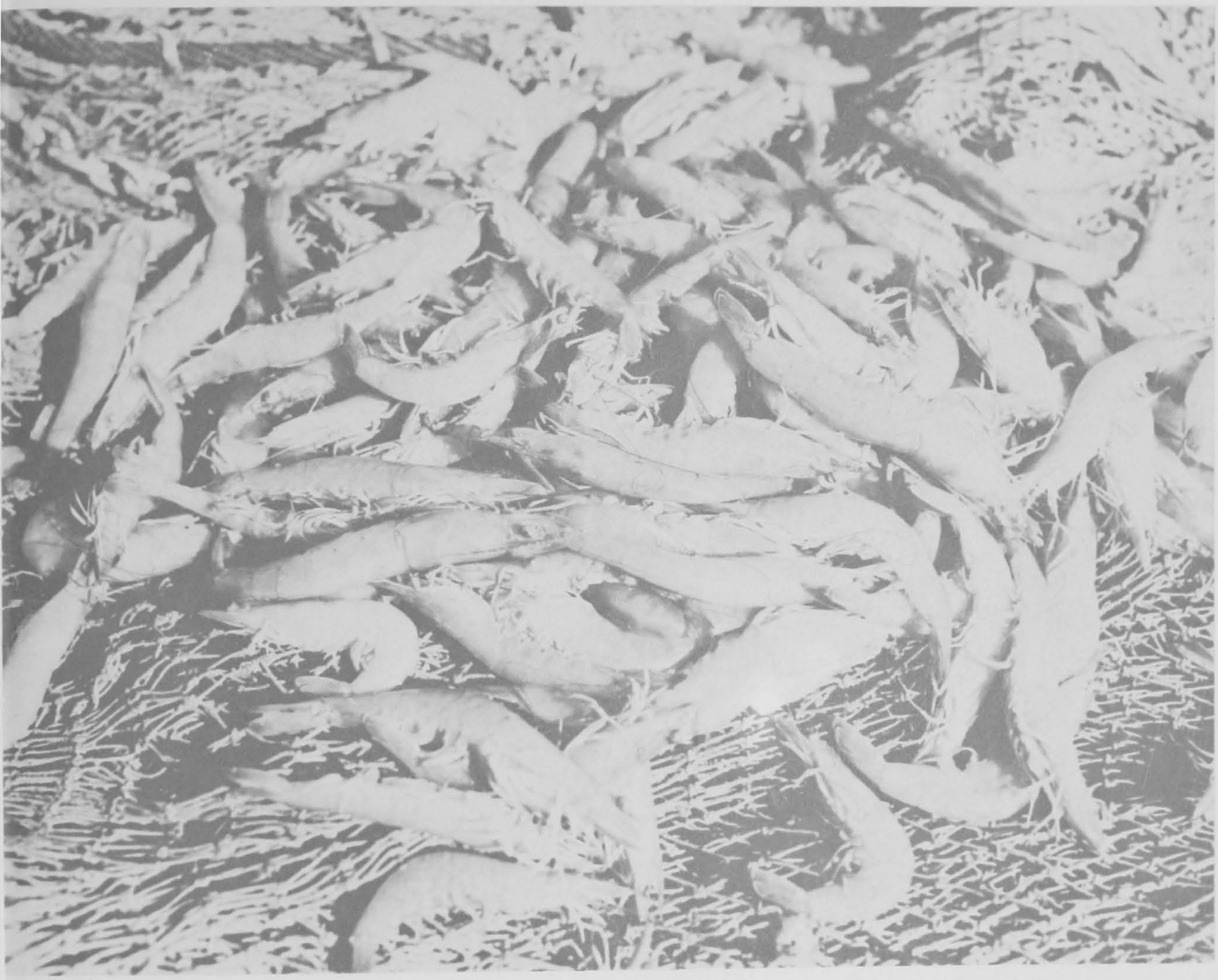
MICROBIOLOGICAL STUDY OF ICED SHRIMP:
Excerpts from the 1965 Iced–Shrimp Symposium



UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
BUREAU OF COMMERCIAL FISHERIES

