated to be the causative agent in over 70% of the cases of gastro-enteritis in Japan, according to Sakazaki (1969). It was originally isolated in 1951 by Fujino and his co-workers

from a food poisoning outbreak involving boiled and semi-dried young sardines (as cited by Sakazaki, 1969). Vibrio parahaemolyticus can be readily isolated from sea water and sea fish during the summer months off the coast of Japan. It is also during these summer months that the food poisoning outbreaks occur. These generally result from the ingestion of raw sea fish, sea fish insufficiently cooked, and salted vegetables. A limited number of human feeding studies by Japanese investigators confirm that ingestion of viable Vibrio parahaemolyticus causes gastroenteritis in humans.

Vibrio parahaemolyticus can be isolated from the coastal waters and sediments here in the United States (Baross and Liston, 1968 and Ward, 1968). The organism can also be found in sea fish, particularly the molluscan shellfish of the Pacific Northwest United States (Baross and Liston, 1970). In addition, Vibrio parahaemolyticus has been isolated from processed meat of the Chesapeake Bay blue crabs (Fishbein, Mehlman and Pitcher, 1970).

Despite isolations from waters and shellfish, the incidence of food poisoning in the United States does not parallel that in Japan. The low incidence of food poisoning due to Vibrio parahaemolyticus in the United States might be explained by several factors: 1. The organism was not looked for in food poisoning outbreaks until recently. 2. Americans depend on fish for their diet

to a lesser extent than do the Japanese. 3. Americans consume much less raw fish than do the Japanese.

Vibrio parahaemolyticus, because of its halophilic nature, requires sodium chloride for growth. In many of the Japanese food poisoning outbreaks the incriminated foods were salted. Perhaps the addition of salt to these foods has protected the organism.

This study was initiated to provide information concerning the survival of this organism during heating, refrigeration, and freezing. There were two main objectives: 1. To determine the survival of Vibrio parahaemolyticus over a period of time in Trypticase soy broth (a complex laboratory medium) and in fish homogenate during heating, refrigeration, and freezing. 2. To determine the effect of sodium chloride concentration on the survival of Vibrio parahaemolyticus in Trypticase soy broth (a complex laboratory medium) and fish homogenate during heating, refrigeration, and freezing.

REVIEW OF LITERATURE

Twenty years have passed since the enteropathogenic, halophilic Vibrio parahaemolyticus was first isolated by Fujino and his co-workers (Sakazaki, 1969). Much work has been done on the organism in Japan by Sakazaki and his co-workers and Zen-Yoji and his co-workers. In the United States Baross and Liston have made progress in isolating and studying Vibrio parahaemolyticus found in the Pacific Northwest and others have worked to a limited extent in their areas. Many questions concerning this organism still remain unanswered at this time.

Taxonomy of Vibrio parahaemolyticus

In 1951 Fujino and his co-workers isolated what they called Pasteurella parahaemolytica from autopsy materials from a food poisoning outbreak involving boiled and semi-dried young sardines. Little attention was given to the organism until 1956 when Takikawa and Fujisawa isolated a halophilic organism from another gastroenteritis outbreak. Takikawa, on the basis of his studies, named the organism Pseudomonas enteritis (as cited by Sakazaki, 1969). In 1962, Miyamoto, Nakamura, and Takizawa proposed a new genus Oceanomonas in which they felt the food poisoning organism should be included. In 1963, Sakazaki and his co-workers reported an extensive study of the morphological,

physiological, and biochemical nature of the cultures of this organism and concluded that they belonged to the genus Vibrio. They proposed the name Vibrio parahaemolyticus which has come to be the accepted name for this organism.

Temperature Stress

There seems to be little information concerning temperature stress and survival of Vibrio parahaemolyticus. The earliest reported work in this area occurred when Temmyo (1966) studied the prevention of food poisoning outbreaks caused by Vibrio parahaemolyticus in Japan. Peptone water as well as raw saurels and mackerels were used for testing low temperature survival. He noted that it was practically impossible to avoid the primal contamination due to the prevalence of the bacterium in the sea water. However, holding at -2 and -18°C experimentally killed Vibrio parahaemolyticus in seafoods as well as in peptone water containing 3% sodium chloride. In general, Temmyo found that the resistance of the organism to low temperatures and freezing was rather low, but was influenced by the bacterial count and by the length of storage of the samples. Inoculum levels of 10^2, 10^3, 10^4, 10^5, and 10^6 organisms per ml were used for his first studies in 3% sodium chloride peptone water and 10^2 and 10^5 organisms per ml for his studies in seafood. In addition, Temmyo found that Vibrio parahaemolyticus could be killed if

heated in a peptone solution containing 3% sodium chloride for ten minutes at 55°C for inoculum levels of 10^3 and 10^4 cells per ml. Heating for five minutes at 60°C killed the organisms at all inoculum levels ranging from 10^3 to 10^7 organisms per ml.

In 1967, Asakawa did a laboratory study on the low temperature sensitivity of Vibrio parahaemolyticus. In laboratory media he found survival best at 0°C followed by -20°C. A temperature of -10°C caused a much greater rate of decline in numbers than -20°C. He also inoculated raw tuna meat and found that survival was about the same at -20 as at -10°C and that survival was greater at both than at 0°C.

Much of the work pertaining to temperature stress and survival of Vibrio parahaemolyticus has been done in fisheries research at the University of Washington by Liston and his associates. In 1967, they (Liston et al., 1967) studied the effects of freezing Vibrio parahaemolyticus at -10°C and -24°C and heating at 60°C. In this study two strains of the organism were used and were frozen in fish homogenate and heated in phosphate buffer and in fish homogenate. They concluded from these preliminary studies that the organism appeared to be more readily inactivated by freezing and heating than other food poisoning organisms.

In a summary report on Vibrio parahaemolyticus in

1968, Liston, Chan, and Baross stated that the organism tended to die out fairly rapidly when held below 5°C in the laboratory. However, the organism was found to survive under retail conditions since it could be repeatedly isolated from commercial shellfish samples by Liston and his co-workers (1969). In addition Liston reported that in the laboratory the organism would not grow below 8°C.

Survival of Vibrio parahaemolyticus in fish homogenate during storage at low temperatures was reported by Matches, Liston, and Daneault in 1971. The fish homogenate used was heat sterilized prior to inoculation with Japanese strains of Vibrio parahaemolyticus. They found greatest survival of the organism at 0.6°C than at lower temperatures. This is in agreement with the work of Temmyo (1966) on raw saurels and mackerels mentioned earlier but not with work done by Asakawa (1967). Asakawa worked with inoculated tuna meat and found that survival of Vibrio parahaemolyticus was about the same at -20 and -10°C and that survival was greater at these temperatures than at 0°C.

Effect of Sodium Chloride

One particularly important factor is the sodium chloride requirement of Vibrio parahaemolyticus. Since it is a halophilic organism, it requires at least some sodium chloride for growth. Studies on growth have shown that Vibrio parahaemolyticus will grow with from 0.5-9%

sodium chloride in the growth medium (Sakazaki, 1969).

Very little work has been done on survival of the organism with varying amounts of sodium chloride. Temmyo (1966) found the organism could be killed by placing it in distilled water for a short period of time. He also studied the effects of 0.5, 1, 3, 5, and 7% sodium chloride concentrations on the survival of Vibrio parahaemolyticus in saurel extracts stored at -2°C. At the end of eight days of storage, samples with 10^5 cells per ml originally were still positive for all sodium chloride concentrations. In contrast, samples with an original inoculum of 10^2 cells per ml were all negative after eight days. Samples taken daily during the eight-day period at the 10^2 cells per ml inoculum level led Temmyo to conclude that the greater the sodium chloride concentration the longer the survival of the organism. At -18°C Temmyo studied the effects of 0.5, 3, 5, and 7% sodium chloride on the survival of Vibrio parahaemolyticus in saurel extracts for six days. Survivors were indicated by growth after removal from the freezer, thawing, adjusting to 3% sodium chloride, and incubating at 37°C for 24 hr. With a large inoculum level, all tubes were positive after six days of storage. With a low inoculum level, saurel extract with 0.5% sodium chloride was negative after four days, in 3% after three days, in 5% after six days, and in 7% after five days. Temmyo thus noted that sodium chloride concentration could

enhance survival of Vibrio parahaemolyticus during low temperature storage. His work also indicated an interaction between sodium chloride and storage time.

Other growth conditions besides the sodium chloride concentration are critical as evidenced by the work of Freitas Leitão (1970). He studied the heat resistance of Vibrio parahaemolyticus (ATCC-17802) in sodium chloride peptone water and compared recovery media after heating. From his work, he concluded that Colwell's Vibrio maintenance medium was significantly better than Trypticase soy agar with 2.5% sodium chloride or brain heart infusion agar with 2.5% sodium chloride.

Variation of Strains

Serological typing and biochemical tests are used to determine strain variation. In general there is not a great variability among the 52 serotypes of Vibrio parahaemolyticus, according to Zen-Yoji et al. (1970).

The main variability is the hemolytic nature of the strain. Until 1965 it was thought that only the hemolytic strains of Vibrio parahaemolyticus were pathogenic to humans; but in 1965 none of the strains isolated as causative agents in Japanese outbreaks were hemolytic. Fishbein, Twedt and Olson (1969) and Colwell (1970) reported that all the strains of Vibrio parahaemolyticus tested showed hemolysis. Fishbein, Twedt and Olson (1969) noted

that hemolysis could be inhibited by the addition of 0.1% glucose to the blood agar medium. One problem may be that hemolysis is readily lost with laboratory storage of cultures (Baross, 1971). Zen-Yoji et al. (1970) concluded that further work in this area is still needed.

In 1970, Zen-Yoji et al. reported that the strains of Vibrio parahaemolyticus could be placed into twelve groups on the basis of the somatic O antigen. On the basis of the capsular antigen (K-antigen), there are at present 52 types. For routine serotyping only the K-antigen is necessary, according to Sakazaki (1969). This study is the most recent and reports that results of serological typing of the isolates of Vibrio parahaemolyticus from food poisoning cases in Japan show an annual replacement in the principal strain causing the gastroenteritis. Several different K-type strains could be isolated from a single outbreak and two strains could even be isolated from the same patient. In addition, new strains seem to suddenly appear annually, and thus new serotypes are added to the 52 types already in existence.

MATERIALS AND METHODS

Cultures

Three strains of Vibrio parahaemolyticus were obtained for use in this study. The strain number, antigenic type, original isolation, and source of each strain are given in Table 1.

Table 1. Cultures of Vibrio parahaemolyticus Used in the Study.

Strain	Antigenic Type	Original Isolation	Source
T-3765-1	03, K7	Japanese food poisoning patient-1969	H. Zen-Yoji Tokyo - to Laboratories for Medical Sciences Tokyo, Japan
SB04-422	02, K3	Oyster from Seabeck, Wash. outbreak-1969	J. Baross U. of Washington Seattle, Wash.
ATCC 17802	--	Shirasu food poisoning in Japan-1953	J. Baross U. of Washington Seattle, Wash.

Cultures were maintained on 3% sodium chloride Trypticase soy agar slants in screw-capped tubes and were stored at room temperature.

Gram stain, amylase production on salt-water starch agar (Food and Drug Administration, 1969), gelatinase production on Liston's maintenance medium containing

1% Trypticase (BBL), 3% gelatin, 2% sodium chloride, and 1.5% agar (Baross and Liston, 1970), and β-hemolysis on sheep red blood cell agar (Food and Drug Administration, 1969) were the criteria to confirm that the organism under study was Vibrio parahaemolyticus. β-hemolytic activity is readily lost upon laboratory storage of cultures (Baross, 1971). As tests were repeated monthly, β-hemolysis grew weaker for strains SB04-422 and 17802 and was lost for strain T-3765-1.

Recovery Media

In preliminary experiments nutrient agar (BBL) (Becton, Dickinson and Company) with 3% sodium chloride, Trypticase soy agar (BBL) with 3% sodium chloride and Liston's maintenance medium (Baross and Liston, 1970) were compared for recovery of stressed cells. Cells were stressed by heating, refrigeration, and freezing. Experimentation showed no appreciable differences in the recovery of stressed cells, so Trypticase soy agar with 3% sodium chloride was chosen for use as the recovery medium. Trypticase soy agar containing 6% sodium chloride was used in addition to the 3% sodium chloride Trypticase soy agar for recovery of heated Vibrio parahaemolyticus cells in 6% sodium chloride medium to study the effects, if any, of a change in osmotic environment on the cells.

Colwell's Vibrio maintenance medium (Colwell et al.,

1968) was used as an additional recovery medium in the fish homogenate experiments, since work by Freitas Leitão (1970) indicated its superiority over other recovery media for stressed Vibrio parahaemolyticus cells. The composition of Colwell's Vibrio maintenance medium is 2.4% NaCl, 0.07% KCl, 0.53% $MgCl_2 \cdot 6H_2O$, 0.70% $MgSO_4 \cdot 7H_2O$, 1% proteose peptone (Difco Laboratories), 0.3% yeast extract, and 1.5% agar.

All plating was done by the pour plate method and plates were incubated at 37°C, the temperature considered optimum for the organism (Sakazaki, 1969), for 24 hr and colonies counted.

Experiment I

Trypticase soy broths with sodium chloride concentrations of 0, 3, 6, 9, and 12% by weight were used as the menstrua for heating at 48 ± 1°C and for storage at 5 ± 1°C, -5 ± 1°C, and -18 ± 1°C. Five milliliter ampoules were used as containers for heating and storage, each containing four milliliters of inoculum. On one experimental day, bacterial suspensions with all five sodium chloride concentrations were prepared for one strain. The order in which the bacterial suspensions were prepared was determined by the use of a random numbers table. A slant of Vibrio parahaemolyticus grown on 3% sodium chloride Trypticase soy agar for 48 hr at 25°C was washed with the

designated salt concentration of Trypticase soy broth. The culture suspension was adjusted with the same concentration of sodium chloride in Trypticase soy broth to approximately 60% transmittance in a spectophotometer at 620 nm. This resulted in approximately 10^8 cells per ml. Two serial dilutions were made in the same salt concentration of Trypticase soy broth so that the final dilution contained 10^4 cells per ml. This final dilution was then dispensed aseptically to 18 sterile ampoules. The choice of the inoculum level of approximately 10,000 cells per ml was based on a reported recovery of 10,000 vibrios per gram in clams in the summer months (Food and Drug Administration, 1969).

The ampoules were sealed in a flame and then four were stored in a freezer at -18 ± 1°C, four were stored in a beaker of pre-chilled water at 5 ± 1°C and four were stored in a refrigerated bath (Forma Scientific, Model 2095) containing ethylene glycol and water and maintained at -5 ± 1°C. One ampoule from each temperature was removed at the end of 24 hr, 9 days, 16 days, and 30 days of storage and plated for recovery of the organism. Frozen ampoules were thawed quickly in a 40°C water bath.

Of the six ampoules remaining, one ampoule was used for a zero count. This ampoule was opened and plated after all low temperature samples of that sodium chloride concentration were stored and high temperature samples

were being heated. The remaining five were placed in a water bath (Thelco Model 83, Precision Scientific Company) maintained at 48 ± 1°C. One ampoule was removed at each time interval: 0.5 min, 5 min, 10 min, and 20 min. A 40-min sampling time was added for 6, 9, and 12% sodium chloride broth. Ampoules were placed in water at room temperature for cooling, opened, and then plated. Dilutions were made with 0.1% peptone water with the designated sodium chloride concentration. The sodium chloride concentration remained constant throughout the entire experiment from washing the slant until plating on 3 or 6% sodium chloride Trypticase soy agar. This was done so as to diminish osmotic effects on the cells and also because the constant salt concentration would parallel a food system. Time from washing the slant until heating was kept constant at 20 min.

The time from placing the ampoules into the heated water bath, refrigerator, refrigerated bath, and freezer until temperature equilibrium was reached was measured with a potentiometer (Brown Electronik Model No. 153X60P4-X-62F4) and termed lag time. The time for heated ampoules to reach room temperature when cooled in a water bath and the time for thawing of frozen ampoules was also measured.

Decimal reduction time (D value) is defined as the time required to destroy 90% of the cells (Stumbo, 1965).

D values were calculated for the various sodium chloride broths at 48 ± 1°C by the use of the following formula (Stumbo, 1965):

$$D = \frac{t}{\log a - \log b}$$

D = decimal reduction time
t = time of heating in minutes
a = initial number of cells
b = survivors at time t

In the calculations t was 20 min, a represented the cell count at 0.5 min and b represented the cell count at 20 min.

The statistical design consisted of a 5 x 4 factorial for the 48 ± 1°C replicated three times for each strain and a 5 x 4 x 3 factorial for the low temperature studies also replicated three times for each strain. For balance the design excluded the 40 min values. A paired t-test was used for comparison of recovery on 3% sodium chloride and 6% sodium chloride Trypticase soy agar for cells heated in 6% sodium chloride broth. Computer program *BMDO5V-General Linear Hypothesis was used for the factorial analyses and program *SIPS for the paired t-test. Least significant differences were used for within comparisons. The .05 level of significance was chosen for all tests.

Experiment II

Using aseptic technique, samples were taken from freshly killed sturgeon, black rockfish, and ling cod

and were frozen prior to the preparation of the fish homogenate. At the time of sampling one part of fish by weight was diluted with four parts of 3% sodium chloride solution and blended three minutes at low speed in an electric blender. The homogenate was then plated, using the pour plate technique, in plate count agar and 3% sodium chloride Trypticase soy agar. One plate of each medium was incubated at 37°C and at room temperature. The samples were considered to be usable if less than five colonies appeared on the 3% sodium chloride Trypticase soy agar plate and less than ten on the plate count agar plate after 24 hours incubation at both temperatures.

The fish homogenate used in the experimental series was prepared with one part fish to four parts of the designated sodium chloride solution and blended for three minutes at low speed in an electric blender. One batch of each sodium chloride concentration (0, 3, 6, 9, and 12%) was prepared. The fish consisted of two parts sturgeon, one part black rockfish, and one part ling cod. One hundred gram portions of the homogenate were frozen in plastic bags and on the day prior to the experiment one bag of each sodium chloride concentration was removed and emptied into a sterile quart jar and then placed in the refrigerator maintained at 5 $\pm$ 1°C to thaw. On the day of experimentation, 100 grams of the designated sterile sodium chloride solution was added to each jar to obtain

a final dilution of one part of fish to nine parts of solution. This dilution was necessary for ease of pipeting and thus greater accuracy in sampling.

The inoculum was prepared as in Experiment I. After adjusting to 60% transmittance, two milliliters of a 10^4 dilution were added to the fish homogenate to obtain a final inoculum level of approximately 10,000 cells per gram of homogenate. The homogenate was then blended for three minutes at low speed in an electric blender to insure even inoculation and dispensed in five-milliliter portions to sterile ampoules. Heating and storage were carried out under the same conditions as in Experiment I, except that a 40-min sample was added for all strains at all sodium chloride concentrations except zero. An 80-min sampling time was added to the heated series for strains SB04-422 and 17802 for all sodium chloride concentrations except zero. Time from washing the slant until heating was kept constant at 25 min. In addition to the 3% sodium chloride Trypticase soy agar, Colwell's maintenance medium (Colwell et al., 1968) was used for plating.

Lag times and D values were done as in Experiment I.

The statistical analysis was the same as Experiment I except that in Experiment II there were two replications instead of three. The 5 x 4 x 3 factorial for Experiment II became a 5 x 4 x 2 factorial. For balance in the fish homogenate analysis at 48 $\pm$ 1°C, 40 and 80 min values

were excluded from the design. Program *ANOVA4 was used for analysis of the survival of Vibrio parahaemolyticus in fish homogenate at 48 ± 1°C since these data were complete. Paired t-tests were also used for comparison of cell recovery on 3% sodium chloride Trypticase soy agar and Colwell's Vibrio maintenance medium. Logarithmic transformations of viable cells per ml were used in all calculations.

RESULTS AND DISCUSSION

The purpose of this study was to provide information concerning the survival of Vibrio parahaemolyticus during heating, refrigeration, and freezing. There were two main objectives: 1. To determine the survival of Vibrio parahaemolyticus over a period of time in Trypticase soy broth (a complex laboratory medium) and in fish homogenate during heating, refrigeration, and freezing. 2. To determine the effect of sodium chloride concentration on the survival of Vibrio parahaemolyticus in Trypticase soy broth (a complex laboratory medium) and fish homogenate during heating, refrigeration, and freezing.

The temperature, sodium chloride concentration, and the system the organism was suspended in were thought to be of primary concern.

Temperature

Most seafoods consumed in the United States are at least slightly cooked, so it was desirable to study the survival of Vibrio parahaemolyticus during heating. On the basis of preliminary studies, 48 ± 1°C was chosen as the experimental temperature at which studies of the effects of sodium chloride concentrations on Vibrio parahaemolyticus during heating were done.

Foods to be served and food samples for microbiological examination are often refrigerated and so it was

desirable to obtain information on the survival of the organism under these conditions. A temperature of 5 ± 1°C was chosen so that the organism would not grow during storage.

A temperature just below the freezing point of water was desirable to determine the resistance of Vibrio parahaemolyticus to low temperatures with varying amounts of sodium chloride. At -5 ± 1°C the samples should freeze; however, an occasional sample (one out of 20 and usually 0% sodium chloride samples) was frozen and the rest experienced supercooling.

Samples were also stored at -18 ± 1°C to study the effects of freezing on the viability of Vibrio parahaemolyticus in varying sodium chloride concentrations. This was done in an effort to learn about the survival of the organism in the frozen state which might have implications for storage recommendations for food samples from food poisoning outbreaks.

Sodium Chloride Concentration

It was originally thought by the experimenter that as the sodium chloride concentration increased so would the survival of Vibrio parahaemolyticus. According to Stumbo (1965), low concentrations of sodium chloride tended to increase the temperature resistance of microorganisms. Since Vibrio parahaemolyticus is a halophilic organism

it was thought perhaps even higher sodium chloride concentrations would increase the temperature resistance of this organism. Sodium chloride concentrations were thus chosen to cover a wide range. A series with no sodium chloride which would not support growth was used as well as 3, 6, and 9% sodium chloride concentrations in which the organism would usually grow, 3% sodium chloride being optimum for growth. The 12% sodium chloride concentration was chosen as one in which the organism would not normally grow, but which perhaps would be protective to the cells.

Systems

A basic system to study the effect of temperature and sodium chloride concentration on the survival of Vibrio parahaemolyticus was desired so that findings could be attributed to temperature, sodium chloride concentration, or to an interaction of the two. For comparison purposes with the work of others, including Liston and his co-workers (1967), it was necessary to use a basic system. For these reasons, Trypticase soy broth with varying sodium chloride concentrations was chosen for one experiment.