Circular 284

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Microbiological Study of Iced Shrimp: Excerpts from the 1965 Iced-Shrimp Symposium

by

B. J. CARROLL, G. B. REESE, and B. Q. WARD

Circular 284

Washington, D.C.
May 1968

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington, D.C. 20402 - Price 35 cents

CONTENTS

FOREWORD

In 1965, the staff of the Bureau of Commercial Fisheries Technological Laboratory, Pascagoula, Miss., gave a symposium for members of the iced-shrimp industry. The industry representatives participated actively in discussions, asked a great variety of questions, and made practical suggestions from their experience. Barest essentials of the bacteriological findings presented at the symposium have been published in the annual report of the Technological Laboratory at Pascagoula, fiscal year 1965 (Fish and Wildlife Circular 251). The present report includes very nearly all of the bacteriological material, as well as a representative selection of the histological presentations of that symposium. Essentials of chemical results presented in the symposium were also published in the annual report of 1965.

Microbiological Study of Iced Shrimp: Excerpts from the 1965 Iced-Shrimp Symposium

By

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ABSTRACT

The presentations of studies of microbiology and summarized results of the abridged histological studies are reported. How marine and land bacteria cause spoilage under refrigerated conditions is explained. Total numbers of bacteria, the changing makeup of bacterial populations, and organoleptic grades are given for pink, white, and brown shrimps through 14 days of iced storage. The effects of thorough washing are contrasted to those of average washing and the superior quality of well-washed shrimp in the second week of iced storage is shown by lower bacterial counts and the prolongation of grade. Practical recommendations, based upon experimental observations or reports in the literature, are offered. A series of photomicrographs show how the tissues of shrimps of all three species disintegrate in much the same way during 14 days of iced storage. A selection of suggested references for additional reading is provided.

INTRODUCTORY REMARKS ON MICROBIOLOGY

We have no new solutions for controlling microbial spoilage. We feel, however, that we are gaining a clear understanding of how shrimp spoil and are beginning to separate the various factors that contribute to spoilage. We have found that certain developments, such as plate count fluctuations, population changes, histological changes, and decrease in grade, appear regularly and that these developments are closely related. We also have found that microbiological observations dovetail neatly with the results of chemical studies. When bacteriology, chemistry, histology, and organoleptic evaluations fit together to produce a coherent picture of spoilage, we feel that such a picture is worthy of attention.

In the presentation of illustrations, the following major points will be developed:

1. Bacterial counts, as such, are not necessarily a definitive indication of quality, because we must take into account such factors as shrimp species, bottom characteristics, and time.

2. The makeup of the bacterial populations undergoes a periodic drastic change: certain species drop from a dominant position to one of relative insignificance; other species, previously few in number, suddenly increase enormously. Thus, the makeup of populations is probably more significant than is the total number of bacteria present when a count is made.

3. Finally, all iced or fresh shrimp spoil eventually, regardless of the care taken. Even shrimp touched only by the net and never allowed to come in contact with man, commercial ice, or the vessel will show changes in their bacterial population and become unfit for consumption despite low counts of bacteria.

In relation to Point 2--the makeup of populations--we might ask the question: Why do populations of bacteria shift or change? The only way that a species of bacteria can continue to exist as a species is to reproduce rapidly and to endure the extremes of an environment over which it has little influence. Reproduction times in hours, or even days, are realistic when dealing with bacteria that grow on iced materials. Even so, this rate of reproduction is very rapid--a fact that explains the reason for washing shrimp. Every bacterium that is washed off represents an enormous number of cells that will not be present several days later. If a bacterial cell were to divide only once a day for 2 weeks, it could give rise to over 4,000 cells; if only twice a day, tens of millions would result.

With a favorable environment, a bacterium will produce tremendous numbers like itself in a short time; with an unfavorable environment, the bacteria begin to die or to assume

an inert form. In time, when the environment again becomes favorable, the survivors may increase rapidly again. Populations of bacteria increase and decrease continuously in nature but not in shrimp samples preserved with ice. The temperature is set with melting ice, so the bacteria that prefer higher temperatures decline in numbers. We set the salinity as one of ever-decreasing salt concentration as melting ice washes the salt away. Without the influx of salt water, the salt-dependent bacteria find conditions increasingly unfavorable and, finally, impossible. In a closed evironment, foods are not replenished. When organisms that can use only a few types of food exhaust their limited supply, they die and are replaced by other bacteria that can use either food untouched by the earlier group or the waste products of that group.

In general, marine or estuarine microorganisms are probably better adapted to cold than are most land forms. To survive, salt-water organisms must live and reproduce at temperatures that are usually cool. Many land forms endure cold but must wait for warm temperatures before they reproduce. A few land forms, however, can function even at a few degrees below freezing.

Icing immediately establishes conditions favoring marine or estuarine microorganisms and land forms that can function at low temperatures in the presence of some salt. At sea, and to a lesser extent even in estuaries, the major sources of land bacteria are the boat, gear, and crew. With the passage of time, melting ice progressively reduces the overall salinity of the shrimp. True marine microbes disappear because they are unable to tolerate lowered salinity. Estuarine microorganisms, however, regularly endure changes in salinity and may become dominant in iced, aseptically collected shrimp.

We have not been able to complete the lengthy procedures necessary to determine the actual species involved in population shifts in iced shrimp, nor to pinpoint, from the long list of possible causes, the main cause of each shift. Our initial observations, however, lead us to believe that our findings on populations will be in fair agreement with those reported by other scientists.

Some of you may be familiar with the counts reported by such scientists as O. B. Williams or M. Green. Later in the program, you may wonder with respect to Point 1--bacterial counts--why our counts are so much higher than theirs are. We believe that the difference in experimental design explains the difference in the results. Williams washed his shrimp and counted bacteria in the wash water, or else he considered only the contents of the gut of the shrimp. Green used frozen shrimp; however, we know that freezing may reduce counts by as much as 85 percent. The difference in the results then is not as great as it might seem. Reports of Camber, Vance, and Alexander are more in line with our own.

With respect to Point 3--the eventual spoilage of all shrimp--we have some curious results. Somewhat later, the chemists will discuss and compare their chemical analyses of "aseptic" and commercial shrimp. You will be told in the discussion of histology that "aseptic" shrimp are no more resistant to body disintegrations than are regular commercial shrimp. Thus, shrimp, as caught, normally have a microbial flora that is fully capable of destroying them. Our results indicate that this flora alone, although its component microbes are never very numerous, is associated with the destruction of a shrimp in only half again the time required to destroy its commercial counterpart, which has been exposed to all manner of extraneous contamination. Of course, we cannot claim that all of this damage is brought about by the normal bacterial flora, because some part of the damage, and probably a considerable part, may be caused by the actions of the shrimp's own enzymes. The bacteria and the enzymes, however, must be wholly responsible for the damage.

Later you will be presented with recommendations on how to extend the storage life of shrimp. These suggested procedures probably will not be entirely new to you but may have certain new aspects that are of interest. In the two areas of study--(1) population changes during storage and (2) spoilage from natural microbial flora and from native enzymes of shrimp--the following questions must be answered: (1) What factors influence the microbial flora and how do these factors function, (2) what changes do the microbial flora cause in the shrimp, and (3) of these changes, which are due to bacterial action and which to the shrimp's own enzymes? If research could provide a clear understanding of these questions, we might be able to find new approaches to the problem of modifying or halting the deterioration of shrimp. The road would be long and probably quite tedious and expensive to follow, but the research could prove extremely rewarding.

BACTERIAL RESULTS ON ICED GULF SHRIMP

Aims of the Study

The overall purposes of our recent bacteriological study were (1) to determine, beginning no more than 8 hours after capture of the shrimp (inclusive of their heading and washing) and continuing for 2 weeks, the daily increase in bacterial load of iced headless shrimp; (2) to determine the differences in bacterial loads of well-washed as opposed to average-washed shrimp; (3) to compare, bacteriologically, the

three commercial species--whites, pinks, and browns--of penaeid shrimp found in the Gulf; (4) to relate bacterial buildup of damage to connective tissue in the stored shrimp; and (5) to determine the initial bacterial load of white and brown shrimps collected as nearly aseptically as practical, and to compare the daily increase in this load with the well- and average-washed shrimp of the same species.