Since Vibrio parahaemolyticus is involved in food poisoning outbreaks from sea foods, it seemed desirable to study the conditions for survival of the organism in a sea food system as well. Raw fish, prepared to have a minimum number of contaminating microorganisms, was chosen as

opposed to heat sterilized fish because it more nearly simulated the actual situation prior to an outbreak. In addition it was thought that substances present in the fish might be more protective to the organism in the raw state than after heat treatment.

Strains

Three strains of Vibrio parahaemolyticus were chosen for these studies because the original intent of the experiment was not to look at strain differences. Little work has been done on variability of the organism and Zen-Yoji et al. (1970) reported in general there was not a great variability in biochemical characteristics among the 52 serotypes. Strains T-3765-1, SB04-422, and ATCC 17802 were chosen for several reasons. This group represented both Japanese and American cultures of the organism, a culture from food as well as two from food poisoning patients, and a culture carried for a period of time in the laboratory as well as recently isolated cultures. (See Table 1, p. 11.)

Experiment I

This experiment was concerned with the effect of temperature and sodium chloride concentration on the survival of Vibrio parahaemolyticus in Trypticase soy broth.

Lag times are reported for the heated samples so

that one knows when 48 ± 1°C was reached. The lag times for refrigeration and for the freezer give an indication of the cooling rate. Lag time for Trypticase soy broth in the heated water bath was 1.3 min, in the refrigerator 2.7 min, and in the refrigerated bath 3.3 min. In the freezer there was a rapid decrease to below zero in ten minutes and then a slow cooling curve was observed until 68 min after being stored, when the temperature reached -15°C. A very slow cooling curve continued until 106 min after storage, when the temperature stabilized at -18°C. The time after removal from the heated bath until room temperature was reached by cooling in water was 1.3 min. Thawing of the frozen ampoules in a 40°C water bath required 2.7 min.

The analyses of variance for the effect of heating at 48 ± 1°C on the survival of Vibrio parahaemolyticus in Trypticase soy broth with varying levels of sodium chloride are given in Table 2. The level of sodium chloride and the sampling times were statistically significant at the .05 level for all three strains. For strains T-3765-1 and 17802, the sodium chloride x sampling time interaction was significant and in both cases subdividing revealed that the 0% sodium chloride x time interaction was significant.

Table 2. Statistical Analysis of the Effect of Heating at 48 ± 1°C on the Survival of Vibrio parahaemolyticus in Trypticase Soy Broth with Varying Levels of Sodium Chloride.

Source of Variation	Degrees of Freedom	Mean Square[a]	F Value[b]
Strain T-3765-1			
Replications	2	.08	.82
NaCl	4	10.54	104.70*
Time	3	2.65	26.31*
NaCl x Time	12	.57	5.65*
0% NaCl x Time	3	1.18	11.73*
3% NaCl x Time	3	.19	1.89
6% NaCl x Time	3	.50	4.98
9% NaCl x Time	3	.40	4.01
Error	34	.10	
Strain SB04-422			
Replications	2	1.42	4.82
NaCl	4	11.01	37.40*
Time	3	2.92	9.92*
NaCl x Time	12	.22	.75
0% NaCl x Time	3	.48	1.62
3% NaCl x Time	3	.02	.08
6% NaCl x Time	3	.36	1.24
9% NaCl x Time	3	.02	.06
Error	38	.29	
Strain 17802			
Replications	2	.03	.15
NaCl	4	17.12	90.13*
Time	3	7.00	36.86*
NaCl x Time	12	.67	3.55*
0% NaCl x Time	3	2.24	11.82*
3% NaCl x Time	3	.30	1.58
6% NaCl x Time	3	.05	.27
9% NaCl x Time	3	.10	.53
Error	33	.19	

a. Logarithmic transformations of viable cells per ml.

b. * indicates significance at the .05 level.

Points on the figures are averages from three replications except where data are missing (Appendix). Sampling times were 0.5, 5, 10, and 20 min for all sodium chloride concentrations plus 40 min sampling time for 6, 9, and 12% sodium chloride broths. Averages were the best way to illustrate the data, but considerable variability existed around each point, especially at 0% sodium chloride concentration. For example, ten minutes of heating in 9% sodium chloride broth resulted in counts of survivors of $16x10^3$, $49x10^2$, and $28x10^2$. Counts of survivors in 6% sodium chloride broth had the least variability of all sodium chloride concentrations, especially during heating. Thus 6% sodium chloride appeared to be best for cell stability. (Data for all replications can be found in Appendix Tables 1-5.)

Figure 1 illustrates the survival of Vibrio parahaemolyticus T-3765-1, a strain isolated from a Japanese food poisoning patient, in Trypticase soy broth at 48 ± 1°C. The initial cell count was approximately 10,000 cells per ml. However, at 0% sodium chloride the organism was rapidly killed due to effects other than heating. The very sharp drop in survivors for 0% sodium chloride broth should be noted throughout the experiment. This also resulted in often rather erratic results. Least significant differences of sodium chloride concentrations for all strains at 48 ± 1°C confirm that survival in 0% sodium

chloride broth is significantly different from survival in all other sodium chloride broths. From Figure 1, broths with 6 and 9% sodium chloride appear to be optimum for survival of this strain. Least significant differences (LSD tests) supported this observation and showed no statistically significant difference between the survival in 6 and 9% sodium chloride broths. The D values in Table 3 are also much greater for 6 and 9% broths for T-3765-1.

Table 3. Decimal Reduction Time Values in Minutes for Vibrio parahaemolyticus at 48 ± 1°C (D_{48}).

Medium	Sodium Chloride Concentration	Strains T-3765-1	SB04-422	17802
	%			
Broth[a]	0	8.51[b]	13.8	7.35
	3	13.4	23.0	13.2
	6	118	64.5	11.9[b]
	9	74.1	17.5	15.6
	12	25.3	20.0	13.8
Fish[b]	0	95.2	15.6	28.6
	3	90.9	143	31.2
	6	33.9	400	41.7
	9	57.1	222	16.8
	12	50.0	23.8	16.8

a. All are based on three replications except those footnoted b.

b. All are based on two replications.

For SB04-422, a strain of Vibrio parahaemolyticus isolated from oysters in the United States, 6% sodium chloride broth is also optimum for survival at 48 ± 1°C as shown in Figure 2. The D value in Table 3 is also largest for 6% broth.

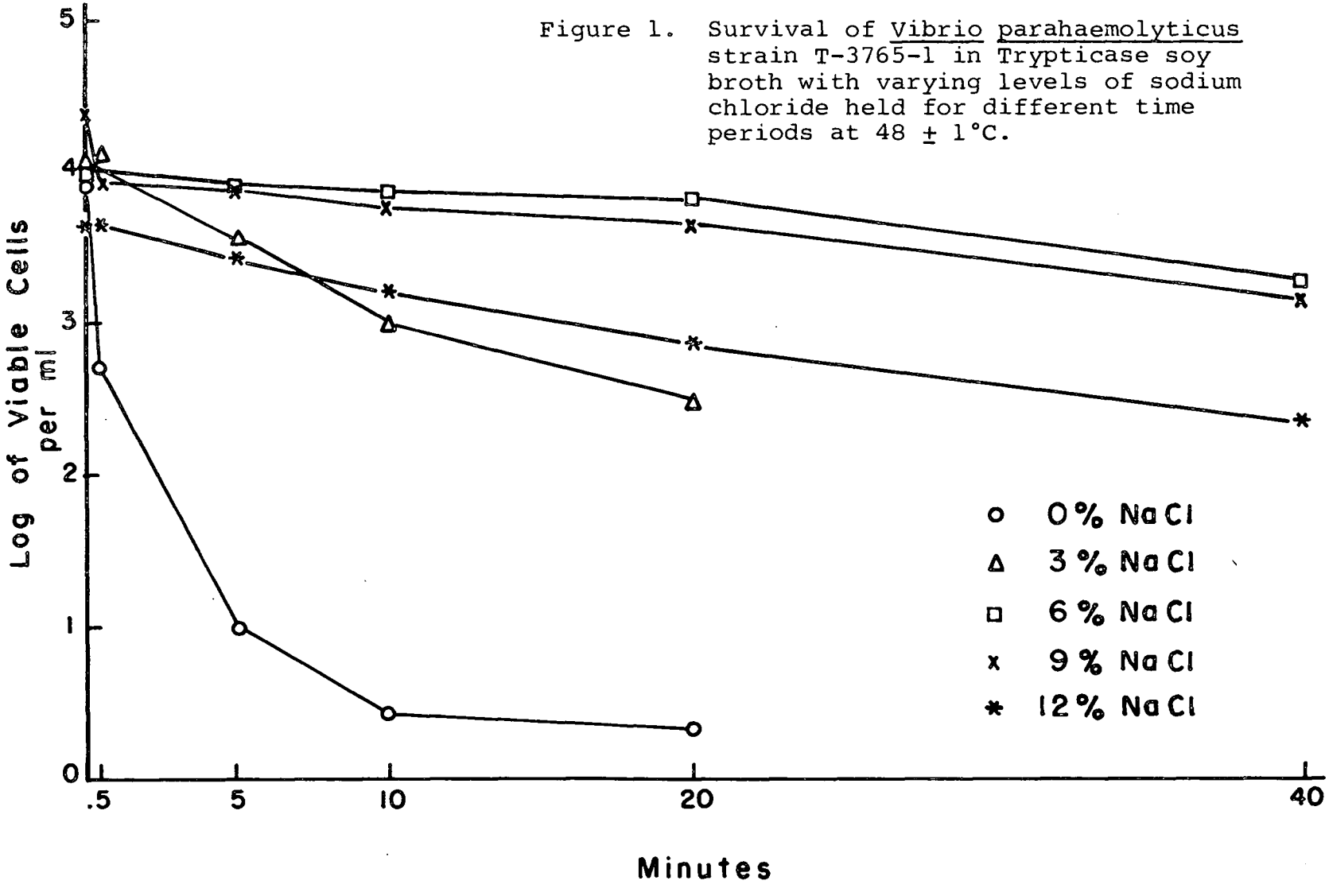

Figure 1. Survival of Vibrio parahaemolyticus strain T-3765-1 in Trypticase soy broth with varying levels of sodium chloride held for different time periods at 48 ± 1°C.

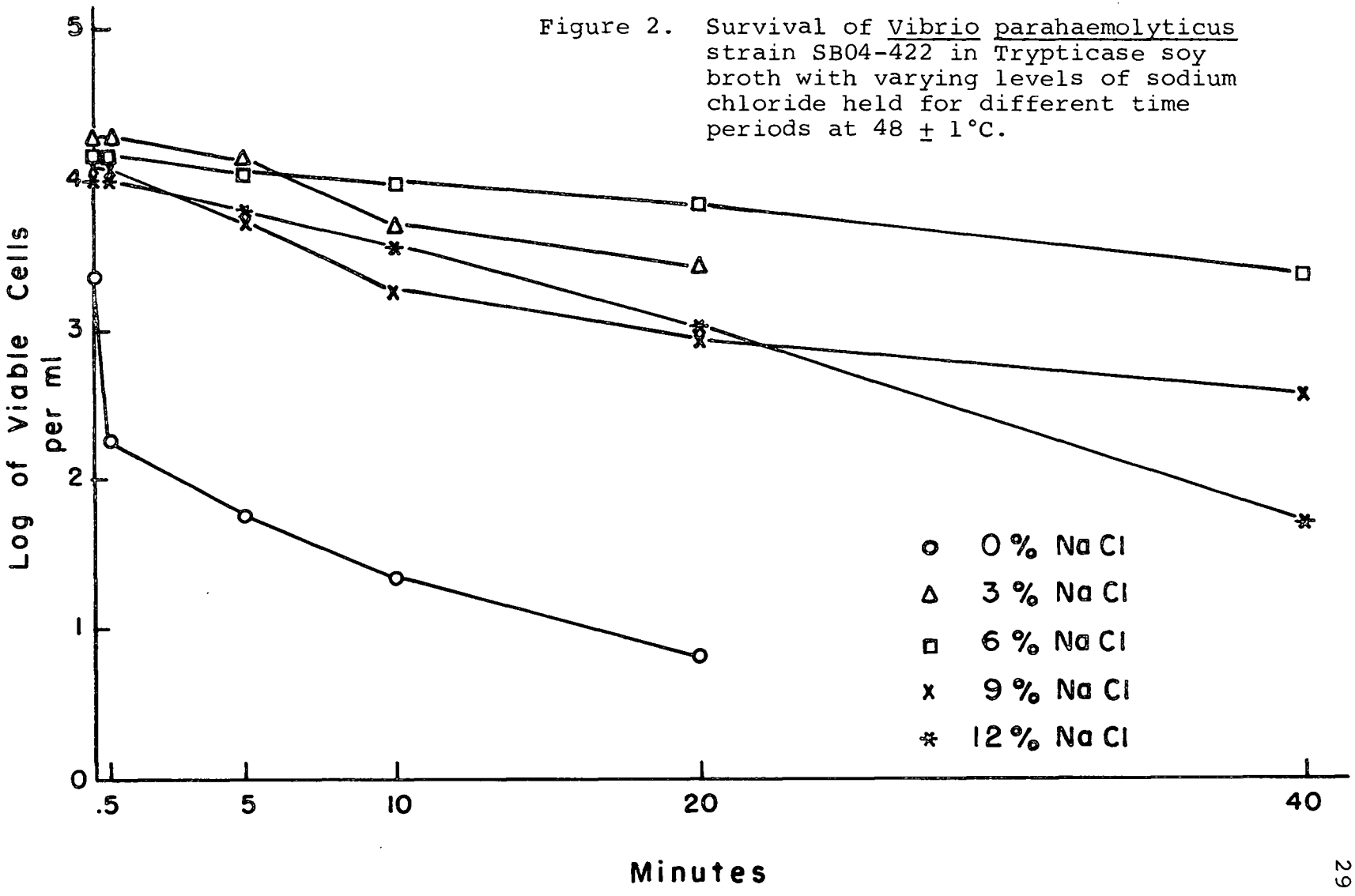

Figure 2. Survival of Vibrio parahaemolyticus strain SB04-422 in Trypticase soy broth with varying levels of sodium chloride held for different time periods at 48 ± 1°C.

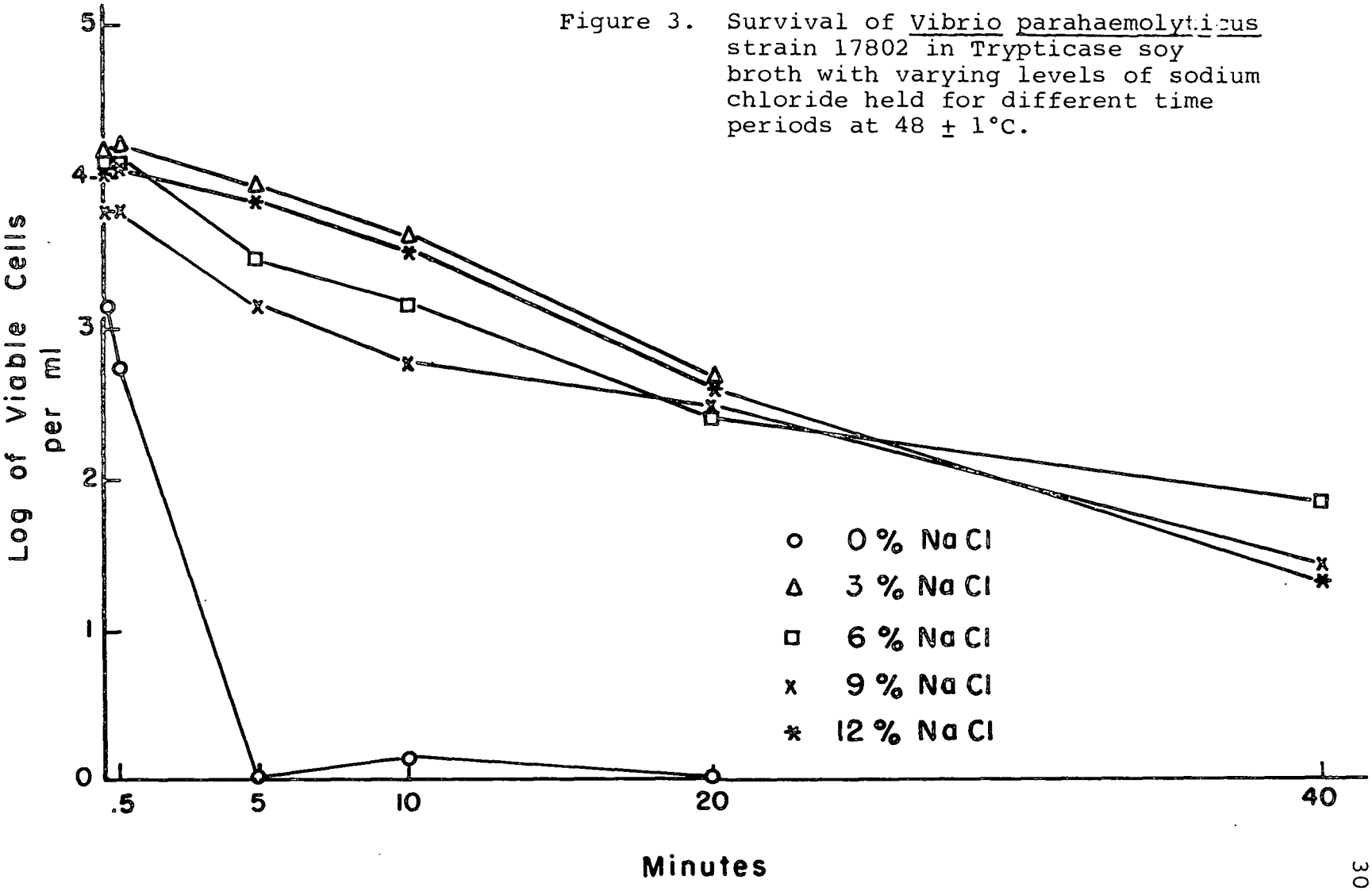

Figure 3. Survival of Vibrio parahaemolyticus strain 17802 in Trypticase soy broth with varying levels of sodium chloride held for different time periods at 48 ± 1°C.

Survival in 6% sodium chloride broth is significantly different from all other sodium chloride concentrations except 3% (LSD tests).

Figure 3 illustrates the greater sensitivity of 17802, a strain of Vibrio parahaemolyticus isolated from one of the first Japanese food poisoning outbreaks, to heating at 48 ± 1°C at all sodium chloride concentrations. Broths of 3 and 12% sodium chloride were significantly protective to this strain during heating at 48 ± 1°C. This difference does not appear in the D values.

Studies of heating Vibrio parahaemolyticus at 48 ± 1°C in Trypticase soy broth with varying amounts of sodium chloride indicated that 6 and 9% sodium chloride Trypticase soy broths gave significantly higher recoveries of strain T-3765-1, while 6% sodium chloride broth was most protective for strain SB04-422. This is shown in Figures 1 and 2 and is also apparent from the D values in Table 3. Survival in 3 and 12% sodium chloride broth was significantly higher for strain 17802. Plating on 3% sodium chloride Trypticase soy agar gave significantly (.05 level) higher counts for recovery of cells heated in 6% sodium chloride broth than did plating on 6% sodium chloride Trypticase soy agar. The sodium chloride level for the injured cells appears to be important.

Analyses of variance for the effect of 5 ± 1, -5 ± 1, and -18 ± 1°C on the survival of each of the three strains

of Vibrio parahaemolyticus in Trypticase soy broth with varying levels of sodium chloride are given in Table 4. Effects of sampling times and sodium chloride concentrations were statistically significant for all three strains. There was a significant difference in survival due to storage temperatures for strain T-3765-1. LSD tests showed survival to be significantly lower at -5 ± 1°C for this strain. A 5°C x sodium chloride interaction was significant only for strain 17802.

Survival of Vibrio parahaemolyticus in Trypticase soy broth at 5 ± 1°C is illustrated in Figures 4-6. A trend for better survival of strain T-3765-1 in 6 and 9% broths for nine days can be seen in Figure 4. LSD tests showed that 0 and 3% sodium chloride concentrations had significantly lower counts of survivors. None of the sodium chloride concentrations effectively stabilized survival since samples of all concentrations at 30 days had counts lower than 30 organisms per ml (log less than 1.5). Figure 5 illustrates that strain SB04-422 tended to survive better with time in 9 and 12% sodium chloride broths. Broths of 0 and 3% sodium chloride again had significantly lower numbers of survivors as evidenced by LSD tests. However, after 30 days of storage these samples contained only 30 organisms per ml. The sensitivity of strain 17802 is again illustrated in Figure 6. The sharp slopes for all but 3% sodium chloride broth for nine days at 5 ± 1°C

Table 4. Statistical Analysis of the Effect of 5 ± 1°C, -5 ± 1°C, and -18 ± 1°C on the Survival of Vibrio parahaemolyticus in Trypticase Soy Broth with Varying Levels of Sodium Chloride.

Source of Variation	Degrees of Freedom	Mean Square[a]	F Value[b]
Strain T-3765-1			
Replications	2	1.01	2.67
Temperature	2	7.20	19.10*
Time	3	36.75	97.51*
NaCl	4	17.86	47.39*
5°C x Time	3	1.59	4.21
5°C x NaCl	4	1.06	2.81
-5°C x Time	3	.41	1.09
-5°C x NaCl	4	.31	.82
Error	151	.38	
Strain SB04-422			
Replications	2	.20	.20
Temperature	2	.98	1.00
Time	3	48.98	49.58*
NaCl	4	22.24	22.51*
5°C x Time	3	1.11	1.12
5°C x NaCl[c]			
-5°C x Time[c]			
-5°C x NaCl[c]			
Error	162	.99	
Strain 17802			
Replications	2	.75	1.63
Temperature	2	1.23	2.66
Time	3	60.07	130.32*
NaCl	4	5.21	11.29*
5°C x Time	3	1.00	2.17
5°C x NaCl	4	1.95	4.23*
-5°C x Time	3	1.50	3.24
-5°C x NaCl	4	.75	1.62
Error	141	.46	

a. Logarithmic transformations of viable cells per ml.

b. * indicates significance at the .05 level.

c. Not computed.