Methods

Shrimp.--Brown and white shrimps were taken in waters of Louisiana and Mississippi, relatively close to the Pascagoula laboratory. Pink shrimp were caught in Florida waters and returned to the laboratory in ice. In all instances, commercial shrimping vessels and ordinary commercial gear, operated by experienced commercial vessels' crews, were used. All catches were handled as nearly alike as possible, each species being washed ("good" and "average" wash), headed, iced, and returned to the laboratory, where bacterial plate counts were made regularly through 2 weeks of storage in crushed ice in commercial wooden boxes. The makeup of each bacterial population was also regularly followed.

Washing.--Admittedly, "good" and "average" when applied to washing are subjective terms, but we tried to use as "average" a wash that most industry people would consider neither uncommonly rigorous nor uncommonly lax. Obvious mud and incidental trash was removed, but the catch was not closely examined for such materials. In a "good" wash, water draining from baskets had to be clear, and obvious mud and trash had to be absent even upon close inspection. Good washing is one essential factor in extending the storage life of the product. The type of bottom from which the shrimp are taken should influence the type and extent of washing given the product. A shrimp caught on a muddy bottom requires a much better wash than does a shrimp caught on a clear sandy bottom.

"Aseptic" samples.--A member of our staff collected parts of many catches to ensure the reliability of "aseptic" samples. Using sterile tongs and scissors, he headed the samples and placed them into sterile glass jars containing sterile ice cubes. The jars containing these shrimp were stored in regular wooden shrimp boxes packed with crushed ice. At no time were these shrimp allowed to come into contact with the collector's hands, the vessel, or any other possible source of extraneous contamination other than, unavoidably, the trawl itself. Thus, the number of, and type of, microorganisms present on these samples should represent overwhelmingly those that are indigenous to the shrimp, mud, or water. The remainders of these catches were then used as washed and well-washed samples.

Comparison with previous reports.--In the past, numerous bacteriological studies have been published. Those concerned with the bacteriology of iced shrimp dealt with:

1. The external flora of Gulf white shrimp (Williams, Rees, and Campbell, 1952; Williams, Campbell, and Rees, 1952).
2. The intestinal flora of white shrimp (Williams and Rees, 1952).
3. Changes during storage of white shrimp (Campbell and Williams, 1952).
4. The bacteriology of Gulf coast shrimp (Green and Fieger, 1947). (We are not certain what species of shrimp were used.)
5. "Black spot" in pink shrimp--for over 2-1/2 weeks the scientists followed the increase in bacteria on the shrimp (Camber, Vance, and Alexander, 1957).

In general, our results agree with most of the data in the preceding publications; our counts of bacteria, however, are considerably higher, except for those obtained by Camber, Vance, and Alexander (1957). These workers made direct microscopic counts of bacteria and then reported them in a manner that permits their data to be compared with ours. We believe that our data differ from those of some of the other scientists because our way of preparing samples enabled us to recover bacteria from the entire sample and because we had a different culture methodology (such as the use of sea water in our media or the incubation of our samples at a lower temperature).

Total plate count.--We used the standard total-plate-count method to determine daily the number of bacteria per gram of shrimp. The procedure used in making a total plate count is as follows: 50 grams of headless shrimp with shell intact was placed in a sterile Waring[1] blendor jar containing 450 cc. of sterile phosphate buffer and blended for 2 minutes. From this blended portion, further dilutions were made as needed. Each dilution was plated by pipetting 1.0 cc. of the dilution into an empty petri dish and pouring sterile melted Tryptone Glucose Extract Agar into the plate, then mixing and allowing the agar to solidify. The medium was prepared with both distilled and filtered sea water. Once inoculated, the plates were divided into two groups--one being incubated at 25° C. (77° F.); the other, at 37° C. (98.6° F.). The plates were incubated at these temperatures for 48 hours. Then they were removed and the colonies were counted. The total plate count for each sample was determined by averaging data from duplicate plates of the same dilution.

The total plate count method of determining numbers of bacteria present in or on a substance is based on the assumption that each

[1] Use of trade names does not imply endorsement.

colony developed from a single cell (trapped when the melted agar gelled). This method gave a reasonably good estimate of the actual number of cells originally present. We found that plates containing the agar medium prepared with sea water, when incubated at 25° C., gave consistently higher counts, which indicated a more nearly complete recovery of the bacteria present. All of the graphs presented here will be based on these 25° C. salt-water plates.

Population composition.--Since each type of bacterium can be identified only by a rather large number of tests, and the tests required differ from species to species, we believe that only persons primarily concerned with bacteria rather than shrimp quality would be interested in detailed information of actual steps. We followed the generally established procedures as outlined in Bergey's Manual of Determinative Bacteriology.

Tissue damage.--The study of tissues is so different from the study of bacteria that it has been assigned a section, "Histological Findings." Development of changes here reported, however, can be related to the tissue changes noted in that subsequent section.

Counts of Bacteria

First, let us offer an explanation for Figures 1 and 2. Figures 1 and 2 cover the period 0 to 15 days; line graphs show the first and second weeks' counts in thousands (these are usually shown in millions). Millions cannot be drawn to a scale of thousands, and thousands barely show on a scale of millions. Therefore, we preferred to use two graphs rather than a logarithmic scale. Figure 3 is for the first 7 days and shows the counts in millions. Figures 4 to 6 cover the period 0 to 15 days and show the counts in millions.

From a bacteriological standpoint, the first important generalization that can be made concerning shrimp is that they are highly variable. This variability is probably due to one or more of such factors as size of shrimp, type of bottom the shrimp inhabit, their intestinal flora, and contact with extraneous contaminants. A prime example of the effect produced by the type of bottom they inhabit can be seen in a comparison of bacterial counts for brown or white shrimp, which inhabit muddy bottoms, with those for pink shrimp, which inhabit clear sandy bottoms (figs. 1, 2, and 3). The variability in bacterial counts manifests itself throughout the storage life of the shrimp (figs. 2, 4, 5, and 6).

The bacterial counts on the white or brown shrimp were initially higher than those on the pinks, and reached much higher numbers throughout the study than did those of the pinks. The simplest explanation for these differences relates to the type of bottom the shrimp came from--a muddy bottom tends to have a much higher bacterial count per gram of sediment than does a sandy bottom.

Changes in Populations of Bacteria

Campbell and Williams (1952) followed microbial population changes in iced shrimp (table 1). Micrococcus, Bacillus, and Flavobacterium decreased steadily, and Achromobacter increased steadily. During the study we isolated about 200 typical colony types. Although we did not have time to complete a taxonomic study on our isolates, we feel that our preliminary examination shows that our isolates follow the same general pattern described by Campbell and Williams (1952). These major changes in species populations were reflected throughout this investigation by sharp declines in total counts, which usually preceded the development of even higher counts.

The first major change in the population of bacteria occurred in our white and pink shrimps between the 4th and 5th days (fig. 1&2). These changes can probably be attributed to the loss of micrococci. This conclusion is supported by a drastic reduction in the micrococci appearing on stained smears and the almost total absence of typical colony types of micrococci. Campbell and Williams (1952) also found a sharp decline in this genus between the 4th and 8th days (table 1). In our brown shrimp, this change was delayed somewhat, for it did not occur until the 7th and 8th days (figs. 3&4 and 6&7). We cannot offer any reason for the delay.

The second major change in population occurred between the 10th and 11th days in our well-washed white shrimp and well-washed brown shrimp (figs. 4 and 6). The same change occurred on the 9th and 10th days in the well-washed pink shrimp (fig. 5). This change in population corresponds to the sharp drop in Flavobacterium indicated by Campbell and Williams (1952; table 1). This change might logically be attributed to the reduction of the salinity of the shrimp since Flavobacterium species are perhaps better adapted to higher salinities than are the other species present.