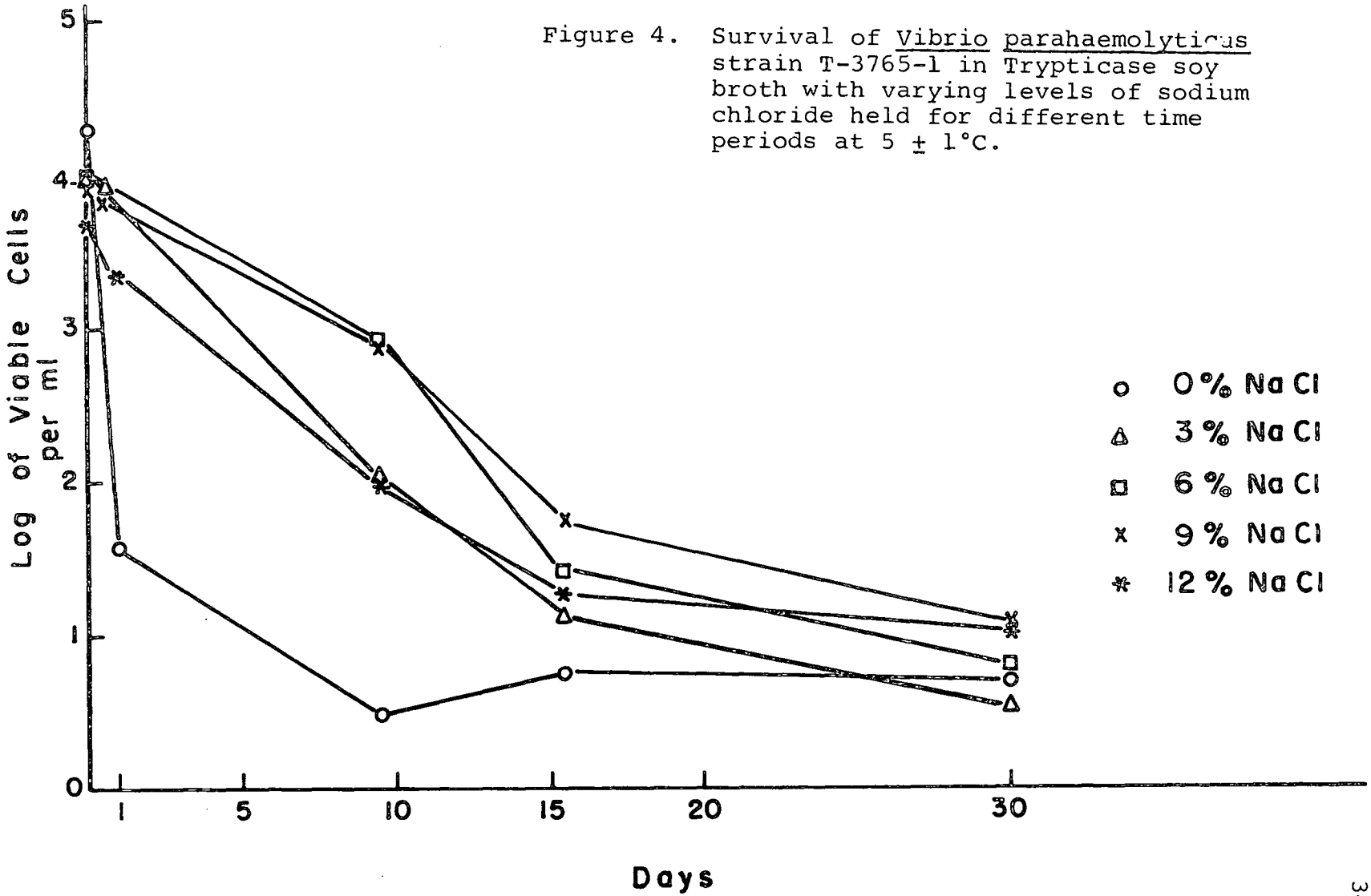

Figure 4. Survival of Vibrio parahaemolyticus strain T-3765-1 in Trypticase soy broth with varying levels of sodium chloride held for different time periods at 5 ± 1°C.

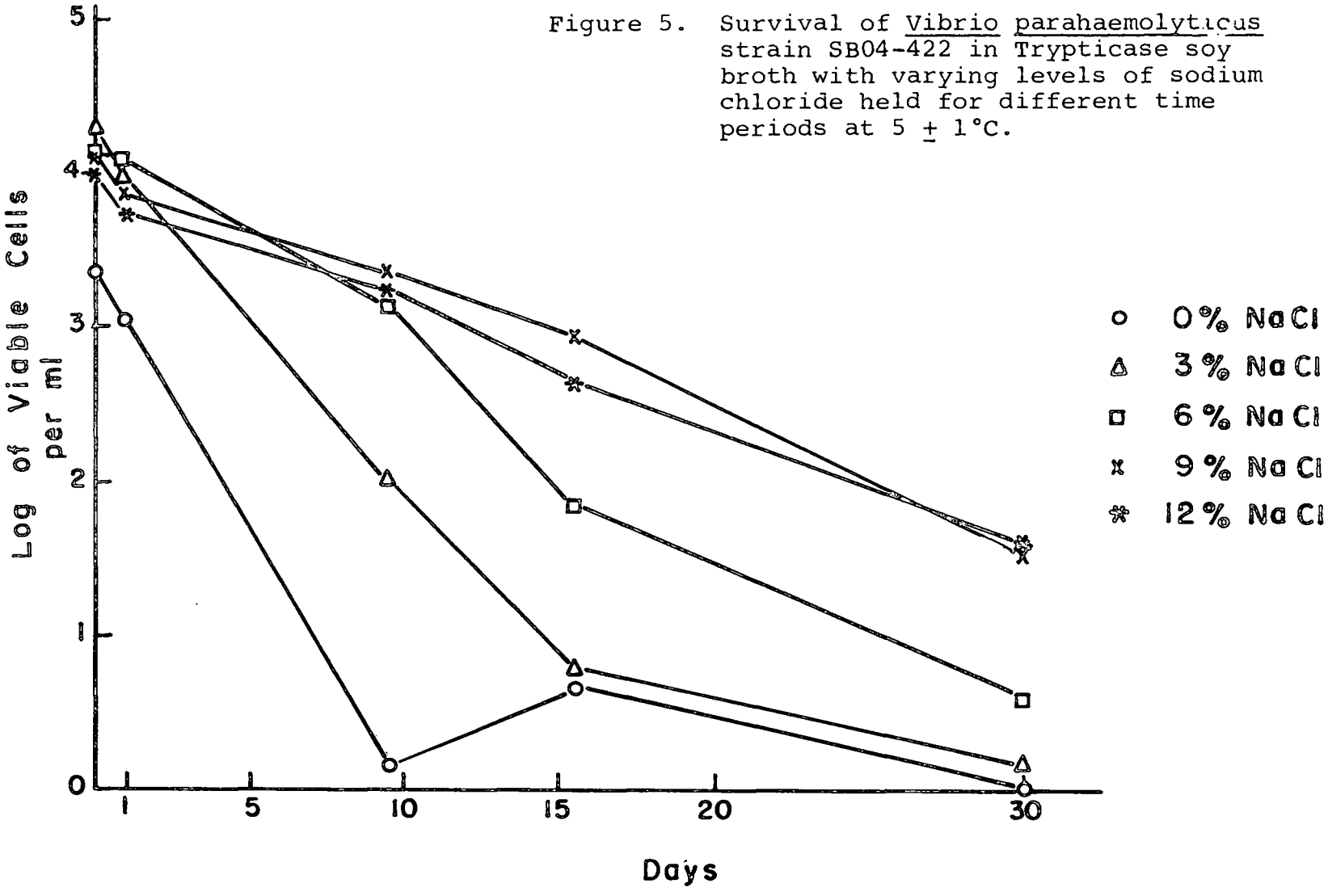

Figure 5. Survival of Vibrio parahaemolyticus strain SB04-422 in Trypticase soy broth with varying levels of sodium chloride held for different time periods at 5 ± 1°C.

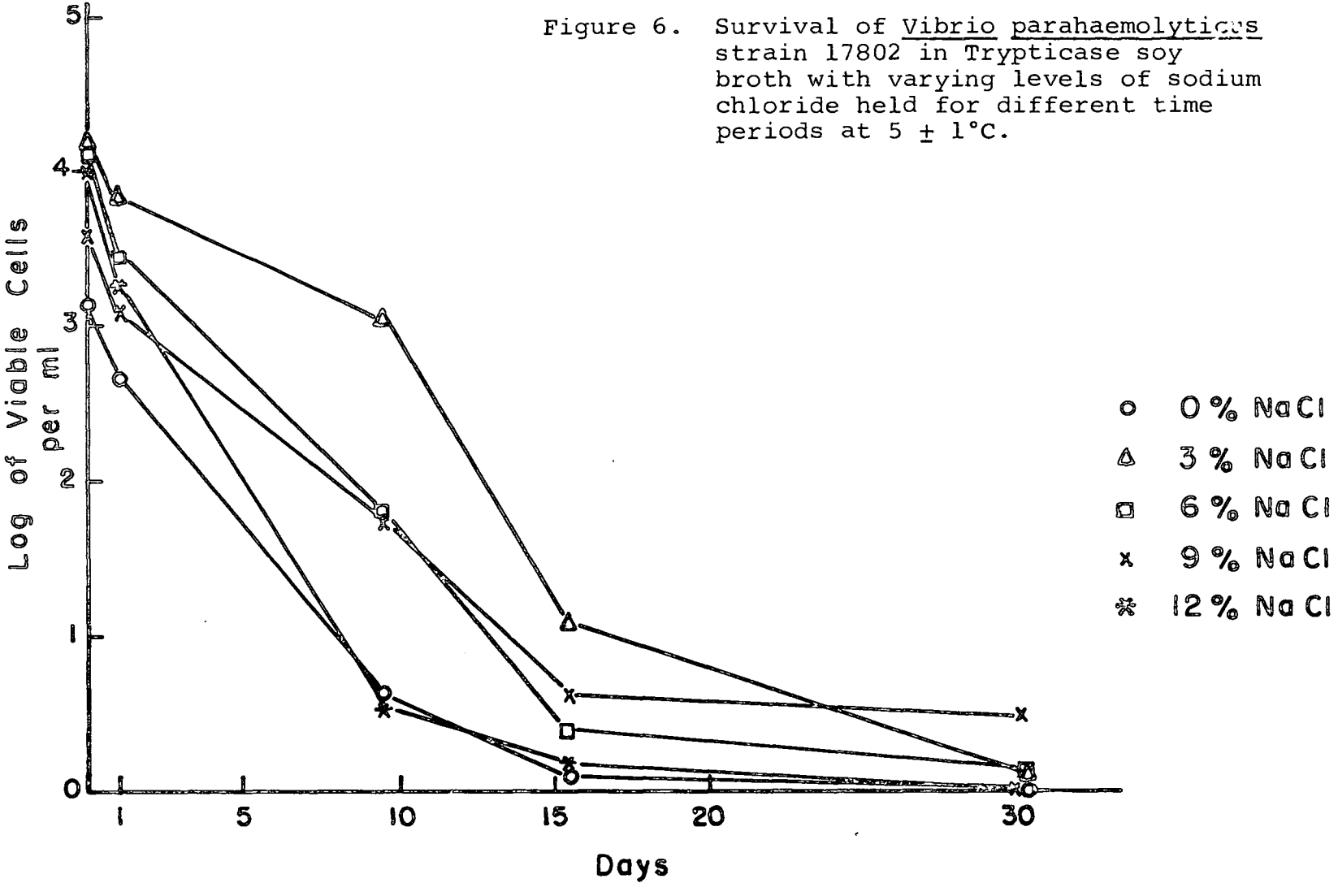

Figure 6. Survival of Vibrio parahaemolyticus strain 17802 in Trypticase soy broth with varying levels of sodium chloride held for different time periods at 5 ± 1°C.

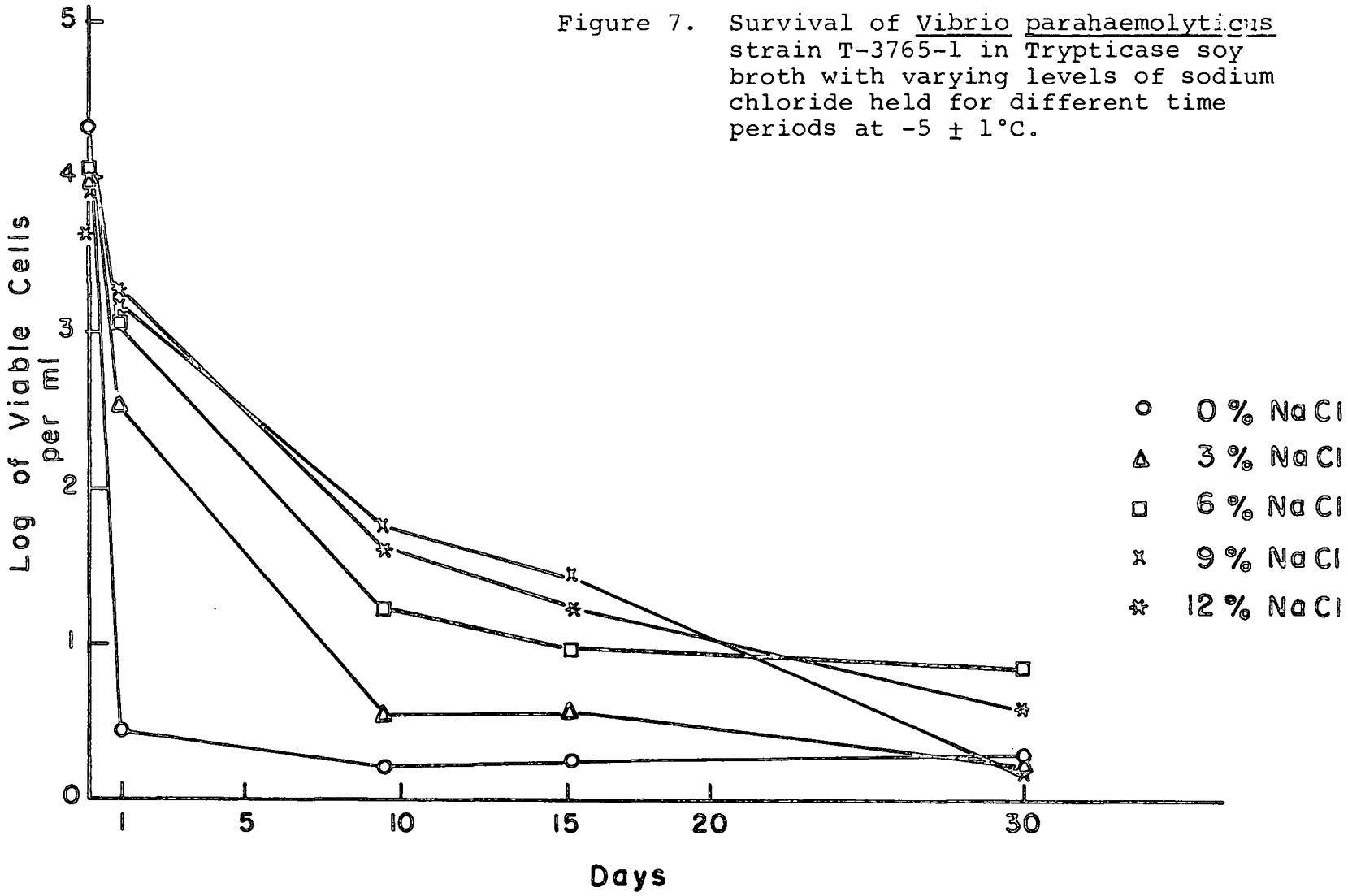

Figure 7. Survival of Vibrio parahaemolyticus strain T-3765-1 in Trypticase soy broth with varying levels of sodium chloride held for different time periods at -5 ± 1°C.

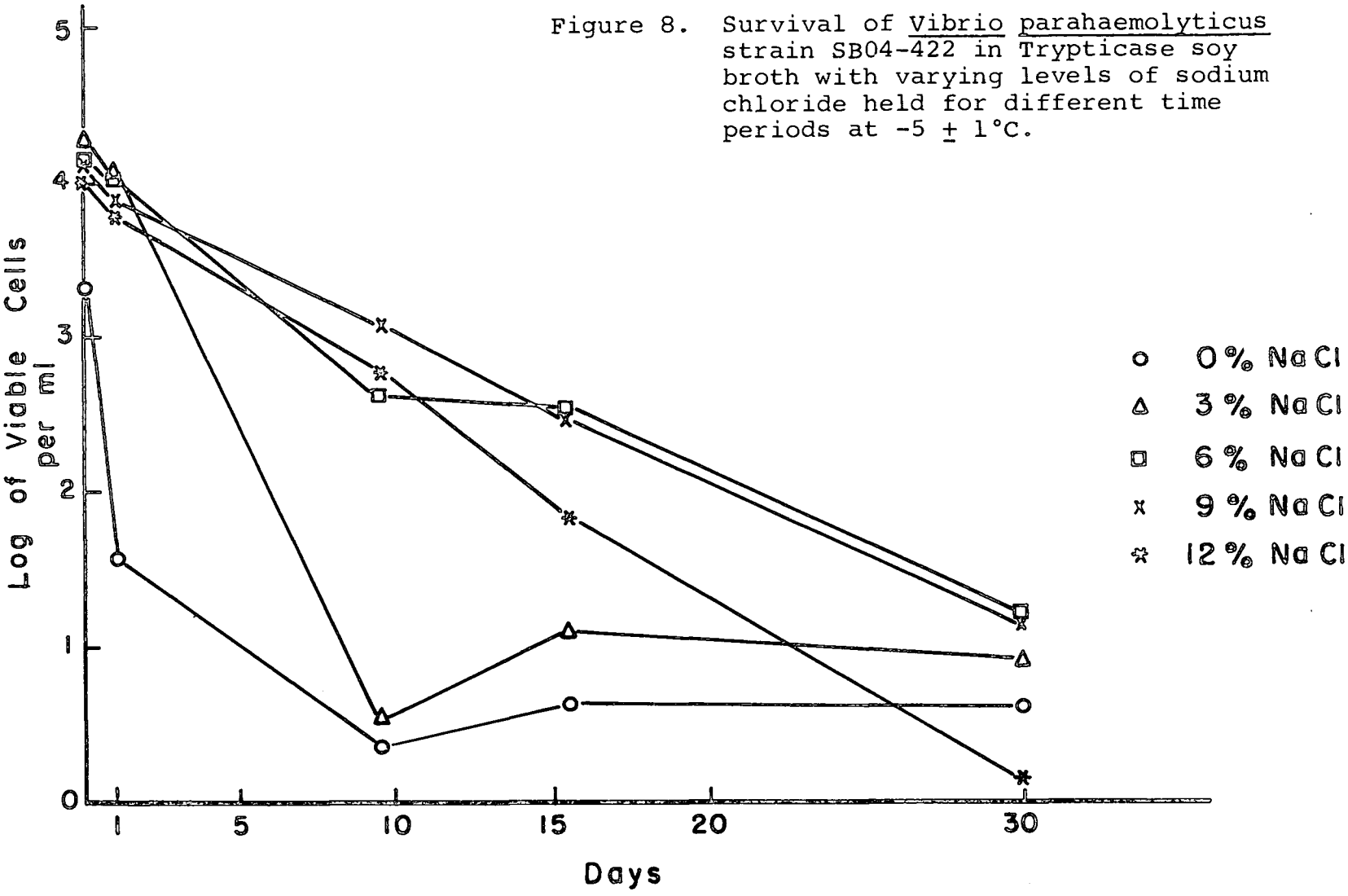

Figure 8. Survival of Vibrio parahaemolyticus strain SB04-422 in Trypticase soy broth with varying levels of sodium chloride held for different time periods at -5 ± 1°C.

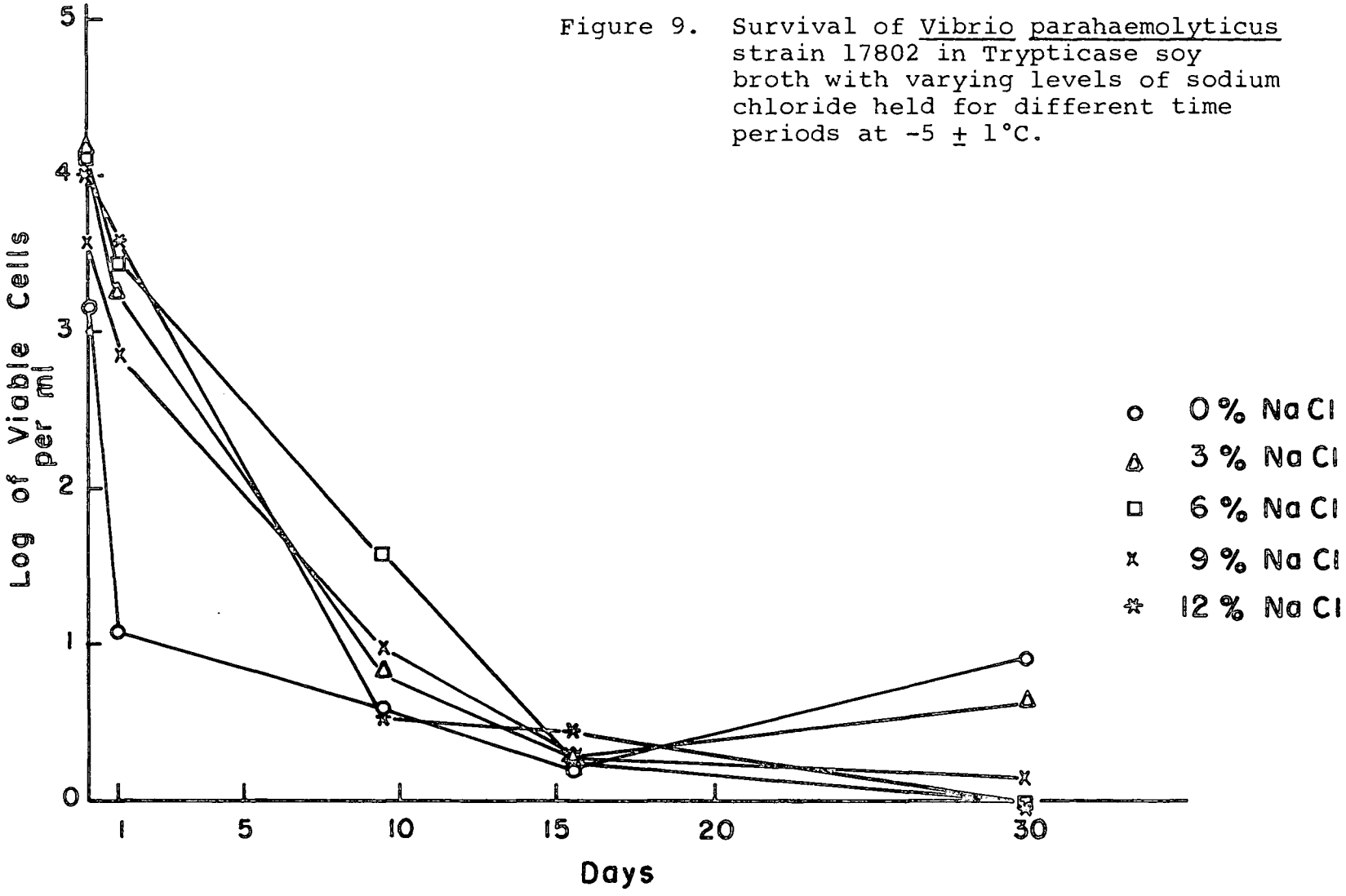

Figure 9. Survival of Vibrio parahaemolyticus strain 17802 in Trypticase soy broth with varying levels of sodium chloride held for different time periods at -5 ± 1°C.

are quite apparent when compared with Figure 5 for strain SB04-422. Broths with 0% sodium chloride gave significantly lower recoveries for strain 17802. From these data, it appears that refrigeration at 5°C will greatly reduce the numbers of Vibrio parahaemolyticus in a sample.

Figures 7-9 illustrate the survival of Vibrio parahaemolyticus at -5 ± 1°C. For one strain, of the low temperatures tested, -5 ± 1°C appeared to be the most lethal to the organism regardless of sodium chloride concentration. For strain T-3765-1 survival at -5 ± 1°C was significantly lower than survival at 5 ± 1 and -18 ± 1°C. This finding is similar to that of Asakawa (1967) who found that -10°C caused a much greater rate of decline in numbers of organisms than -20°C. Comparisons can not be made with the findings of Liston et al. (1967) who studied survival at -10°C and -24°C in phosphate buffer because of the lack of detail in their report. Figure 7 indicates that 9 and 12% sodium chloride broths were slightly better for survival of this strain than the other sodium chloride concentrations up through 16 days of storage. Beyond 16 days of storage, sodium chloride appears not to be protective. The only significantly lower counts were at 0 and 3% sodium chloride concentrations on the basis of LSD tests. SB04-422 seemed the most resistant of the strains tested at -5 ± 1°C (Figure 8). The sensitivity of strain 17802 can be seen from Figure 9. Counts were 30 cells

per ml or less after only nine days of storage.

Comparison of Figures 10-12 on the survival of Vibrio parahaemolyticus in broth at -18 ± 1°C again illustrates the sensitivity of strain 17802. For two strains, 6, 9, and 12% sodium chloride Trypticase soy broths gave greater survival (.05 level) than did lower sodium chloride concentrations. For strain 17802 only 12% sodium chloride broth appeared to be somewhat protective to the cells through 16 days of storage (Figure 12). Temmyo's (1966) studies at -18°C showed that sodium chloride concentration in saurel extracts could enhance the survival of Vibrio parahaemolyticus during low temperature storage.

The greatest protective effect on Vibrio parahaemolyticus cells due to sodium chloride concentration appeared to be at 48 ± 1°C. Refrigeration and freezing reduced the number of organisms per milliliter regardless of the sodium chloride concentration, although in the presence of sodium chloride viable cells still remained at the end of the sampling time.

Experiment II

This experiment was concerned with the effect of temperature and sodium chloride concentration on the survival of Vibrio parahaemolyticus in fish homogenate.

The time from placing the ampoules in the heated water bath (48 ± 1°C), refrigerated bath (-5 ± 1°C), and

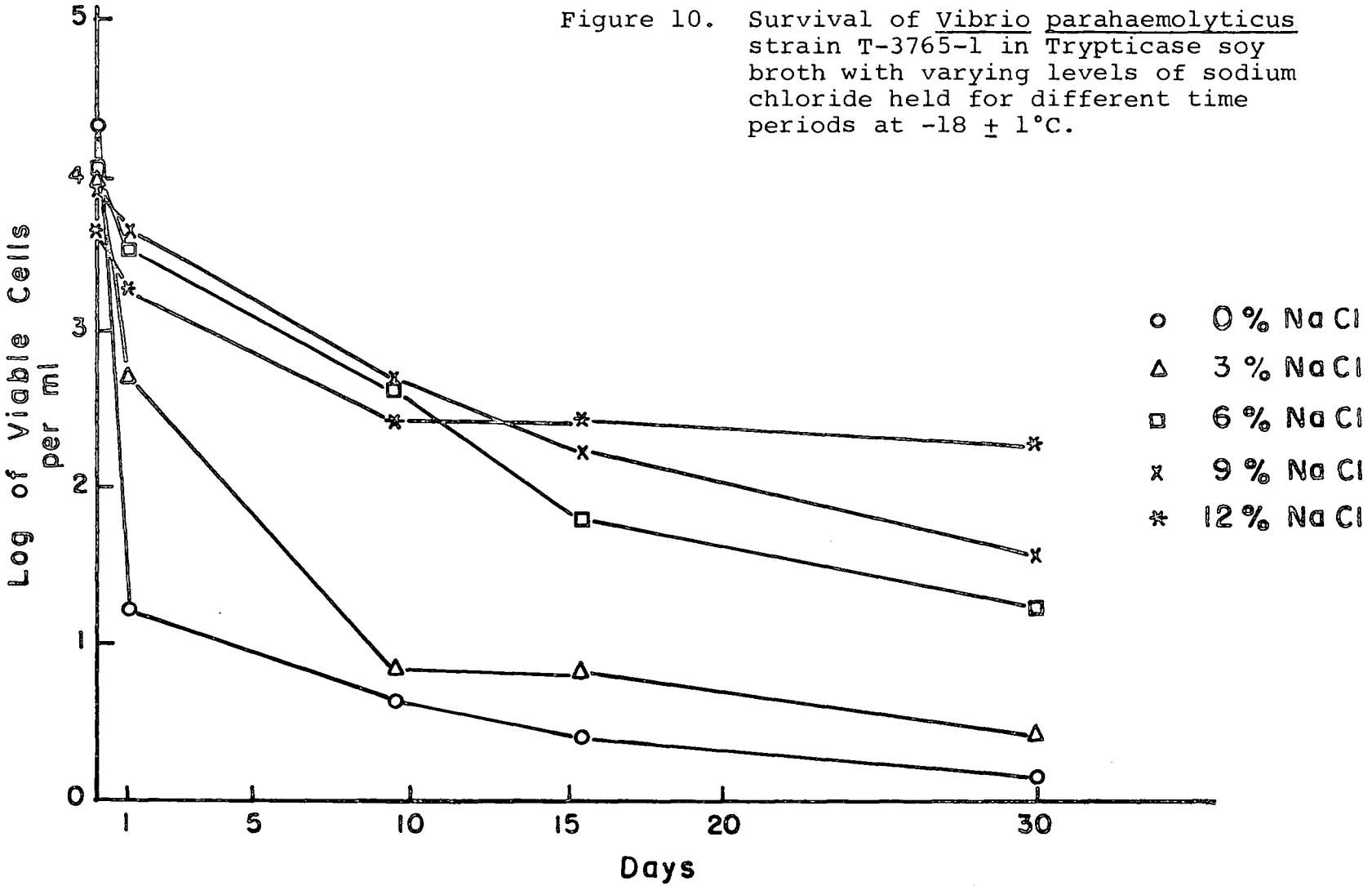

Figure 10. Survival of Vibrio parahaemolyticus strain T-3765-1 in Trypticase soy broth with varying levels of sodium chloride held for different time periods at -18 ± 1°C.

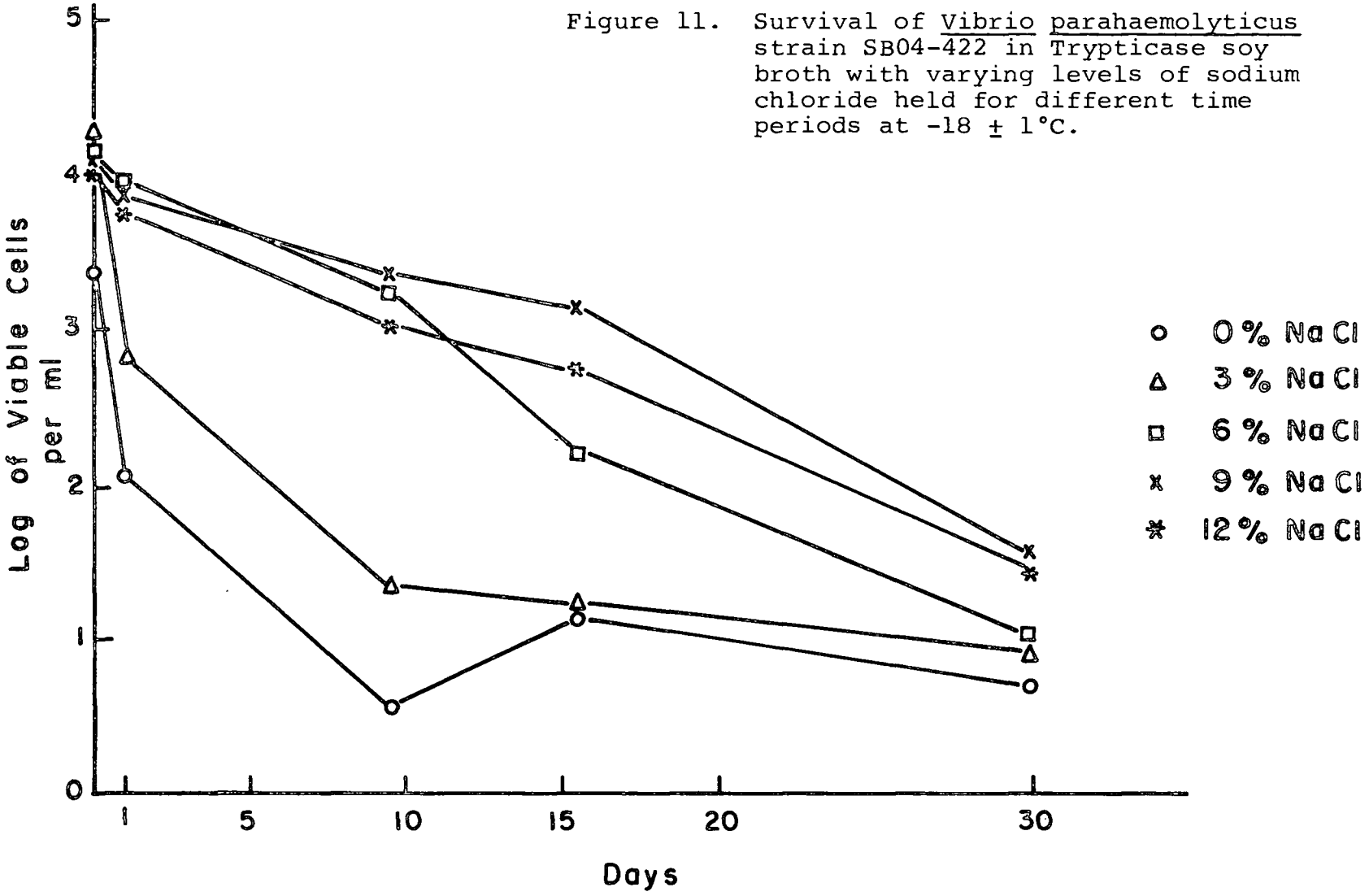

Figure 11. Survival of Vibrio parahaemolyticus strain SB04-422 in Trypticase soy broth with varying levels of sodium chloride held for different time periods at -18 ± 1°C.

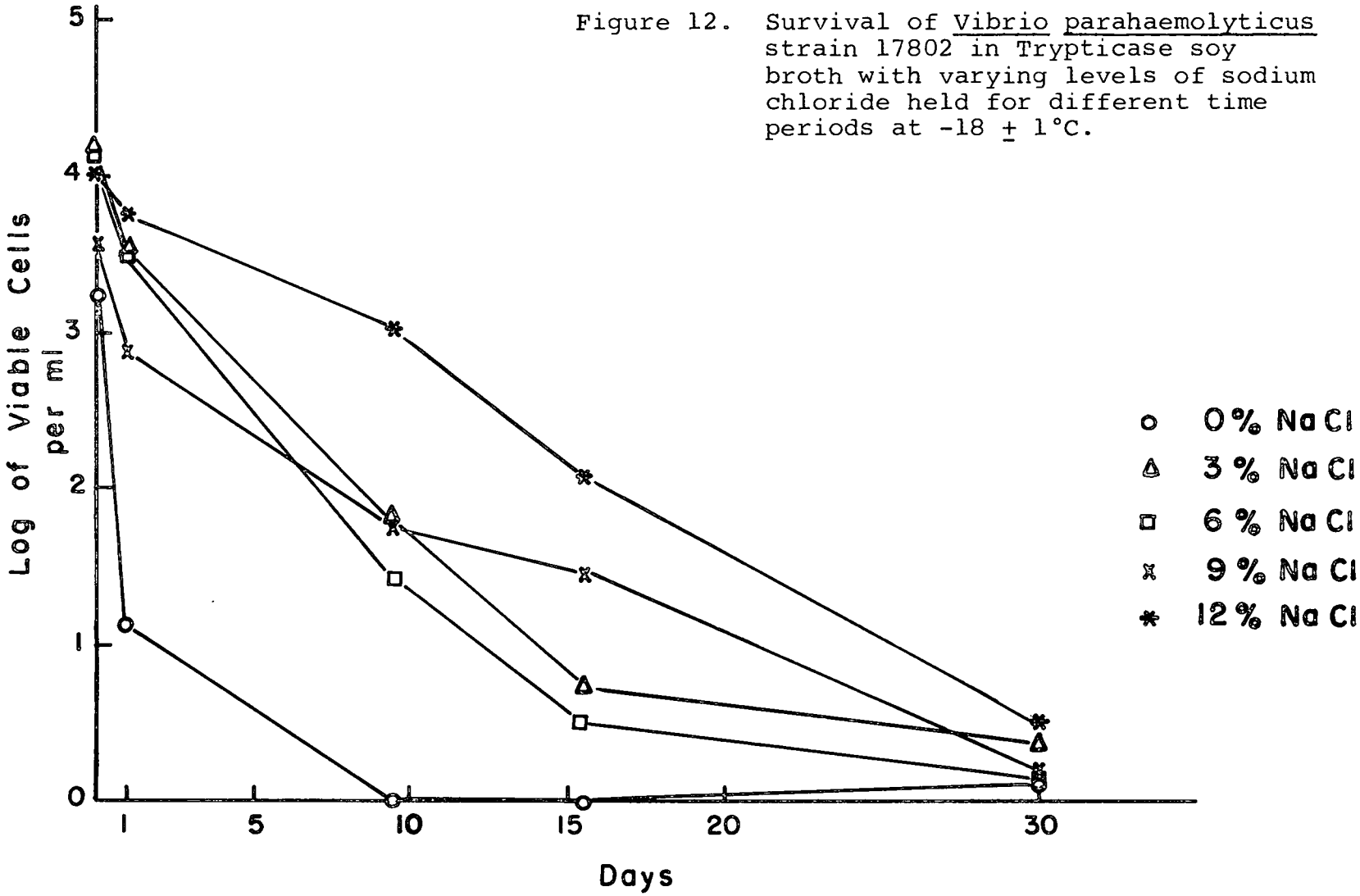

Figure 12. Survival of Vibrio parahaemolyticus strain 17802 in Trypticase soy broth with varying levels of sodium chloride held for different time periods at -18 ± 1°C.

freezer (-18 ± 1°C) until the desired temperature was reached, was again measured and termed lag time. Lag time at 48 ± 1°C was four minutes at all sodium chloride concentrations. This means that effective heating of the samples began at four minutes after the ampoules were placed in the water bath. At -5 ± 1°C, lag time ranged from 3.6 min at 0% sodium chloride concentration to 4.7 min at 6% sodium chloride where it then leveled off and remained the same for fish homogenates with 9 and 12% sodium chloride. In the freezer at -18 ± 1°C, -3°C was reached in ten minutes. A slow cooling curve to near the freezer temperature occurred between 63 and 80 min depending on the sodium chloride concentration (higher sodium chloride concentrations required longer times). Finally, a very slow cooling curve was observed until equilibrium was reached at 108 min. In general, lag times for the fish homogenate were longer than those for the broth. Frozen ampoules required 2.7 min on the average to reach room temperature when thawed in a 40°C water bath. The lower sodium chloride concentrations always thawed faster than the higher sodium chloride concentrations. Ampoules cooled to room temperature after removal from the 48 ± 1°C water bath required approximately two minutes.

Data from samples stored at 5 ± 1°C had to be eliminated from this experiment because growth of gram-positive cocci from the fish homogenate occurred. The

colonies of the cocci were usually pinpoint in size. When uninoculated fish was stored, numbers of 10^3 per ml for 3, 6, and 9% sodium chloride fish homogenates were reached after nine days of storage. Counts of 10^2 per ml for 0 and 12% were obtained after nine days of storage.

Uninoculated fish samples were also stored at -5 ± 1°C and -18 ± 1°C to study background counts. After 24 hr at -5 ± 1°C counts averaged 70 cells per ml. These counts decreased after 16 days of storage to 20 organisms per ml. Similar studies at -18 ± 1°C revealed counts averaging 35 cells per ml after 24 hr, decreasing to 20 cells per ml after 16 days of storage. Since the initial counts of inoculated samples were approximately 10,000 cells per ml, the contaminating microorganisms were a small part of the total, unless the temperature during the experimental period permitted their multiplication as occurred at 5 ± 1°C.

Analyses of variance for survival of Vibrio parahaemolyticus at 48 ± 1°C in fish homogenate with varying levels of sodium chloride are given in Table 5. The levels of sodium chloride were statistically significant for all three strains; sampling time was significant for strains SB04-422 and 17802. Sodium chloride x time interaction was significant only for strain SB04-422.

Curves on figures are based on averages of two replications for each sodium chloride concentration at each

Table 5. Statistical Analysis of the Effect of Heating at 48 ± 1°C on the Survival of *Vibrio parahaemolyticus* in Fish Homogenate with Varying Levels of Sodium Chloride.

Source of Variation	Degrees of Freedom	Mean Square[a]	F Value[b]
Strain T-3765-1			
Replications	1	.30	2.97
Salt	4	2.88	28.47*
Time	3	.23	2.26
Salt x Time	12	.02	0.17
Error	19	.10	
Strain SB04-422			
Replications	1	.10	2.46
Salt	4	7.46	185.29*
Time	3	.41	10.27*
Salt x Time	12	.14	13.38*
Error	19	.04	
Strain 17802			
Replications	1	.20	2.13
Salt	4	4.49	48.83*
Time	3	1.33	14.87*
Salt x Time	12	.07	.76
Error	19	.09	

a. Logarithmic transformations of viable cells per ml.

b. * indicates significance at the .05 level.

sampling time, except where data are missing (Appendix). Sampling times for 0 and 3% sodium chloride were not carried beyond 20 min for strain T-3765-1 and not beyond 40 min for the other two strains. Variability of counts was less in the fish homogenate series than in Experiment I. For example, in 3% fish homogenate heated at 48 ± 1°C for ten minutes, 82 x 10^2 and 90 x 10^2 were recorded for two

separate replications. (Data for all replications can be found in Appendix Tables 6-9.)

Heating of Vibrio parahemolyticus in fish homogenate with varying amounts of sodium chloride appeared to confer some resistance to the cells as can be seen in Figures 13-15. Without additional sodium chloride, fish homogenate had a stabilizing effect on the survival of Vibrio parahaemolyticus with time at 48 ± 1°C as compared with Trypticase soy broth. This can be seen from the figures as well as from the D values in Table 3. Survival in 0% sodium chloride fish homogenate was significantly lower than at all other sodium chloride levels for all strains tested. Counts of survivors of strain T-3765-1 at 20 min appeared to be quite stable in 3-12% sodium chloride fish homogenate. Similar results can be seen in Figure 14 for strain SB04-422. Survival counts were significantly higher for 3% sodium chloride fish homogenate than the 9% concentration of sodium chloride. Strain SB04-422 in 12% sodium chloride had significantly lower survival counts than at all other sodium chloride concentrations. Fish homogenate with 3% sodium chloride appeared optimum for survival. The D values in Table 3 are quite large for 3, 6, and 9% sodium chloride fish homogenate for both strains with the exception of 6% sodium chloride for strain T-3765-1. D values are slightly biased for the fish homogenate, since numbers of cells at 0.5 min were used for the initial

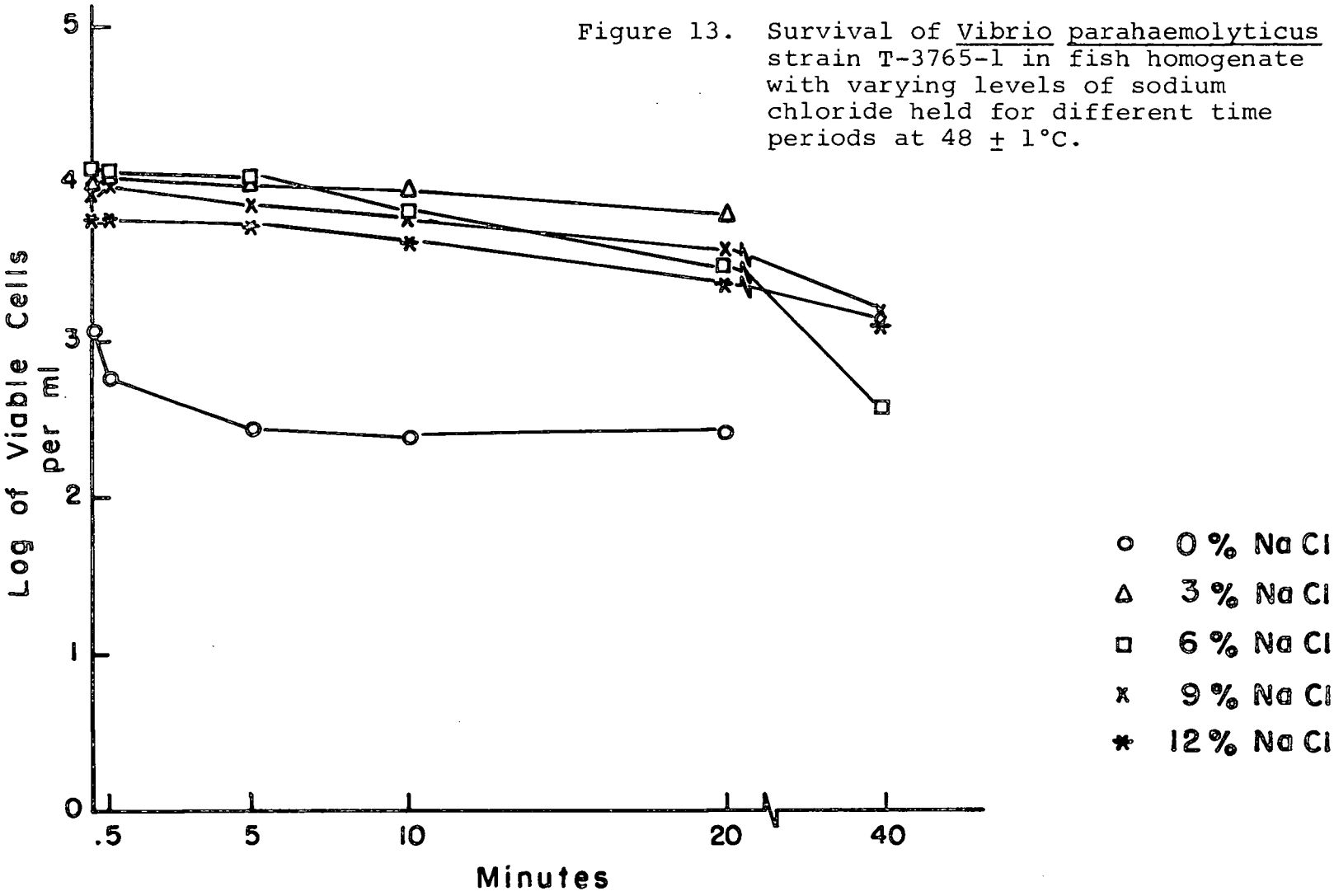

Figure 13. Survival of Vibrio parahaemolyticus strain T-3765-1 in fish homogenate with varying levels of sodium chloride held for different time periods at 48 ± 1°C.