The third and last major change in population occurred between the 12th and 15th days in all three species (figs. 4, 5, and 6). This change seems to represent the points where the number of pseudomonads is reduced and the number of Achromobacter is increased. In this period, Achromobacter becomes the dominant type of microorganism in the spoilage pattern.

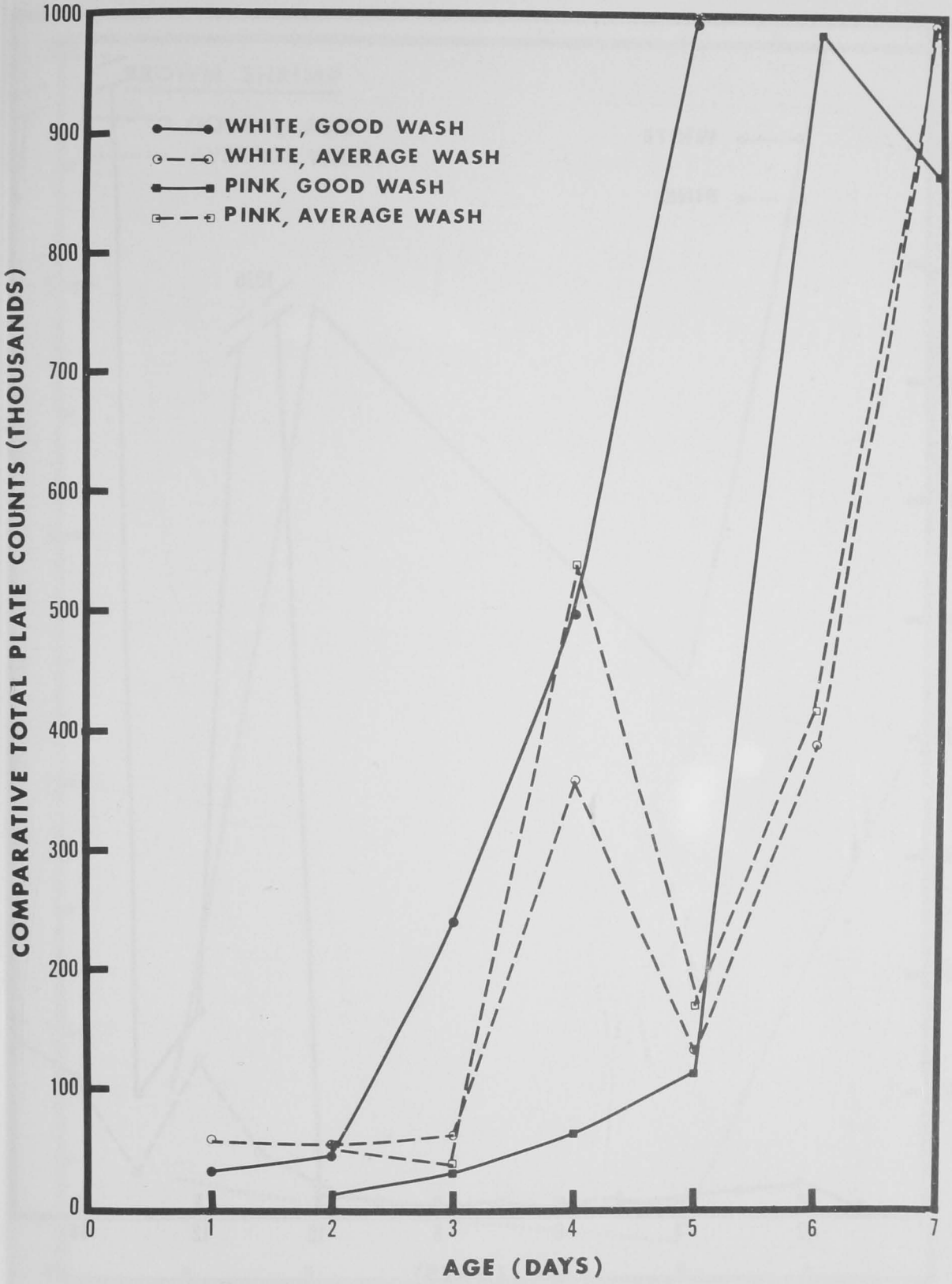


Figure 1.--Comparative total plate counts during the first 7 days of iced storage of pink and white shrimp on salt-water media at 25° C.