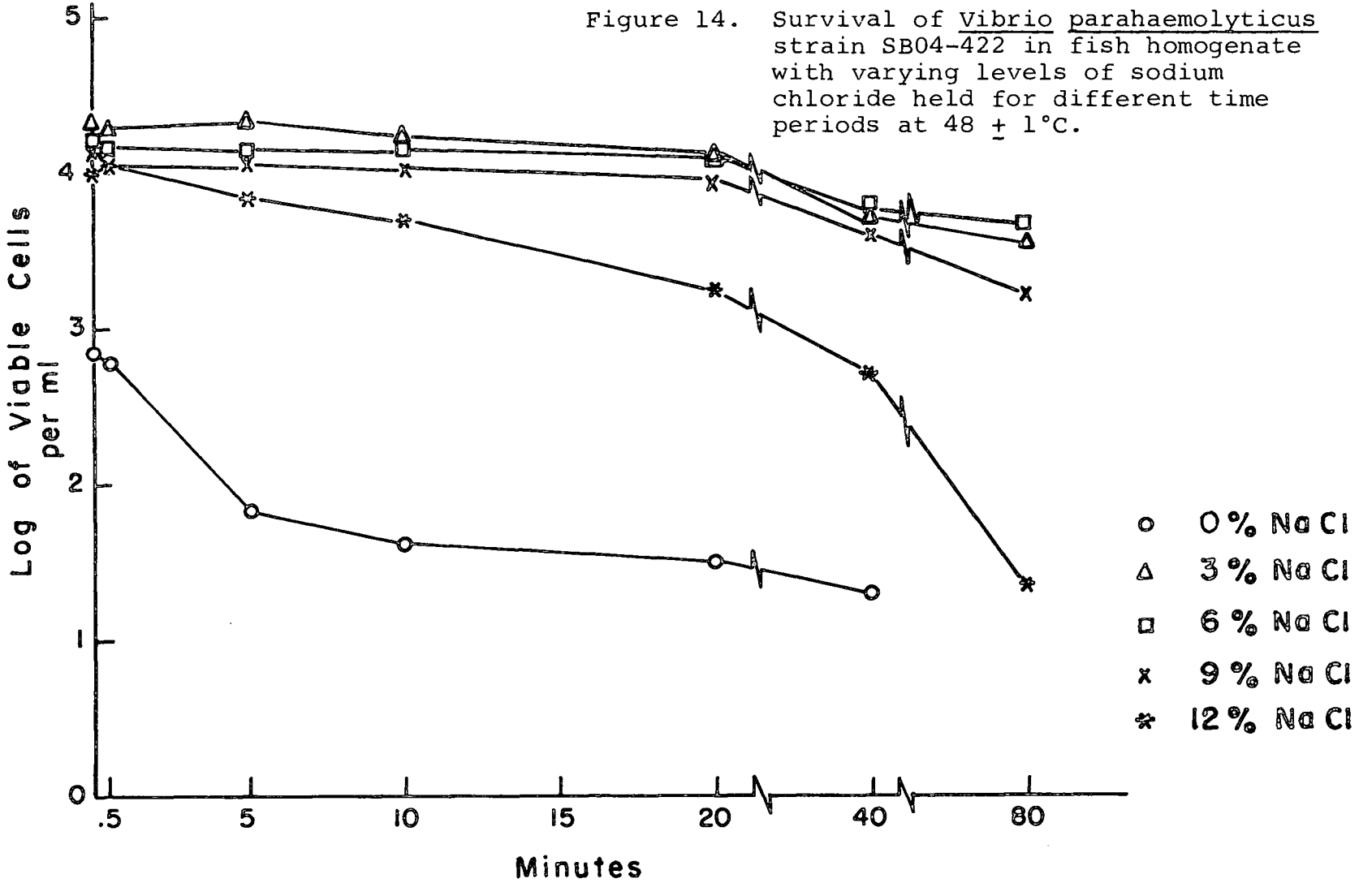

Figure 14. Survival of Vibrio parahaemolyticus strain SB04-422 in fish homogenate with varying levels of sodium chloride held for different time periods at 48 ± 1°C.

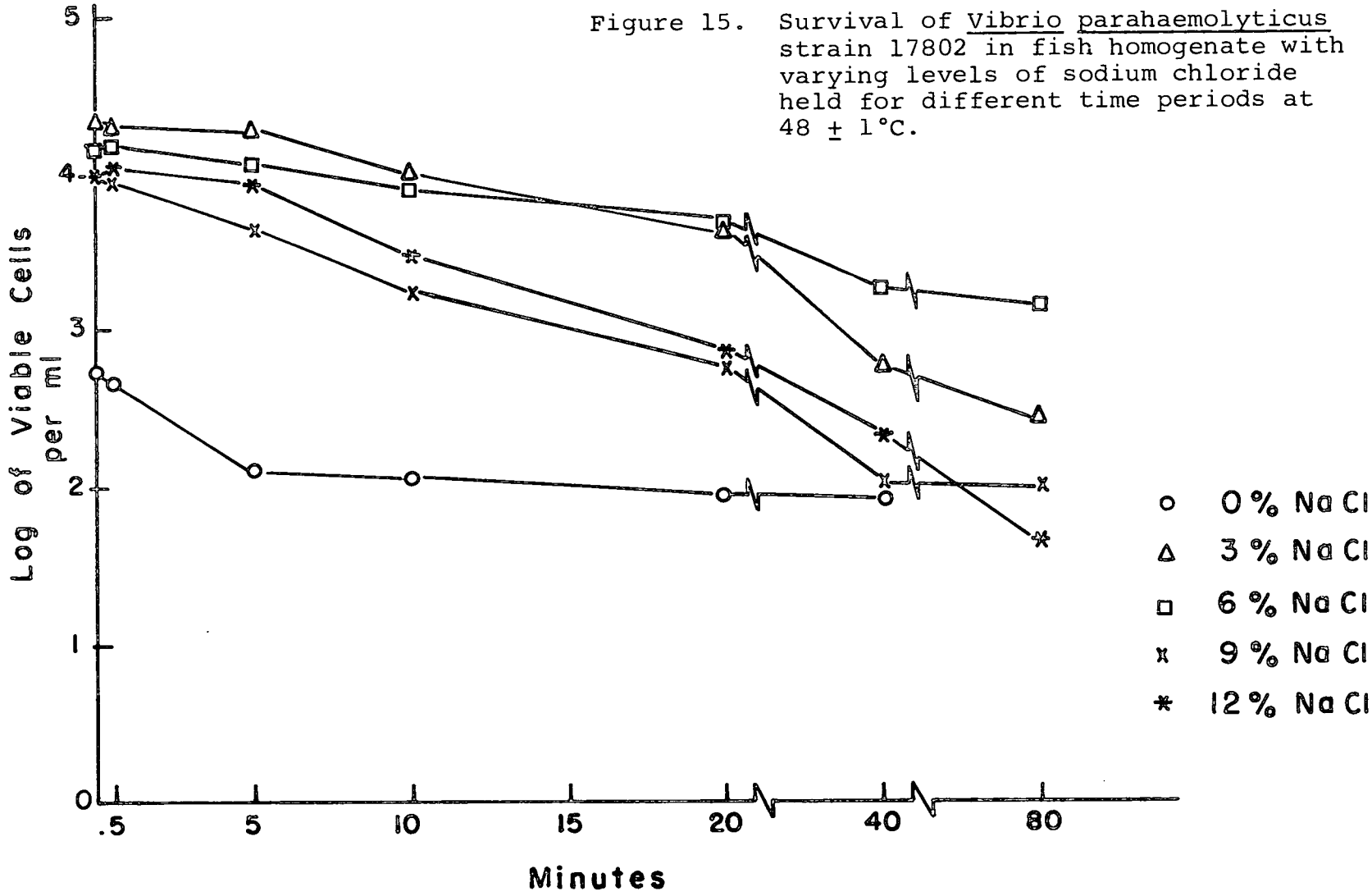

Figure 15. Survival of Vibrio parahaemolyticus strain 17802 in fish homogenate with varying levels of sodium chloride held for different time periods at 48 ± 1°C.

counts and 48 ± 1°C was not reached until four minutes after placement in the heated water bath. Figure 15 illustrates again the sensitivity of strain 17802 regardless of sodium chloride concentration. Sodium chloride concentrations of 3 and 6% significantly enhance resistance to heat. This was also apparent from the D values in Table 3.

Studies of heating Vibrio parahaemolyticus at 48 ± 1°C showed that fish homogenate with no additional sodium chloride could be protective, although survival counts at this concentration were significantly lower than when sodium chloride was present. In general, sodium chloride concentrations of 3, 6, and 9% appeared to enhance survival. Comparisons could not be made with the heating of Vibrio parahaemolyticus in fish homogenate at 60°C, reported by Liston et al. (1967), because of the lack of detail.

Plating on 3% rather than 6% sodium chloride Trypticase soy agar gave significantly higher counts for recovery of cells heated in 6% sodium chloride fish homogenate. Again, the sodium chloride level for the injured cells appears to be important. The paired t-test was also used to compare recovery counts in 3% sodium chloride Trypticase soy agar and Colwell's Vibrio maintenance medium at all sodium chloride concentrations for each strain. For T-3765-1 no statistically significant difference of recovery counts was found for the two media. Strains

SB04-422 and 17802 showed a statistically significant difference in media at the .05 level. Both strains had higher recovery counts on 3% sodium chloride Trypticase soy agar. This is in contrast to the work of Freitas Leitão (1970). He found recovery counts for strain 17802 to be higher on Colwell's Vibrio maintenance medium than on Trypticase soy agar with 2.5% sodium chloride. Perhaps the contrast of these findings with his lies in the difference of the sodium chloride concentration used in the recovery medium.

Statistical analyses of the survival of Vibrio parahaemolyticus in fish homogenate with varying levels of sodium chloride at -5 ± 1°C and -18 ± 1°C are given in Table 6. Sampling times and levels of sodium chloride were significant for all strains. Time x sodium chloride interactions were significant for two strains. Replication was a significant factor for strain 17802. Survival at these storage temperatures did not differ significantly.

Effects of sodium chloride were strain dependent. LSD comparisons showed that 12% sodium chloride was best for survival of strain T-3765-1, 6 and 9% for strain SB04-422, and 3 and 6% sodium chloride for strain 17802.

Survival of Vibrio parahaemolyticus at -5 ± 1°C in fish homogenate with varying sodium chloride concentrations is illustrated in Figures 16-18. Figures 19-21 indicate the survival of Vibrio parahaemolyticus at -18 ± 1°C in

Table 6. Statistical Analysis of the Effect of -5 ± 1°C and -18 ± 1°C on the Survival of *Vibrio parahaemolyticus* in Fish Homogenate with Varying Levels of Sodium Chloride.

Source of Variation	Degrees of Freedom	Mean Square[a]	F Value[b]
Strain T-3765-1			
Replications	1	.09	.31
Temperature	1	.28	.98
Time	3	11.29	40.05*
NaCl	4	1.87	6.64*
Temperature x Time	3	.69	2.45
Temperature x NaCl	4	.27	.95
.5 min x NaCl	4	1.16	4.10
5 min x NaCl	4	.04	.15
10 min x NaCl	4	.22	.79
Error	50	.28	
Strain SB04-422			
Replications	1	.03	.45
Temperature	1	.52	7.78
Time	3	17.55	263.03*
NaCl	4	2.56	38.41*
Temperature x Time	3	.58	8.78*
Temperature x NaCl	4	.51	7.59*
.5 min x NaCl	4	2.10	31.56*
5 min x NaCl	4	.26	3.86
10 min x NaCl	4	.40	6.02*
Error	50	.07	
Strain 17802			
Replications	1	1.10	10.28*
Temperature	1	.13	1.20
Time	3	9.21	85.89*
NaCl	4	2.39	22.26*
Temperature x Time	3	.13	1.23
Temperature x NaCl	4	.03	.30
.5 min x NaCl	4	.65	6.06*
5 min x NaCl	4	.05	.47
10 min x NaCl	4	.15	1.40
Error	50	.11	

a. Logarithmic transformations of viable cells per ml.

b. * indicates significance at the .05 level.

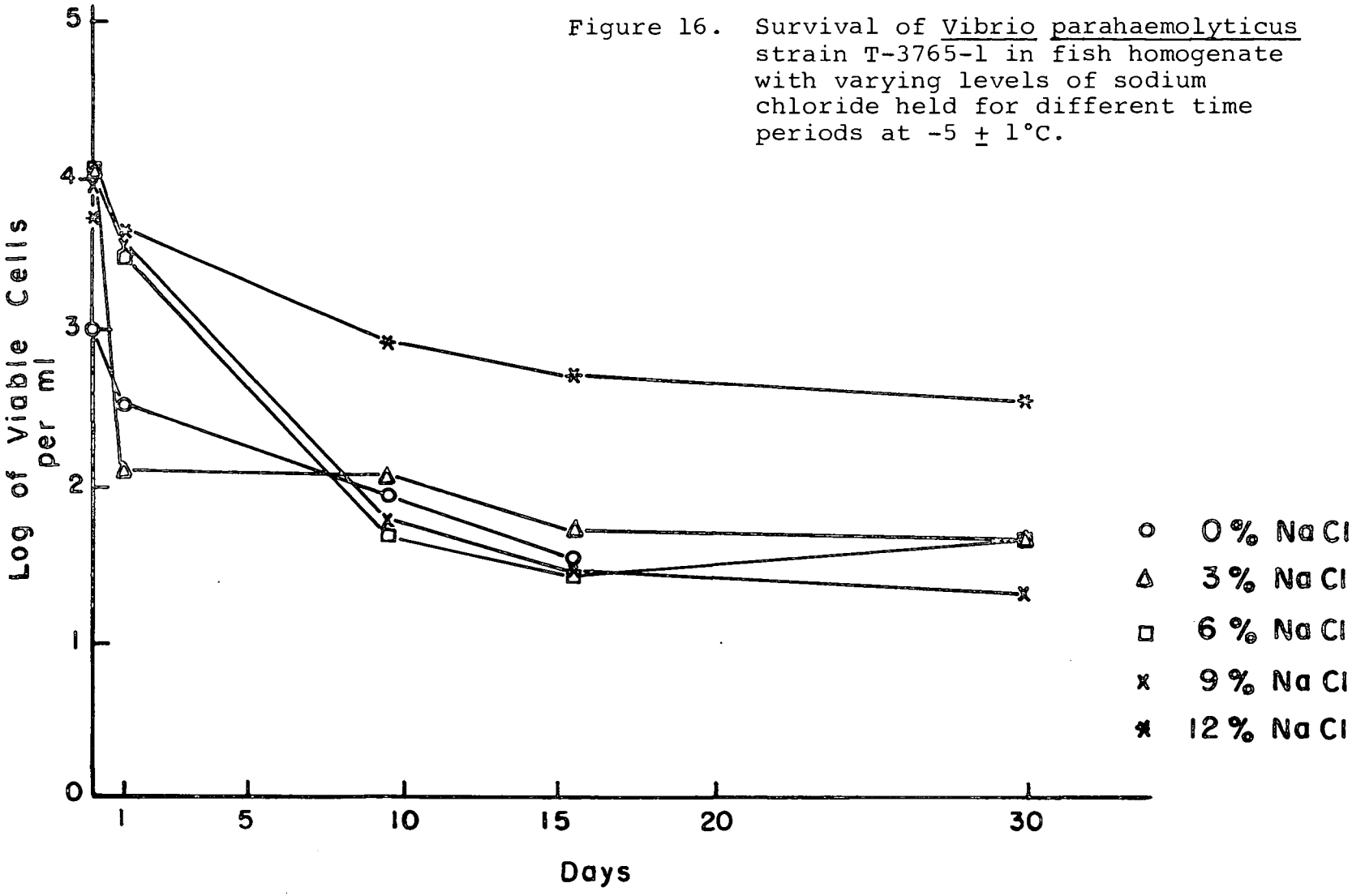

Figure 16. Survival of Vibrio parahaemolyticus strain T-3765-1 in fish homogenate with varying levels of sodium chloride held for different time periods at -5 ± 1°C.

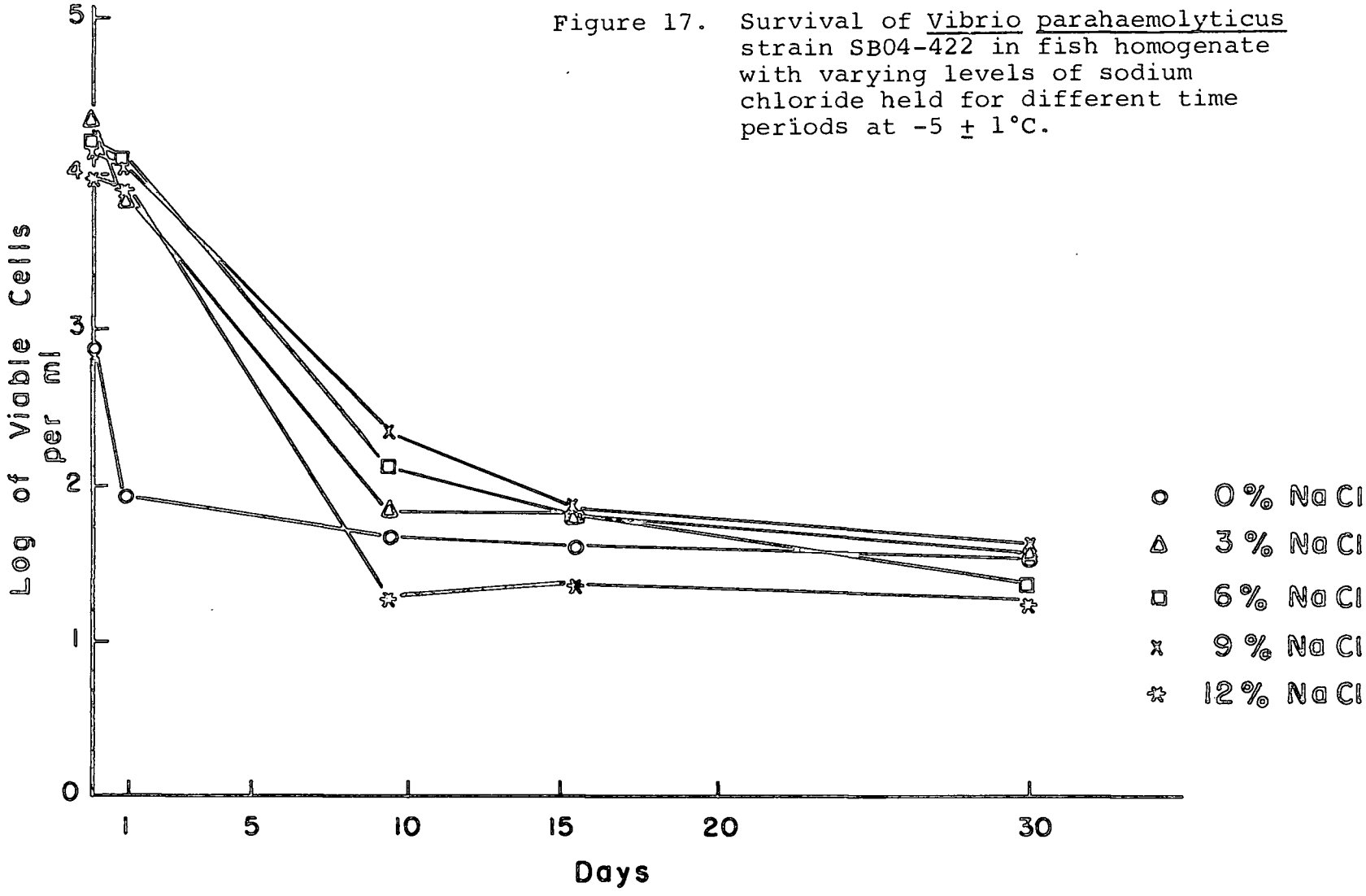

Figure 17. Survival of Vibrio parahaemolyticus strain SB04-422 in fish homogenate with varying levels of sodium chloride held for different time periods at -5 ± 1°C.