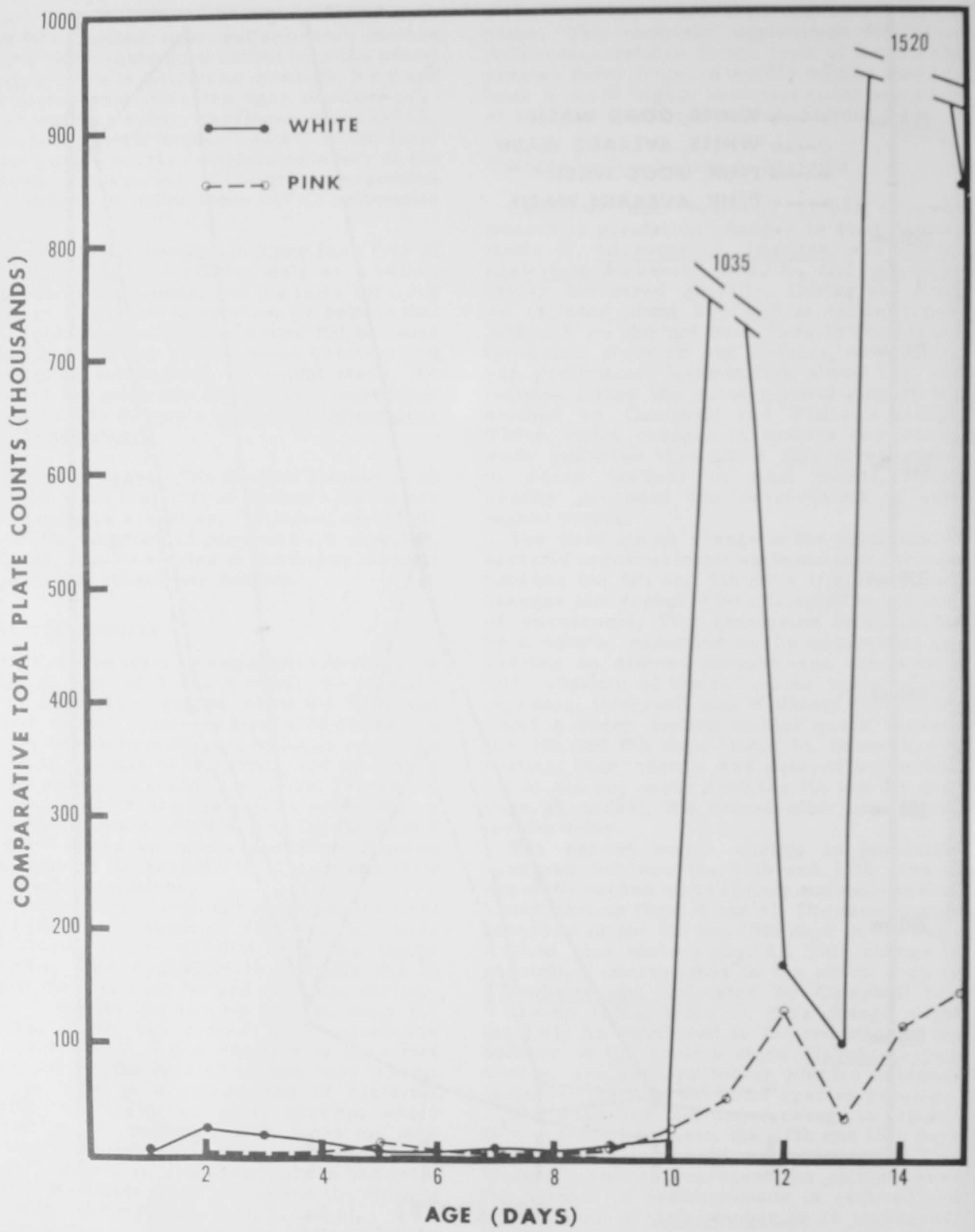


Figure 2.--Total plate counts of "aseptically" collected white and pink shrimp homogenates on sea-water media at 25° C.