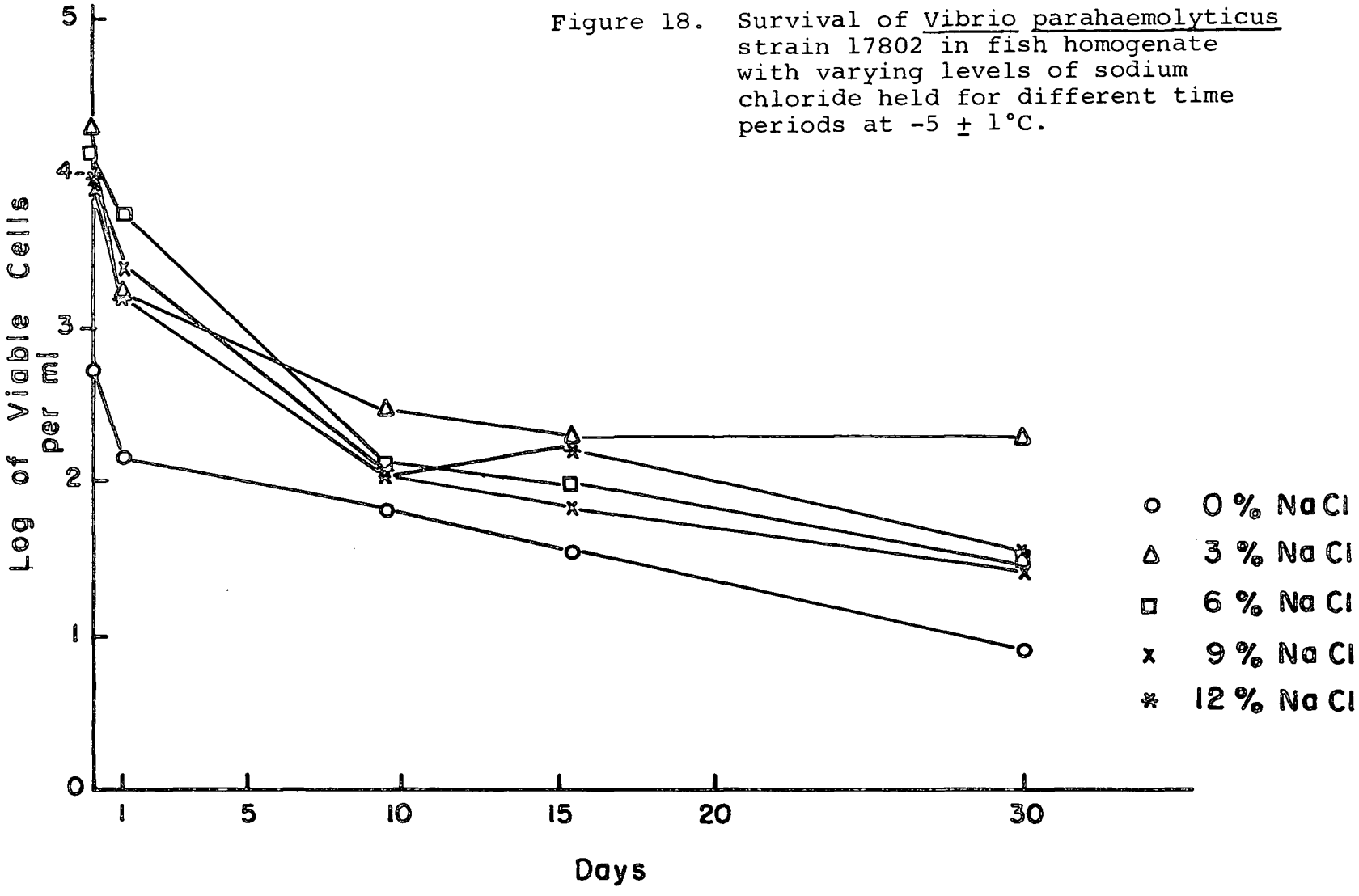

Figure 18. Survival of Vibrio parahaemolyticus strain 17802 in fish homogenate with varying levels of sodium chloride held for different time periods at -5 ± 1°C.

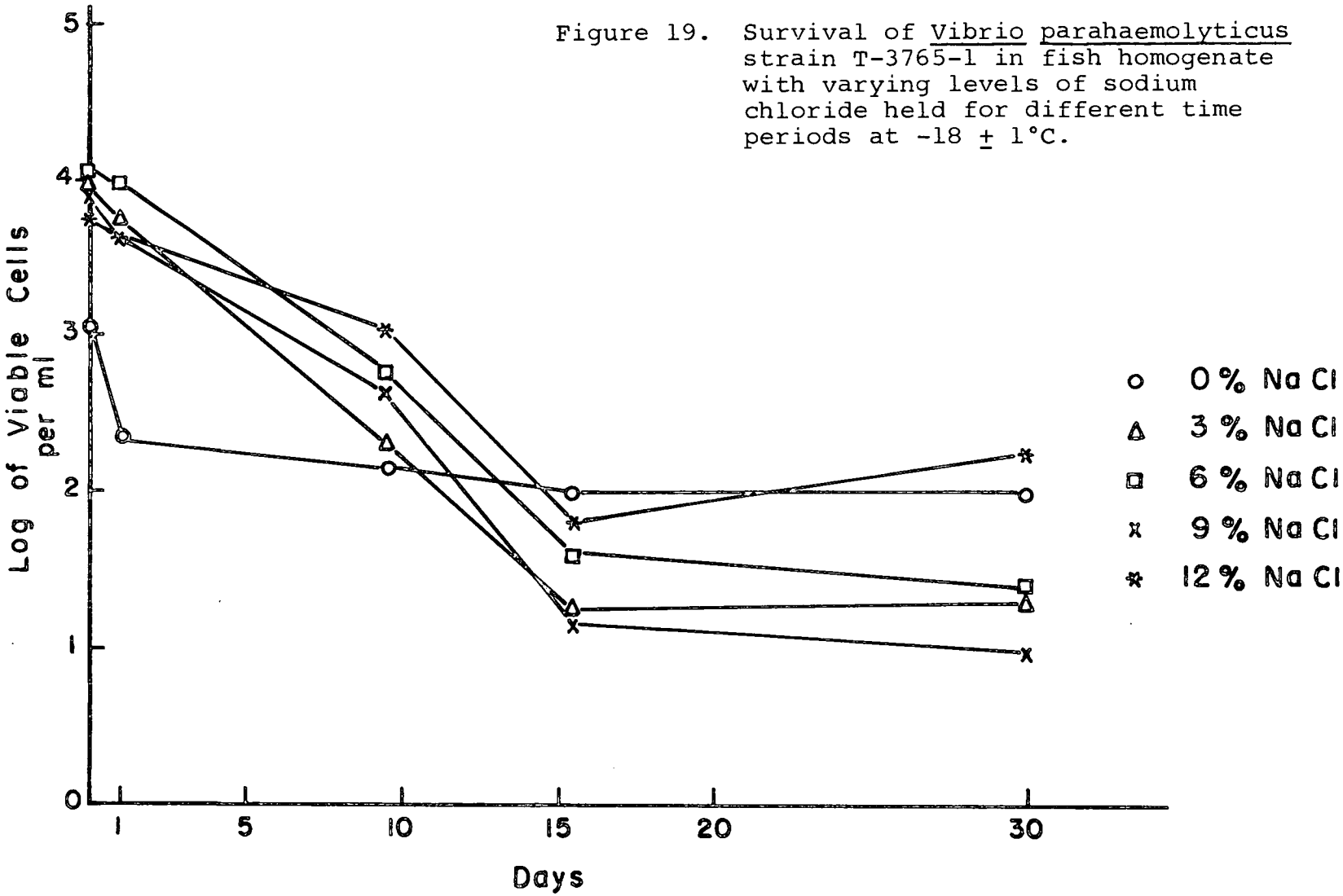

Figure 19. Survival of Vibrio parahaemolyticus strain T-3765-1 in fish homogenate with varying levels of sodium chloride held for different time periods at -18 ± 1°C.

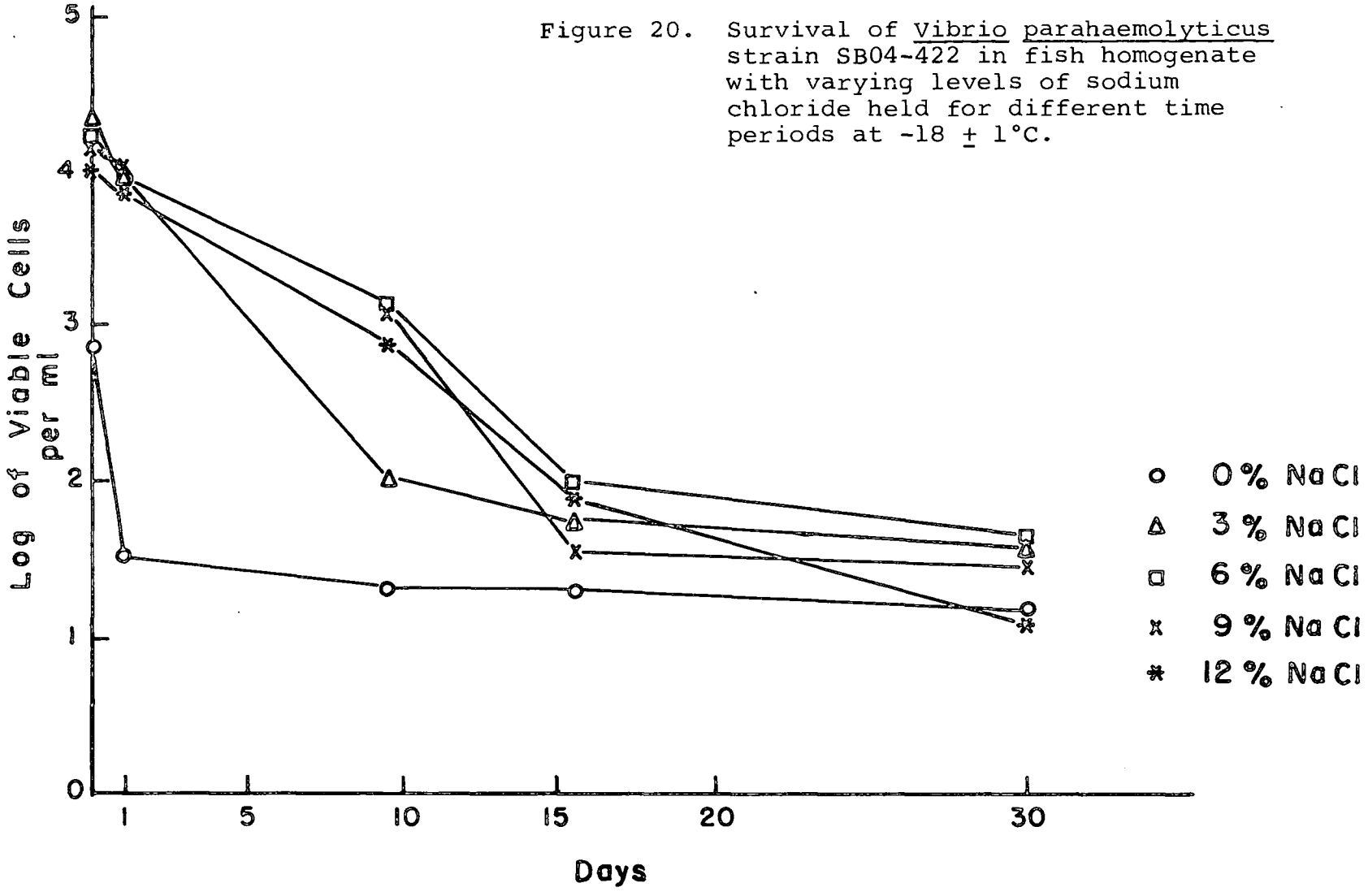

Figure 20. Survival of Vibrio parahaemolyticus strain SB04-422 in fish homogenate with varying levels of sodium chloride held for different time periods at -18 ± 1°C.

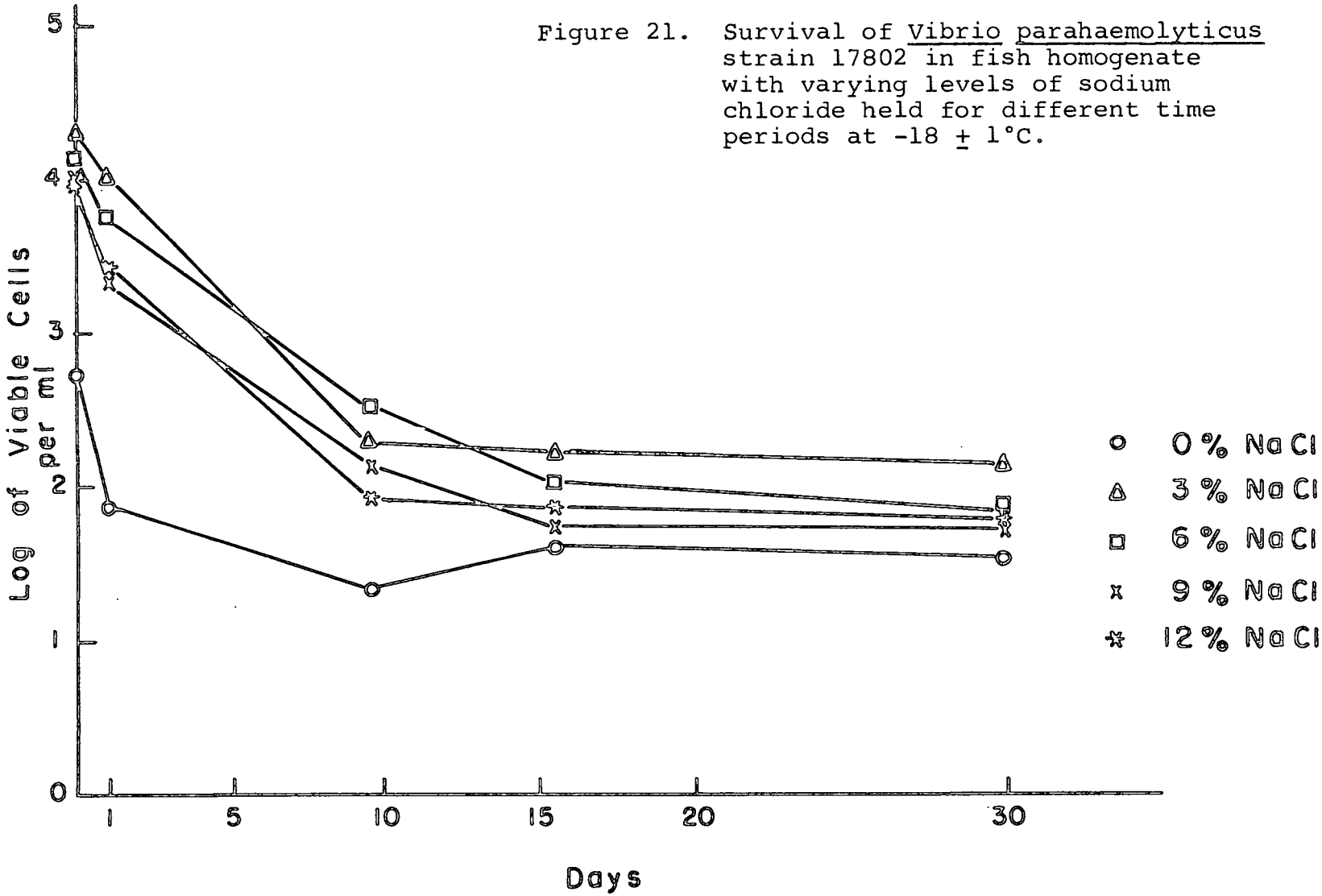

Figure 21. Survival of Vibrio parahaemolyticus strain 17802 in fish homogenate with varying levels of sodium chloride held for different time periods at -18 ± 1°C.

fish homogenate with varying levels of sodium chloride. The decline in numbers of survivors was steady to 16 days of storage, at which time low numbers remained at both temperatures.

The experimental findings from this study of the survival of Vibrio parahaemolyticus in fish homogenate at -5 ± 1°C and -18 ± 1°C are in agreement with Temmyo (1966) who found that resistance of Vibrio parahaemolyticus to -2 and -18°C was low, and with Asakawa (1967) who found survival of the organism in tuna meat to be low at -10 and -20°C. Sodium chloride concentrations appeared to give little protection at -5 or -18°C. As was noted earlier, there was no significant difference in survival at the two temperatures. Temmyo (1966) found that sodium chloride appeared to give some protection to Vibrio parahaemolyticus in saurel extracts stored for eight days at -2°C and six days at -18°C. The discrepancy may be in the storage times. Storage times of 16 and 30 days at -5 ± 1°C and -18 ± 1°C, as in this study, may completely erase any protection of sodium chloride which might exist for short storage periods.

Differences in recovery counts on 3% sodium chloride Trypticase soy agar and Colwell's Vibrio maintenance medium were not significant at the .05 level for any of the strains at -5 ± 1°C or -18 ± 1°C.

The results of these experiments show that the

medium in which Vibrio parahaemolyticus is heated or stored greatly affects the survival of the organism. Even a complex laboratory medium such as Trypticase soy broth is not as protective to the cells as the fish homogenate. Food systems have been shown to be protective to other organisms such as staphylococci and salmonellae. Therefore, if information on survival of Vibrio parahaemolyticus in a food system is desired, a food system must be used for the studies.

Strain 17802 appeared to be more sensitive than the other strains used in these studies. For example, in 9% sodium chloride broth at ten minutes, strain 17802 had $11x10^2$ survivors while strain T-3765-1 had $16x10^3$ and strain SB04-422 had $14x10^3$. Since one of the three strains used in this study appeared to be more temperature sensitive, perhaps strain differences should be further examined because of the implications they might have on future research with Vibrio parahaemolyticus.

These studies have shown that thorough heating even of salted foods will reduce the numbers of Vibrio parahaemolyticus, if they are present in the food. Often food incriminated in Japanese food poisoning outbreaks attributed to Vibrio parahaemolyticus was salted food that was either raw or had been insufficiently cooked.

Low temperature storage will greatly reduce the numbers of Vibrio parahaemolyticus even in the presence of

sodium chloride. Thus holding seafoods in the frozen state would greatly reduce the Vibrio parahaemolyticus counts, if the organism was present on the food. From the consumer's viewpoint these findings have positive implications. However, since survivors remained, foods that were mishandled might permit multiplication of vibrios. Such reduction in numbers of Vibrio parahaemolyticus by holding at low temperatures indicates that the organism may seldom be detected after samples from food poisoning outbreaks have been frozen and held for a period of time before bacteriological examination. It was originally thought by the experimenter that perhaps sodium chloride was involved in a mechanism for cell protection, since salted foods are often incriminated in Japanese food poisoning outbreaks caused by Vibrio parahaemolyticus. If this were so, sodium chloride could be added to protect the organism during the storage of food samples from food poisoning outbreaks. For short term storage, the results of these experiments clearly show that sodium chloride is protective to Vibrio parahaemolyticus, but at an optimum level which is temperature and strain dependent. So the recommendation for food samples from outbreaks suspect for Vibrio parahaemolyticus could not be made. A method for stabilizing Vibrio parahaemolyticus cells in samples for bacteriological examination is necessary and should be found.

SUMMARY

Survival of Vibrio parahaemolyticus in Trypticase soy broth (a complex laboratory medium) and fish homogenate with 0, 3, 6, 9, and 12% sodium chloride was studied at 48 ± 1°C, 5 ± 1°C, -5 ± 1°C, and -18 ± 1°C. The fish homogenates were composed of sturgeon, black rockfish, and ling cod and consisted of one part fish to nine parts sodium chloride solution in varying concentrations. An inoculum level of 10,000 cells per ml was used for all of the experiments. Sampling times at 48 ± 1°C were 0.5, 5, 10, and 20 min, while 1, 9, 16, and 30 days were the storage periods for the low temperature studies.

Strains T-3765-1, SB04-422, and 17802 of Vibrio parahaemolyticus representing cultures isolated from food as well as from food poisoning patients were used in this study. Strain 17802 was more temperature sensitive than the other strains.

For the Trypticase soy broth experiment, a 5 x 4 factorial at 48 ± 1°C and a 5 x 4 x 3 factorial for the low temperatures study with three replications for each were used for each strain. For the fish homogenate experiment, a 5 x 4 factorial at 48 ± 1°C and a 5 x 4 x 2 factorial for the low temperatures study with two replications for each were statistically analyzed for each strain. Least significant differences (LSD) were used for within

parahaemolyticus in food samples held for bacteriological examination. The presence of sodium chloride does not adequately stabilize the cells of Vibrio parahaemolyticus. The microbiologist must find another method for the stabilization of these cells in food samples from food poisoning outbreaks suspect for Vibrio parahemolyticus.

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APPENDIX

Appendix Table 1. Survival of Vibrio parahaemolyticus in Trypticase Soy Broth at 48 ± 1°C.

Strain	Salt Concentration (%)	Replication	Length of Heating (minutes)	Viable Count (cells per ml)
T-3765-1	0	1	0	no counts
			.5	
			5	
			10	
			20	
	0	2	0	50×10^4
			.5	50×10^1
			5	25
			10	0
			20	0
	0	3	0	96×10^1
			.5	50×10^1
			5	6
			10	8
			20	5
	3	1	0	96×10^2
			.5	97×10^2
			5	20×10^2
			10	68×10^1
			20	72
	3	2	0	98×10^2
			.5	11×10^3
			5	51×10^2
			10	17×10^2
			20	16×10^2
	3	3	0	10×10^3
			.5	13×10^3
			5	48×10^2
			10	87×10^1
			20	42×10^1
	6	1	0	12×10^3
			.5	88×10^2
			5	11×10^3
			10	11×10^3
			20	77×10^2

Appendix Table 1 (Continued)

Strain	Salt Concentration (%)	Replication	Length of Heating (minutes)	Viable Count (cells per ml)
T-3765-1	6	2	0	14×10^3
			.5	16×10^3
			5	12×10^3
			10	11×10^3
			20	10×10^3
	6	3	0	83×10^2
			.5	73×10^2
			5	52×10^2
			10	40×10^2
			20	41×10^2
			40	19×10^2
	9	1	0	17×10^3
			.5	16×10^3
			5	18×10^3
			10	16×10^3
			20	11×10^3
	9	2	0	86×10^2
			.5	69×10^2
			5	55×10^2
			10	49×10^2
			20	37×10^2
			40	22×10^2
	9	3	0	46×10^2
			.5	55×10^2
			5	45×10^2
			10	28×10^2
			20	21×10^1
			40	96×10^1
	12	1	0	27×10^2
			.5	30×10^2
			5	17×10^2
			10	10×10^1
			20	42×10^1
	12	2	0	88×10^2
			.5	95×10^2
			5	37×10^2
			10	22×10^1
			20	88×10^1
			40	15×10^1

Appendix Table 1 (Continued)

Strain	Salt Concentration (%)	Replication	Length of Heating (minutes)	Viable Count (cells per ml)
T-3765-1	12	3	0	43×10^{2}
			.5	38×10^{2}
			5	28×10^{2}
			10	20×10^{2}
			20	13×10^{2}
			40	40×10^{1}
SB04-422	0	1	0	16×10^{2}
			.5	10×10^{2}
			5	11×10^{2}
			10	22×10^{1}
			20	29
	0	2	0	25×10^{2}
			.5	20×10^{1}
			5	2
			10	1
			20	3
	0	3	0	28×10^{2}
			.5	30×10^{1}
			5	10×10^{1}
			10	46
			20	3
	3	1	0	15×10^{3}
			.5	17×10^{3}
			5	76×10^{2}
			10	98×10^{1}
			20	47×10^{1}
	3	2	0	15×10^{3}
			.5	16×10^{3}
			5	12×10^{3}
			10	12×10^{3}
			20	64×10^{2}
	3	3	0	34×10^{3}
			.5	24×10^{3}
			5	21×10^{3}
			10	11×10^{3}
			20	55×10^{2}

Appendix Table 1 (Continued)

Strain	Salt Concentration (%)	Replication	Length of Heating (minutes)	Viable Count (cells per ml)
SB04-422	6	1	0	13×10^3
			.5	14×10^3
			5	13×10^3
			10	12×10^2
			20	88×10^2
			40	73×10^2
	6	2	0	12×10^3
			.5	10×10^3
			5	96×10^2
			10	70×10^2
			20	42×10^2
			40	10×10^2
	6	3	0	22×10^3
			.5	18×10^3
			5	13×10^3
			10	10×10^3
			20	85×10^2
			40	17×10^2
	9	1	0	19×10^3
			.5	19×10^3
			5	17×10^3
			10	13×10^3
			20	11×10^3
			40	72×10^2
	9	2	0	95×10^2
			.5	64×10^2
			5	40×10^2
			10	16×10^2
			20	39×10^1
			40	16×10^1
	9	3	0	12×10^3
			.5	13×10^3
			5	21×10^2
			10	30×10^1
			20	14×10^1
			40	41

Appendix Table 1 (Continued)

Strain	Salt Concentration (%)	Replication	Length of Heating (minutes)	Viable Count (cells per ml)
SB04-422	12	1	0	11×10^3
			.5	14×10^3
			5	88×10^2
			10	74×10^2
			20	27×10^2
			40	20×10^1
	12	2	0	95×10^2
			.5	73×10^2
			5	49×10^2
			10	20×10^2
			20	69×10^1
			40	23
	12	3	0	10×10^3
			.5	10×10^3
			5	47×10^2
			10	28×10^2
			20	55×10^1
			40	29
17802	0	1	0	38×10^2
			.5	36×10^2
			5	0
			10	0
			20	0
	0	2	0	95×10^1
			.5	10×10^1
			5	0
			10	3
			20	1
	0	3	0	70×10^1
			.5	40×10^1
			5	0
			10	0
			20	0
	3	1	0	17×10^3
			.5	16×10^3
			5	13×10^3
			10	51×10^2
			20	16×10^2

Appendix Table 1 (Continued)

Strain	Salt Concentration (%)	Replication	Length of Heating (minutes)	Viable Count (cells per ml)
17802	3	2	0	11×10^3
			.5	14×10^3
			5	78×10^2
			10	missing
			20	12×10^1
	3	3	0	20×10^3
			.5	18×10^3
			5	70×10^2
			10	33×10^2
			20	59×10^1
	6	1	0	18×10^3
			.5	16×10^3
			5	29×10^2
			10	46×10^1
			20	70
			40	30
	6	2	0	15×10^3
			.5	14×10^3
			5	29×10^2
			10	41×10^2
			20	20×10^1
			40	25
	6	3	0	99×10^2
			.5	11×10^3
			5	30×10^2
			10	19×10^2
			20	14×10^2
			40	51×10^1
	9	1	0	
			.5	
			5	missing
			10	
			20	
			40	

Appendix Table 1 (Continued)

Strain	Salt Concentration (%)	Replication	Length of Heating (minutes)	Viable Count (cells per ml)
17802	9	2	0	16×10^3
			.5	12×10^3
			5	31×10^2
			10	11×10^2
			20	14×10^2
			40	37
	9	3	0	24×10^2
			.5	27×10^2
			5	71×10^1
			10	33×10^1
			20	69
			40	12
	12	1	0	77×10^2
			.5	84×10^2
			5	52×10^2
			10	36×10^2
			20	12×10^2
			40	13
	12	2	0	89×10^2
			.5	95×10^2
			5	40×10^2
			10	11×10^2
			20	79
			40	37
	12	3	0	17×10^3
			.5	23×10^3
			5	14×10^3
			10	76×10^2
			20	87×10^1
			40	20

Appendix Table 2. Survival of Vibrio parahaemolyticus in 6% Sodium Chloride Trypticase Soy Broth at 48 ± 1°C.

Strain	Replication	Length of Heating (minutes)	3% STSA[a] (cells per ml)	6% STSA[a] (cells per ml)
T-3765-1	1	0	12×10^3	11×10^3
		.5	88×10^2	91×10^2
		5	11×10^3	97×10^2
		10	11×10^3	94×10^2
		20	77×10^2	76×10^2
	2	0	14×10^3	15×10^3
		.5	16×10^3	14×10^3
		5	12×10^3	12×10^3
		10	11×10^3	12×10^3
		20	10×10^3	10×10^3
	3	0	83×10^2	79×10^2
		.5	73×10^2	66×10^2
		5	52×10^2	51×10^2
		10	40×10^2	50×10^2
		20	41×10^2	41×10^2
		40	19×10^2	18×10^2
SB04-422	1	0	13×10^3	15×10^3
		.5	14×10^3	13×10^3
		5	13×10^3	12×10^3
		10	12×10^3	10×10^3
		20	88×10^2	10×10^3
		40	73×10^2	55×10^2
	2	0	12×10^3	11×10^3
		.5	10×10^3	12×10^3
		5	96×10^2	79×10^2
		10	70×10^2	70×10^2
		20	42×10^2	38×10^1
		40	10×10^2	69×10^1
	3	0	22×10^3	19×10^3
		.5	18×10^3	20×10^3
		5	13×10^3	14×10^3
		10	10×10^3	85×10^2
		20	85×10^2	63×10^2
		40	17×10^2	14×10^2

Appendix Table 2 (Continued)

Strain	Replication	Length of Heating (minutes)	3% STSA[a] (cells per ml)	6% STSA[a] (cells per ml)
17802	1	0	$19x10^3$	$13x10^3$
		.5	$16x10^3$	$16x10^2$
		5	$29x10^2$	$18x10^1$
		10	$46x10^1$	$36x10^1$
		20	$70x10^1$	$60x10^1$
		40	$30x10^1$	$50x10^1$
	2	0	$15x10^3$	missing
		.5	$14x10^3$	missing
		5	$29x10^2$	$26x10^2$
		10	$41x10^2$	$83x10^1$
		20	$20x10^1$	$13x10^1$
		40	25	14
	3	0	$99x10^2$	$11x10^3$
		.5	$11x10^3$	$10x10^3$
		5	$31x10^2$	$29x10^2$
		10	$19x10^2$	$19x10^2$
		20	$14x10^2$	$10x10^1$
		40	$51x10^1$	$37x10^1$

a. Sodium chloride Trypticase soy agar (BBL).

Appendix Table 3. Survival of Vibrio parahaemolyticus in Trypticase Soy Broth at 5 ± 1°C.

Strain	Salt Concentration (%)	Replication	Days of Storage	Viable Count (cells per ml)
T-3765-1	0	1	0	missing
			1	14×10^{1}
			9	0
			16	0
			30	0
	0	2	0	50×10^{4}
			1	16
			9	5
			16	0
			30	0
	0	3	0	96×10^{1}
			1	26
			9	6
			16	6
			30	5
	3	1	0	96×10^{2}
			1	12×10^{2}
			9	23
			16	9
			30	4
	3	2	0	98×10^{2}
			1	65×10^{2}
			9	11×10^{1}
			16	45
			30	3
	3	3	0	10×10^{3}
			1	88×10^{2}
			9	47×10^{1}
			16	7
			30	4
	6	1	0	12×10^{3}
			1	78×10^{2}
			9	87×10^{1}
			16	41
			30	49

Appendix Table 3 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	Viable Count (cells per ml)
T-3765-1	6	2	0	14×10^3
			1	12×10^3
			9	20×10^1
			16	31
			30	1
	6	3	0	83×10^2
			1	90×10^2
			9	39×10^1
			16	14
			30	5
	9	1	0	17×10^3
			1	17×10^3
			9	25×10^2
			16	10×10^1
			30	52
	9	2	0	86×10^2
			1	47×10^2
			9	51×10^1
			16	13×10^1
			30	6
	9	3	0	46×10^2
			1	34×10^2
			9	43×10^1
			16	16
			30	5
	12	1	0	27×10^2
			1	11×10^2
			9	24
			16	4
			30	6
	12	2	0	88×10^2
			1	57×10^2
			9	28×10^1
			16	10×10^1
			30	47
	12	3	0	43×10^2
			1	20×10^2
			9	15×10^1
			16	16
			30	34

Appendix Table 3 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	Viable Count (cells per ml)
SB04-422	0	1	0	16×10^{2}
			1	74×10^{1}
			9	3
			16	0
			30	1
	0	2	0	25×10^{2}
			1	13×10^{2}
			9	0
			16	93
			30	1
	0	3	0	28×10^{2}
			1	15×10^{2}
			9	0
			16	0
			30	0
	3	1	0	15×10^{3}
			1	13×10^{3}
			9	60
			16	6
			30	3
	3	2	0	15×10^{3}
			1	76×10^{2}
			9	82
			16	19
			30	1
	3	3	0	34×10^{3}
			1	14×10^{3}
			9	24×10^{1}
			16	2
			30	1
	6	1	0	13×10^{3}
			1	14×10^{3}
			9	85×10^{1}
			16	34
			30	0
	6	2	0	12×10^{3}
			1	10×10^{3}
			9	14×10^{2}
			16	68
			30	29

Appendix Table 3 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	Viable Count (cells per ml)
SB04-422	6	3	0	22×10^3
			1	17×10^3
			9	21×10^2
			16	14×10^1
			30	2
	9	1	0	19×10^3
			1	13×10^3
			9	34×10^2
			16	12×10^2
			30	21
	9	2	0	95×10^2
			1	55×10^2
			9	15×10^2
			16	78×10^1
			30	51
	9	3	0	12×10^3
			1	67×10^2
			9	20×10^2
			16	62×10^1
			30	31
	12	1	0	11×10^3
			1	85×10^2
			9	45×10^2
			16	17×10^2
			30	50×10^1
	12	2	0	95×10^2
			1	45×10^2
			9	12×10^1
			16	17×10^1
			30	9
	12	3	0	10×10^3
			1	44×10^2
			9	90×10^1
			16	28×10^1
			30	5
17802	0	1	0	38×10^2
			1	49×10^1
			9	19
			16	0
			30	0

Appendix Table 3 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	Viable Count (cells per ml)
17802	0	2	0	95×10^{1}
			1	67×10^{1}
			9	4
			16	2
			30	1
	0	3	0	70×10^{1}
			1	29×10^{1}
			9	0
			16	0
			30	0
	3	1	0	17×10^{3}
			1	94×10^{2}
			9	70×10^{3}
			16	0
			30	0
	3	2	0	11×10^{3}
			1	50×10^{2}
			9	12×10^{1}
			16	30×10^{1}
			30	0
	3	3	0	20×10^{3}
			1	69×10^{2}
			9	14×10^{1}
			16	7
			30	2
	6	1	0	19×10^{3}
			1	31×10^{2}
			9	11×10^{1}
			16	2
			30	1
	6	2	0	15×10^{3}
			1	26×10^{2}
			9	29
			16	2
			30	3
	6	3	0	99×10^{2}
			1	30×10^{2}
			9	81
			16	4
			30	0

Appendix Table 3 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	Viable Count (cells per ml)
17802	9	1	0	
			1	
			9	missing
			16	
			30	
	9	2	0	16×10^3
			1	42×10^2
			9	76×10^1
			16	39
			30	6
	9	3	0	24×10^2
			1	7×10^2
			9	60
			16	2
			30	1
	12	1	0	77×10^2
			1	12×10^2
			9	4
			16	3
			30	1
	12	2	0	89×10^2
			1	92×10^1
			9	3
			16	1
			30	0
	12	3	0	17×10^3
			1	55×10^2
			9	4
			16	0
			30	0

Appendix Table 4. Survival of Vibrio parahaemolyticus in Trypticase Soy Broth at -5 ± 1°C.

Strain	Salt Concentration (%)	Replication	Days of Storage	Viable Count (cells per ml)
T-3765-1	0	1	0	missing
			1	5
			9	0
			16	2
			30	3
	0	2	0	49×10^{4}
			1	0
			9	4
			16	3
			30	0
	0	3	0	96×10^{1}
			1	4
			9	0
			16	0
			30	2
	3	1	0	$96 \times 10^{2}_{1}$
			1	18×10^{1}
			9	2
			16	59
			30	missing
	3	2	0	$98 \times 10^{2}_{1}$
			1	33×10^{1}
			9	1
			16	0
			30	0
	3	3	0	$10 \times 10^{3}_{1}$
			1	63×10^{1}
			9	24
			16	0
			30	4
	6	1	0	$12 \times 10^{3}_{1}$
			1	75×10^{1}
			9	7
			16	6
			30	6

Appendix Table 4 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	Viable Count (cells per ml)
T-3765-1	6	2	0	$14x10^3$
			1	$29x10^2$
			9	$33x10^1$
			16	$14x10^1$
			30	46
	6	3	0	$83x10^2$
			1	$78x10^1$
			9	2
			16	0
			30	0
	9	1	0	$17x10^3$
			1	$29x10^2$
			9	$26x10^1$
			16	$18x10^1$
			30	missing
	9	2	0	$86x10^2$
			1	$15x10^2$
			9	95
			16	26
			30	2
	9	3	0	$46x10^2$
			1	$61x10^1$
			9	7
			16	4
			30	1
	12	1	0	$27x10^2$
			1	$77x10^1$
			9	5
			16	2
			30	6
	12	2	0	$88x10^2$
			1	$71x10^2$
			9	$64x10^1$
			16	$24x10^1$
			30	missing
	12	3	0	$43x10^2$
			1	$97x10^1$
			9	19
			16	12
			30	2

Appendix Table 4 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	Viable Count (cells per ml)
SB04-422	0	1	0	$16x10^2$
			1	12
			9	0
			16	1
			30	0
	0	2	0	$25x10^2$
			1	63
			9	2
			16	16
			30	16
	0	3	0	$28x10^2$
			1	65
			9	7
			16	5
			30	4
	3	1	0	$15x10^3$
			1	$99x10^2$
			9	2
			16	24
			30	7
	3	2	0	$15x10^3$
			1	$11x10^3$
			9	3
			16	16
			30	16
	3	3	0	$34x10^3$
			1	$15x10^3$
			9	6
			16	4
			30	4
	6	1	0	$13x10^3$
			1	$11x10^3$
			9	$18x10^1$
			16	$55x10^1$
			30	13
	6	2	0	$12x10^3$
			1	$91x10^2$
			9	$94x10^1$
			16	$11x10^1$
			30	35

Appendix Table 4 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	Viable Count (cells per ml)
SB04-422	6	3	0	22×10^3
			1	13×10^3
			9	41×10^1
			16	74×10^1
			30	11
	9	1	0	19×10^3
			1	12×10^3
			9	58×10^1
			16	29×10^1
			30	31
	9	2	0	95×10^2
			1	56×10^2
			9	21×10^2
			16	16×10^1
			30	6
	9	3	0	12×10^3
			1	56×10^2
			9	13×10^2
			16	55×10^1
			30	missing
	12	1	0	11×10^3
			1	60×10^2
			9	17×10^2
			16	45×10^1
			30	0
	12	2	0	95×10^2
			1	46×10^2
			9	20×10^1
			16	19
			30	2
	12	3	0	10×10^3
			1	70×10^2
			9	missing
			16	34
			30	missing

Appendix Table 4 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	Viable Count (cells per ml)
17802	0	1	0	38×10^{2}
			1	4
			9	14
			16	1
			30	48
	0	2	0	95×10^{1}
			1	14
			9	2
			16	0
			30	2
	0	3	0	70×10^{1}
			1	34
			9	2
			16	4
			30	6
	3	1	0	17×10^{3}
			1	20×10^{2}
			9	8
			16	4
			30	2
	3	2	0	11×10^{3}
			1	27×10^{2}
			9	6
			16	12
			30	8
	3	3	0	20×10^{3}
			1	93×10^{1}
			9	6
			16	0
			30	5
	6	1	0	19×10^{3}
			1	48×10^{2}
			9	15×10^{1}
			16	1
			30	0

Appendix Table 4 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	Viable Count (cells per ml)
17802	6	2	0	$15x10^3$
			1	$54x10^2$
			9	$20x10^1$
			16	5
			30	0
	6	3	0	$99x10^2$
			1	$88x10^1$
			9	2
			16	1
			30	0
	9	1	0	
			1	
			9	missing
			16	
			30	
	9	2	0	$16x10^3$
			1	$31x10^2$
			9	57
			16	8
			30	1
	9	3	0	$24x10^2$
			1	$61x10^1$
			9	1
			16	0
			30	3
	12	1	0	$77x10^2$
			1	$28x10^2$
			9	22
			16	18
			30	0
	12	2	0	$89x10^2$
			1	$30x10^2$
			9	2
			16	1
			30	0
	12	3	0	$17x10^3$
			1	$72x10^2$
			9	1
			16	0
			30	0

Appendix Table 5. Survival of Vibrio parahaemolyticus in Trypticase Soy Broth at -18 ± 1°C.

Strain	Salt Concentration (%)	Replication	Days of Storage	Viable Count (cells per ml)
T-3765-1	0	1	0	missing
			1	11
			9	1
			16	6
			30	3
	0	2	0	49×10^{4}
			1	17
			9	0
			16	1
			30	0
	0	3	0	96×10^{1}
			1	23
			9	80
			16	0
			30	0
	3	1	0	96×10^{2}
			1	59×10^{1}
			9	2
			16	2
			30	0
	3	2	0	98×10^{2}
			1	31×10^{1}
			9	4
			16	2
			30	2
	3	3	0	10×10^{3}
			1	72×10^{1}
			9	49
			16	76
			30	9
	6	1	0	12×10^{3}
			1	23×10^{2}
			9	80
			16	29
			30	11

Appendix Table 5 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	Viable Count (cells per ml)
T-3765-1	6	2	0	14×10^{3}
			1	64×10^{2}
			9	14×10^{2}
			16	82×10^{1}
			30	30
	6	3	0	83×10^{2}
			1	25×10^{2}
			9	67×10^{1}
			16	11
			30	17
	9	1	0	17×10^{3}
			1	86×10^{2}
			9	43×10^{2}
			16	18×10^{2}
			30	13×10^{1}
	9	2	0	86×10^{2}
			1	54×10^{2}
			9	49×10^{1}
			16	37×10^{1}
			30	34
	9	3	0	46×10^{2}
			1	21×10^{2}
			9	48
			16	7
			30	13
	12	1	0	27×10^{2}
			1	11×10^{2}
			9	21
			16	24
			30	47
	12	2	0	88×10^{2}
			1	65×10^{2}
			9	38×10^{2}
			16	26×10^{2}
			30	58×10^{1}
	12	3	0	43×10^{2}
			1	10×10^{2}
			9	21×10^{1}
			16	29×10^{1}
			30	28×10^{1}

Appendix Table 5 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	Viable Count (cells per ml)
SB04-422	0	1	0	15×10^{2}
			1	21
			9	12
			16	4
			30	3
	0	2	0	24×10^{2}
			1	18×10^{1}
			9	1
			16	12
			30	0
	0	3	0	28×10^{2}
			1	34×10^{1}
			9	4
			16	49
			30	43
	3	1	0	15×10^{3}
			1	22
			9	1
			16	6
			30	0
	3	2	0	15×10^{3}
			1	21×10^{2}
			9	21
			16	13
			30	8
	3	3	0	34×10^{3}
			1	57×10^{2}
			9	57×10^{1}
			16	64
			30	71
	6	1	0	13×10^{3}
			1	61×10^{2}
			9	94×10^{1}
			16	0
			30	11
	6	2	0	12×10^{3}
			1	87×10^{2}
			9	17×10^{2}
			16	36×10^{1}
			30	21×10^{1}

Appendix Table 5 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	Viable Count (cells per ml)
SB04-422	6	3	0	22×10^3
			1	13×10^3
			9	31×10^2
			16	11×10^2
			30	5
	9	1	0	19×10^3
			1	13×10^3
			9	64×10^2
			16	79×10^2
			30	22×10^1
	9	2	0	95×10^2
			1	49×10^2
			9	12×10^2
			16	18×10^1
			30	16
	9	3	0	12×10^3
			1	52×10^2
			9	26×10^2
			16	15×10^2
			30	14
	12	1	0	11×10^3
			1	70×10^2
			9	21×10^2
			16	34×10^2
			30	15×10^2
	12	2	0	95×10^2
			1	52×10^2
			9	86×10^1
			16	21×10^1
			30	4
	12	3	0	10×10^3
			1	65×10^2
			9	65×10^1
			16	26×10^1
			30	4
17802	0	1	0	38×10^2
			1	13
			9	1
			16	1
			30	2

Appendix Table 5 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	Viable Count (cells per ml)
17802	0	2	0	$95x10^1$
			1	4
			9	0
			16	0
			30	1
	0	3	0	$70x10^1$
			1	65
			9	missing
			16	1
			30	1
	3	1	0	$17x10^3$
			1	$59x10^2$
			9	15
			16	1
			30	0
	3	2	0	$11x10^3$
			1	$37x10^2$
			9	$16x10^1$
			16	8
			30	15
	3	3	0	$20x10^3$
			1	$16x10^2$
			9	$11x10^1$
			16	24
			30	1
	6	1	0	$19x10^3$
			1	$73x10^2$
			9	10
			16	1
			30	1
	6	2	0	$15x10^3$
			1	$60x10^2$
			9	63
			16	18
			30	3
	6	3	0	$99x10^2$
			1	$70x10^1$
			9	35
			16	2
			30	1

Appendix Table 5 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	Viable Count (cells per ml)
17802	9	1	0	
			1	
			9	missing
			16	
			30	
	9	2	0	16×10^3
			1	60×10^2
			9	63
			16	18
			30	3
	9	3	0	24×10^2
			1	50×10^1
			9	74
			16	5
			30	0
	12	1	0	77×10^2
			1	35×10^2
			9	40×10^1
			16	37
			30	26
	12	2	0	89×10^2
			1	42×10^2
			9	12×10^2
			16	12×10^1
			30	0
	12	3	0	17×10^3
			1	11×10^3
			9	22×10^2
			16	37×10^1
			30	0

Appendix Table 6. Survival of *Vibrio parahaemolyticus* in Fish Homogenate at 48 ± 1°C.

Strain	Salt Concentration (%)	Replication	Length of Heating (min.)	3% STSA[a] (cells per ml)	VMM[b] (cells per ml)
T-3765-1	0	1	0	$56x10^1$	$45x10^1$
			.5	$10x10^1$	$10x10^1$
			5	$12x10^1$	97
			10	83	84
			20	$11x10^1$	74
	0	2	0	$20x10^2$	$14x10^2$
			.5	$20x10^2$	$15x10^2$
			5	$62x10^1$	$66x10^1$
			10	$71x10^1$	$76x10^1$
			20	$69x10^1$	$69x10^1$
	3	1	0	$95x10^2$	$11x10^3$
			.5	$89x10^2$	$10x10^3$
			5	$78x10^2$	$92x10^2$
			10	$82x10^2$	$74x10^2$
			20	$69x10^2$	$56x10^2$
	3	2	0	$10x10^3$	$11x10^3$
			.5	$12x10^3$	$11x10^3$
			5	$13x10^3$	$81x10^2$
			10	$90x10^2$	$79x10^2$
			20	$56x10^2$	$71x10^2$
	6	1	0	$16x10^3$	$17x10^3$
			.5	$17x10^3$	$16x10^3$
			5	$13x10^3$	$12x10^2$
			10	$58x10^2$	$54x10^2$
			20	$16x10^2$	$13x10^2$
			40	68	$5x10^1$
	6	2	0	$88x10^2$	$86x10^2$
			.5	$84x10^2$	$95x10^2$
			5	$86x10^2$	$87x10^2$
			10	$67x10^2$	$66x10^2$
			20	$58x10^2$	$48x10^2$
			40	$22x10^2$	$19x10^2$
	9	1	0	$81x10^2$	$77x10^2$
			.5	$89x10^2$	$91x10^2$
			5	$62x10^2$	$76x10^2$
			10	$56x10^2$	$45x10^2$
			20	$34x10^2$	$30x10^2$
			40	$19x10^2$	$18x10^2$

Appendix Table 6 (Continued)

Strain	Salt Concentration (%)	Replication	Length of Heating (min.)	3% STSA[a] (cells per ml)	VMM[b] (cells per ml)
T-3765-1	9	2	0	$81x10^2$	$74x10^2$
			.5	$85x10^2$	$69x10^2$
			5	$76x10^2$	$71x10^2$
			10	$62x10^2$	$55x10^2$
			20	$45x10^2$	$48x10^2$
			40	$24x10^2$	$16x10^2$
	12	1	0	$59x10^2$	$63x10^2$
			.5	$67x10^2$	$72x10^2$
			5	$67x10^2$	$70x10^2$
			10	$61x10^2$	$38x10^2$
			20	$32x10^2$	$32x10^2$
			40	$30x10^2$	$25x10^2$
	12	2	0	$62x10^2$	$54x10^2$
			.5	$51x10^2$	$42x10^2$
			5	$42x10^2$	$44x10^2$
			10	$29x10^2$	$29x10^2$
			20	$18x10^2$	$15x10^1$
			40	$76x10^1$	$29x10^1$
SB04-422	0	1	0	$63x10^1$	$59x10^1$
			.5	$40x10^1$	$26x10^1$
			5	$11x10^1$	$14x10^1$
			10	86	51
			20	65	62
			40	43	24
	0	2	0	$86x10^1$	$16x10^1$
			.5	$93x10^1$	$13x10^1$
			5	42	45
			10	20	29
			20	15	15
			40	9	9
	3	1	0	$23x10^3$	$14x10^3$
			.5	$19x10^3$	$18x10^3$
			5	$22x10^3$	$17x10^3$
			10	$16x10^3$	$15x10^3$
			20	$16x10^2$	$14x10^2$
			40	$64x10^2$	$71x10^2$
			80	$55x10^2$	missing

Appendix Table 6 (Continued)

Strain	Salt Concentration (%)	Replication	Length of Heating (min.)	3% STSA[a] (cells per ml)	VMM[b] (cells per ml)
SB04-422	3	2	0	22×10^3	20×10^3
			.5	21×10^3	19×10^3
			5	21×10^3	20×10^3
			10	17×10^3	15×10^3
			20	13×10^2	14×10^2
			40	48×10^2	48×10^2
			80	26×10^2	32×10^2
	6	1	0	15×10^3	17×10^3
			.5	16×10^3	15×10^3
			5	15×10^3	13×10^3
			10	15×10^3	14×10^3
			20	13×10^2	13×10^3
			40	61×10^2	missing
	6	2	0	18×10^3	15×10^3
			.5	15×10^3	13×10^3
			5	13×10^3	13×10^3
			10	14×10^3	11×10^3
			20	14×10^2	12×10^2
			40	52×10^2	43×10^2
			80	49×10^2	36×10^2
	9	1	0	12×10^3	13×10^3
			.5	89×10^2	92×10^2
			5	91×10^2	92×10^2
			10	96×10^2	93×10^2
			20	95×10^2	86×10^2
			40	35×10^2	27×10^2
	9	2	0	19×10^3	17×10^3
			.5	15×10^3	14×10^3
			5	16×10^3	14×10^3
			10	12×10^3	10×10^3
			20	93×10^2	82×10^2
			40	50×10^2	48×10^2
			80	16×10^2	14×10^2
	12	1	0	13×10^3	12×10^3
			.5	15×10^2	12×10^3
			5	82×10^2	93×10^2
			10	70×10^2	69×10^2
			20	35×10^2	32×10^2
			40	10×10^2	12×10^1
			80	42	14×10^1

Appendix Table 6 (Continued)

Strain	Salt Concentration (%)	Replication	Length of Heating (min.)	3% STSA[a] (cells per ml)	VMM[b] (cells per ml)
SB04-422	12	2	0	$80x10^2_3$	$98x10^2_3$
			.5	$10x10^2_2$	$11x10^2_3$
			5	$65x10^2_2$	$62x10^2_2$
			10	$35x10^2_1$	$26x10^2_1$
			20	$90x10^1_1$	$80x10^1_1$
			40	$24x10^1$	$26x10^1$
			80	11	10
17802	0	1	0	$63x10^1_1$	$59x10^1_1$
			.5	$63x10^1_1$	$53x10^1_1$
			5	$12x10^1_1$	$10x10^1_1$
			10	$10x10^1$	$11x10^1$
			20	80	91
			40	77	76
	0	2	0	$44x10^1_1$	$45x10^1_1$
			.5	$33x10^1_1$	$42x10^1_1$
			5	$13x10^1_1$	$12x10^1_1$
			10	$12x10^1_1$	$12x10^1$
			20	$10x10^1$	93
			40	92	84
	3	1	0	$19x10^3_3$	$18x10^3_3$
			.5	$21x10^3_3$	$16x10^3_3$
			5	$18x10^3_3$	$15x10^3_3$
			10	$13x10^3_2$	$11x10^2_2$
			20	$70x10^2_2$	$51x10^2_2$
			40	$15x10^2_1$	$13x10^1_1$
			80	$74x10^1$	$65x10^1$
	3	2	0	$24x10^3_3$	$22x10^3_3$
			.5	$20x10^3_3$	$20x10^3_3$
			5	$21x10^2_2$	$21x10^2_2$
			10	$73x10^2_2$	$84x10^2_2$
			20	$30x10^2_1$	$23x10^1_1$
			40	$26x10^1_1$	$32x10^1_1$
			80	$11x10^1$	$12x10^1$
	6	1	0	$12x10^3_3$	$10x10^3_3$
			.5	$12x10^2_2$	$13x10^2_2$
			5	$90x10^2_2$	$82x10^2_2$
			10	$56x10^2_2$	$63x10^2_2$
			20	$36x10^2_2$	$34x10^2_2$
			40	$18x10^2_2$	$15x10^2_2$
			80	$15x10^2$	$93x10^1$

Appendix Table 6 (Continued)

Strain	Salt Concentration (%)	Replication	Length of Heating (min.)	3% STSA[a] (cells per ml)	VMM[b] (cells per ml)
17802	6	2	0	18×10^3	19×10^3
			.5	19×10^3	17×10^3
			5	16×10^3	15×10^3
			10	12×10^2	12×10^2
			20	67×10^2	62×10^2
			40	18×10^2	20×10^2
	9	1	0	10×10^3	91×10^2
			.5	98×10^2	89×10^2
			5	50×10^2	50×10^2
			10	24×10^1	22×10^1
			20	95×10^1	90×10^1
			40	54×10^1	46×10^1
			80	23×10^1	19×10^1
	9	2	0	83×10^2	76×10^2
			.5	89×10^2	78×10^2
			5	44×10^2	38×10^2
			10	11×10^2	12×10^1
			20	38×10^1	45×10^1
			40	22×10^1	19×10^1
			80	54	49
	12	1	0	29×10^2	26×10^2
			.5	37×10^2	25×10^2
			5	25×10^2	21×10^2
			10	98×10^1	75×10^1
			20	54×10^1	51×10^1
			40	43×10^1	46×10^1
			80	25×10^1	23×10^1
	12	2	0	33×10^3	31×10^3
			.5	32×10^3	30×10^3
			5	31×10^2	31×10^2
			10	89×10^1	97×10^2
			20	94×10^1	11×10^2
			40	12×10^1	80
			80	39	25

a. Sodium chloride Trypticase soy agar (BBL).

b. Vibrio maintenance medium (Colwell, Adeyemo and Kirtland, 1968).

Appendix Table 7. Survival of Vibrio parahaemolyticus in 6% Sodium Chloride Fish Homogenate at 48 ± 1°C.

Strain	Replication	Length of Heating (min.)	3% STSA[a] (cells per ml)	6% STSA[a] (cells per ml)
T-3765-1	1	0	$16x10^3$	$14x10^3$
		.5	$17x10^3$	$17x10^3$
		5	$13x10^2$	$10x10^2$
		10	$58x10^2$	$32x10^1$
		20	$16x10^2$	$74x10^1$
		40	68	28
	2	0	$88x10^2$	$78x10^2$
		.5	$84x10^2$	$66x10^2$
		5	$86x10^2$	$76x10^2$
		10	$67x10^2$	$73x10^2$
		20	$58x10^2$	$34x10^2$
		40	$22x10^2$	$12x10^2$
SB04-422	1	0	$15x10^3$	
		.5	$16x10^3$	
		5	$15x10^3$	missing
		10	$15x10^3$	
		20	$13x10^2$	
		40	$61x10^2$	
	2	0	$18x10^3$	$17x10^3$
		.5	$14x10^3$	$16x10^3$
		5	$13x10^3$	$17x10^3$
		10	$14x10^3$	$15x10^3$
		20	$14x10^2$	$11x10^2$
		40	$52x10^2$	$53x10^2$
		80	$50x10^2$	missing
17802	1	0	$12x10^3$	$10x10^3$
		.5	$12x10^2$	$11x10^2$
		5	$90x10^2$	$81x10^2$
		10	$56x10^2$	$68x10^2$
		20	$36x10^2$	$43x10^2$
		40	$18x10^2$	$13x10^2$
		80	$15x10^2$	$92x10^1$
	2	0	$18x10^3$	$17x10^3$
		.5	$19x10^3$	$17x10^3$
		5	$16x10^3$	$13x10^2$
		10	$12x10^2$	$88x10^2$
		20	$67x10^2$	$47x10^2$
		40	$19x10^2$	$11x10^2$

a. Sodium chloride Trypticase soy agar (BBL).

Appendix Table 8. Survival of Vibrio parahaemolyticus in Fish Homogenate at -5 ± 1°C.

Strain	Salt Concentration (%)	Replication	Days of Storage	3% STSA[a] (cells per ml)	VMM[b] (cells per ml)
T-3765-1	0	1	0	56×10^{1}	45×10^{1}
			1	15×10^{1}	11×10^{1}
			9	53	60
			16	17	8
			30	missing	missing
	0	2	0	20×10^{2}	14×10^{2}
			1	86×10^{1}	87×10^{1}
			9	15×10^{1}	13×10^{1}
			16	73	10×10^{1}
				21	19
	3	1	0	95×10^{2}	11×10^{3}
			1	98	15×10^{1}
			9	76	69
			16	52	37
			30	50	33
	3	2	0	10×10^{3}	11×10^{3}
			1	16×10^{1}	14×10^{1}
			9	20×10^{1}	92
			16	51	59
			30	42	63
	6	1	0	16×10^{3}	17×10^{3}
			1	65×10^{2}	65×10^{2}
			9	48	45
			16	25	12
			30	16	12
	6	2	0	88×10^{2}	86×10^{2}
			1	15×10^{2}	10×10^{2}
			9	50	65
			16	26	28
			30	14×10^{1}	12×10^{1}
	9	1	0	81×10^{2}	77×10^{2}
			1	36×10^{2}	28×10^{2}
			9	32	35
			16	26	13
			30	18	15

Appendix Table 8 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	3% STSA[a] (cells per ml)	VMM[b] (cells per ml)
T-3765-1	9	2	0	81×10^{2}	74×10^{2}
			1	31×10^{2}	22×10^{2}
			9	12×10^{1}	91
			16	28	16
			30	23	48
	12	1	0	59×10^{2}	63×10^{2}
			1	49×10^{2}	48×10^{2}
			9	20×10^{2}	19×10^{2}
			16	17×10^{2}	18×10^{2}
			30	21×10^{2}	22×10^{2}
	12	2	0	62×10^{2}	54×10^{2}
			1	40×10^{2}	36×10^{2}
			9	37×10^{1}	18×10^{1}
			16	15×10^{1}	87
			30	73	23×10^{1}
SB04-422	0	1	0	63×10^{1}	59×10^{1}
			1	11×10^{1}	93
			9	77	60
			16	52	60
			30	missing	missing
	0	2	0	86×10^{1}	16×10^{1}
			1	63	64
			9	28	44
			16	28	33
			30	33	37
	3	1	0	23×10^{3}	14×10^{3}
			1	73×10^{2}	53×10^{2}
			9	50	44
			16	49	63
			30	21	34
	3	2	0	22×10^{3}	20×10^{3}
			1	64×10^{2}	66×10^{2}
			9	92	11×10^{1}
			16	84	97
			30	57	53

Appendix Table 8 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	3% STSA [a] (cells per ml)	VMM [b] (cells per ml)
SB04-422	6	1	0	$15x10^3$	$17x10^3$
			1	$13x10^3$	$10x10^1$
			9	$22x10^1$	$21x10^1$
			16	53	64
			30	14	21
	6	2	0	$18x10^3$	$15x10^3$
			1	$13x10^3$	$12x10^3$
			9	76	$12x10^1$
			16	75	66
			30	36	43
	9	1	0	$12x10^3$	$13x10^3$
			1	$10x10^3$	$10x10^3$
			9	$14x10^1$	$12x10^1$
			16	90	52
			30	58	49
	9	2	0	$19x10^3$	$17x10^3$
			1	$14x10^3$	$13x10^3$
			9	$31x10^1$	$32x10^1$
			16	53	39
			30	28	39
	12	1	0	$13x10^3$	$12x10^3$
			1	$98x10^2$	$62x10^2$
			9	16	23
			16	34	39
			30	3	4
	12	2	0	$80x10^2$	$98x10^2$
			1	$63x10^2$	$64x10^2$
			9	20	30
			16	14	12
			30	9	11
17802	0	1	0	$63x10^1$	$59x10^1$
			1	$13x10^1$	$13x10^1$
			9	66	53
			16	45	40
			30	4	3

Appendix Table 8 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	3% STSA[a] (cells per ml)	VMM[b] (cells per ml)
17802	0	2	0	44×10^{1}	45×10^{1}
			1	17×10^{1}	14×10^{1}
			9	71	25×10^{1}
			16	27	20
			30	16	11
	3	1	0	19×10^{3}	18×10^{3}
			1	10×10^{2}	12×10^{1}
			9	55×10^{1}	48×10^{1}
			16	37×10^{1}	34×10^{1}
			30	35×10^{1}	34×10^{1}
	3	2	0	24×10^{3}	22×10^{3}
			1	26×10^{2}	25×10^{2}
			9	15×10^{1}	14×10^{1}
			16	11×10^{1}	99
			30	11×10^{1}	86
	6	1	0	12×10^{3}	10×10^{3}
			1	61×10^{2}	55×10^{2}
			9	31×10^{1}	46×10^{1}
			16	32×10^{1}	30×10^{1}
			30	36	36
	6	2	0	18×10^{3}	19×10^{3}
			1	53×10^{2}	58×10^{2}
			9	57	58
			16	29	34
			30	24	23
	9	1	0	10×10^{3}	91×10^{2}
			1	29×10^{2}	27×10^{1}
			9	19×10^{1}	20×10^{1}
			16	13×10^{1}	14×10^{1}
			30	59	60
	9	2	0	83×10^{2}	76×10^{2}
			1	20×10^{2}	20×10^{2}
			9	60	43
			16	39	46
			30	13	21

Appendix Table 8 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	3% STSA[a] (cells per ml)	VMM[b] (cells per ml)
17802	12	1	0	$29 \times 10^{2}_{1}$	$26 \times 10^{2}_{1}$
			1	$80 \times 10^{1}_{1}$	$90 \times 10^{1}_{1}$
			9	$18 \times 10^{1}_{1}$	$15 \times 10^{1}_{1}$
			16	18×10^{1}	20×10^{1}
			30	70	63
	12	2	0	$33 \times 10^{3}_{2}$	$31 \times 10^{3}_{2}$
			1	31×10^{2}	32×10^{2}
			9	61	66
			16	missing	missing
			30	16	8

a. Sodium chloride Trypticase soy agar (BBL).

b. Vibrio maintenance medium (Colwell, Adeyemo and Kirtland, 1968).

Appendix Table 9. Survival of *Vibrio parahaemolyticus* in Fish Homogenate at -18 ± 1°C.

Strain	Salt Concentration (%)	Replication	Days of Storage	3% STSA[a] (cells per ml)	VMM[b] (cells per ml)
T-3765-1	0	1	0	56×10^{1}	45×10^{1}
			1	11×10^{1}	58
			9	48	39
			16	31	27
			30	36	42
	0	2	0	20×10^{2}	14×10^{2}
			1	46×10^{1}	50×10^{1}
			9	41×10^{1}	27×10^{1}
			16	30×10^{1}	27×10^{1}
			30	26×10^{1}	27×10^{1}
	3	1	0	95×10^{2}	11×10^{3}
			1	52×10^{2}	63×10^{2}
			9	17×10^{1}	37×10^{1}
			16	11	14
			30	31	18
	3	2	0	10×10^{3}	11×10^{3}
			1	63×10^{2}	60×10^{2}
			9	23×10^{1}	90
			16	29	14
			30	12	13
	6	1	0	16×10^{3}	17×10^{3}
			1	13×10^{3}	12×10^{3}
			9	24×10^{2}	26×10^{2}
			16	79	79
			30	73	76
	6	2	0	88×10^{2}	86×10^{2}
			1	74×10^{2}	51×10^{2}
			9	14×10^{1}	12×10^{1}
			16	20	10
			30	8	8
	9	1	0	81×10^{2}	77×10^{2}
			1	37×10^{2}	54×10^{2}
			9	21×10^{1}	20×10^{1}
			16	10	16
			30	16	24

Appendix Table 9 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	3% STSA[a] (cells per ml)	VMM[b] (cells per ml)
T-3765-1	9	2	0	$81x10^2$	$74x10^2$
			1	$46x10^2$	$40x10^2$
			9	$86x10^1$	$81x10^1$
			16	19	51
			30	5	12
	12	1	0	$59x10^2$	$63x10^2$
			1	$42x10^2$	$46x10^2$
			9	$18x10^2$	$17x10^2$
			16	$14x10^2$	$16x10^2$
			30	$18x10^1$	$16x10^1$
	12	2	0	$62x10^2$	$54x10^2$
			1	$37x10^2$	$34x10^2$
			9	$67x10^1$	$61x10^1$
			16	3	$17x10^1$
			30	$15x10^1$	$11x10^1$
SB04-422	0	1	0	$63x10^1$	$59x10^1$
			1	37	36
			9	24	26
			16	28	25
			30	27	27
	0	2	0	$86x10^1$	$16x10^1$
			1	28	22
			9	19	17
			16	16	21
			30	9	11
	3	1	0	$23x10^3$	$14x10^3$
			1	$81x10^2$	$67x10^2$
			9	60	80
			16	28	31
			30	23	24
	3	2	0	$22x10^3$	$20x10^3$
			1	$12x10^3$	$10x10^3$
			9	$19x10^1$	$21x10^1$
			16	$13x10^1$	$11x10^1$
			30	70	67

Appendix Table 9 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	3% STSA[a] (cells per ml)	VMM[b] (cells per ml)
SB04-422	6	1	0	$15x10^3$	$17x10^3$
			1	$73x10^2$	$88x10^2$
			9	$30x10^2$	$28x10^2$
			16	57	76
			30	49	59
	6	2	0	$18x10^3$	$15x10^3$
			1	$11x10^3$	$96x10^2$
			9	$72x10^1$	$78x10^1$
			16	$19x10^1$	$16x10^1$
			30	41	37
	9	1	0	$12x10^3$	$13x10^3$
			1	$83x10^2$	$10x10^3$
			9	$72x10^1$	$82x10^1$
			16	23	47
			30	32	27
	9	2	0	$19x10^3$	$17x10^3$
			1	$15x10^3$	$15x10^3$
			9	$22x10^2$	$24x10^2$
			16	56	54
			30	26	29
	12	1	0	$13x10^3$	$12x10^3$
			1	$92x10^2$	$86x10^2$
			9	$87x10^1$	$98x10^1$
			16	75	$11x10^1$
			30	12	7
	12	2	0	$80x10^2$	$98x10^2$
			1	$58x10^2$	$65x10^2$
			9	$71x10^1$	$68x10^1$
			16	79	57
			30	13	11
17802	0	1	0	$63x10^1$	$59x10^1$
			1	53	48
			9	19	21
			16	31	33
			30	30	32

Appendix Table 9 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	3% STSA[a] (cells per ml)	VMM[b] (cells per ml)
17802	0	2	0	44×10^{1}	45×10^{1}
			1	89	11×10^{1}
			9	25	19
			16	55	35
			30	41	46
	3	1	0	19×10^{3}	18×10^{3}
			1	12×10^{3}	12×10^{3}
			9	28×10^{1}	21×10^{1}
			16	29×10^{1}	31×10^{1}
			30	23×10^{1}	24×10^{1}
	3	2	0	24×10^{3}	22×10^{3}
			1	93×10^{2}	10×10^{3}
			9	14×10^{1}	15×10^{1}
			16	99	10×10^{1}
			30	92	98
	6	1	0	12×10^{3}	10×10^{3}
			1	39×10^{2}	48×10^{2}
			9	69×10^{1}	73×10^{1}
			16	25×10^{1}	24×10^{1}
			30	24×10^{1}	24×10^{1}
	6	2	0	18×10^{3}	19×10^{3}
			1	93×10^{2}	11×10^{3}
			9	16×10^{1}	21×10^{1}
			16	48	32
			30	22	24
	9	1	0	10×10^{3}	91×10^{2}
			1	25×10^{2}	22×10^{2}
			9	13×10^{1}	14×10^{1}
			16	13×10^{1}	15×10^{1}
			30	13×10^{1}	12×10^{1}
	9	2	0	83×10^{2}	76×10^{2}
			1	18×10^{2}	17×10^{2}
			9	14×10^{1}	11×10^{1}
			16	24	21
			30	23	17

Appendix Table 9 (Continued)

Strain	Salt Concentration (%)	Replication	Days of Storage	3% STSA[a] (cells per ml)	VMM[b] (cells per ml)
17802	12	1	0	29×10^{2}	26×10^{2}
			1	90×10^{1}	70×10^{1}
			9	96	10×10^{1}
			16	11×10^{1}	16×10^{1}
			30	10×10^{1}	19×10^{1}
	12	2	0	33×10^{3}	31×10^{3}
			1	69×10^{2}	69×10^{2}
			9	80	11×10^{1}
			16	49	47
			30	36	32

a. Sodium chloride Trypticase soy agar (BBL).

b. Vibrio maintenance medium (Colwell, Adeyemo and Kirtland, 1968).