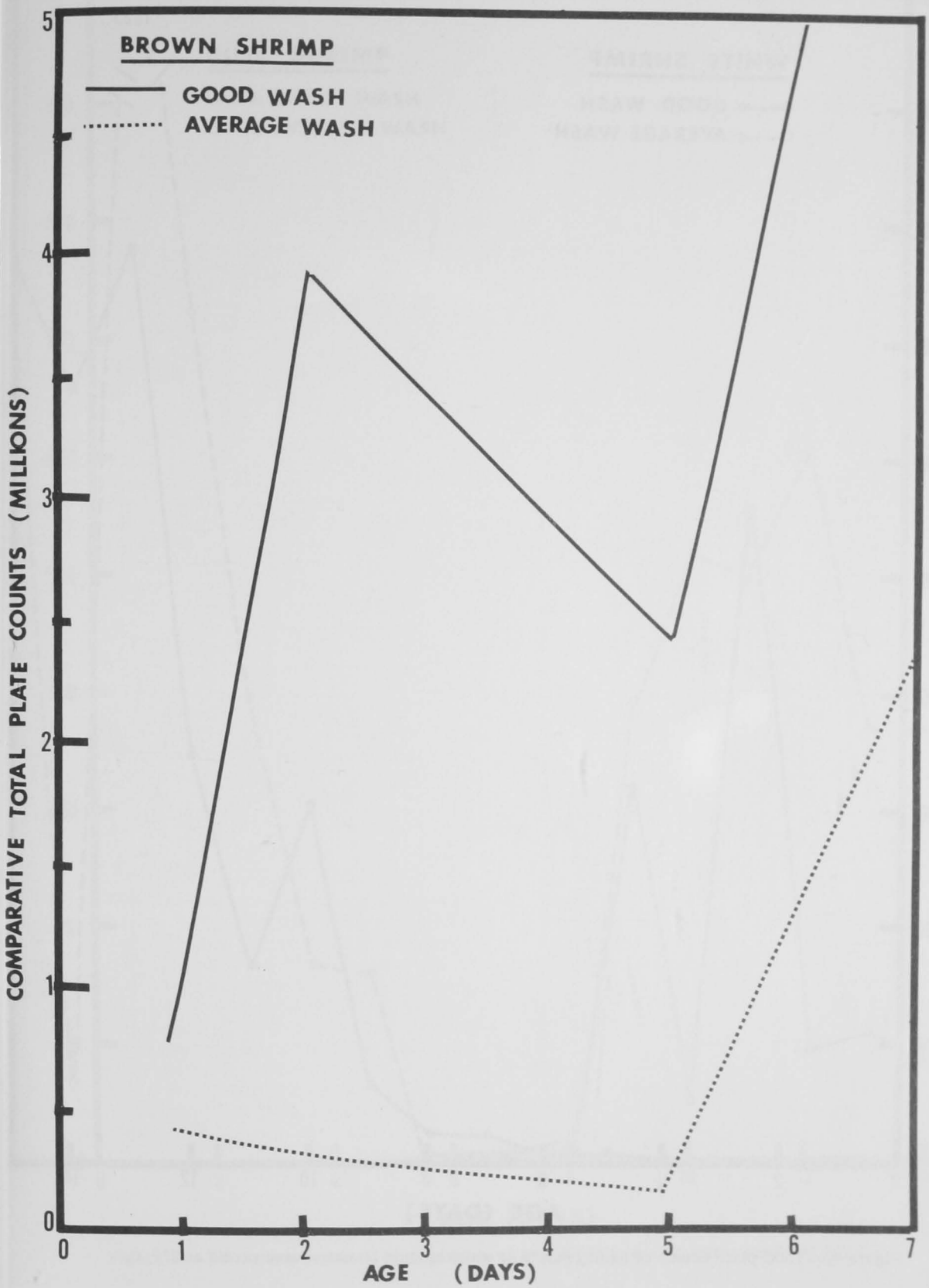


Figure 3.--Comparative total plate counts during first 7 days of iced storage of brown shrimp on salt-water media at 25° C.

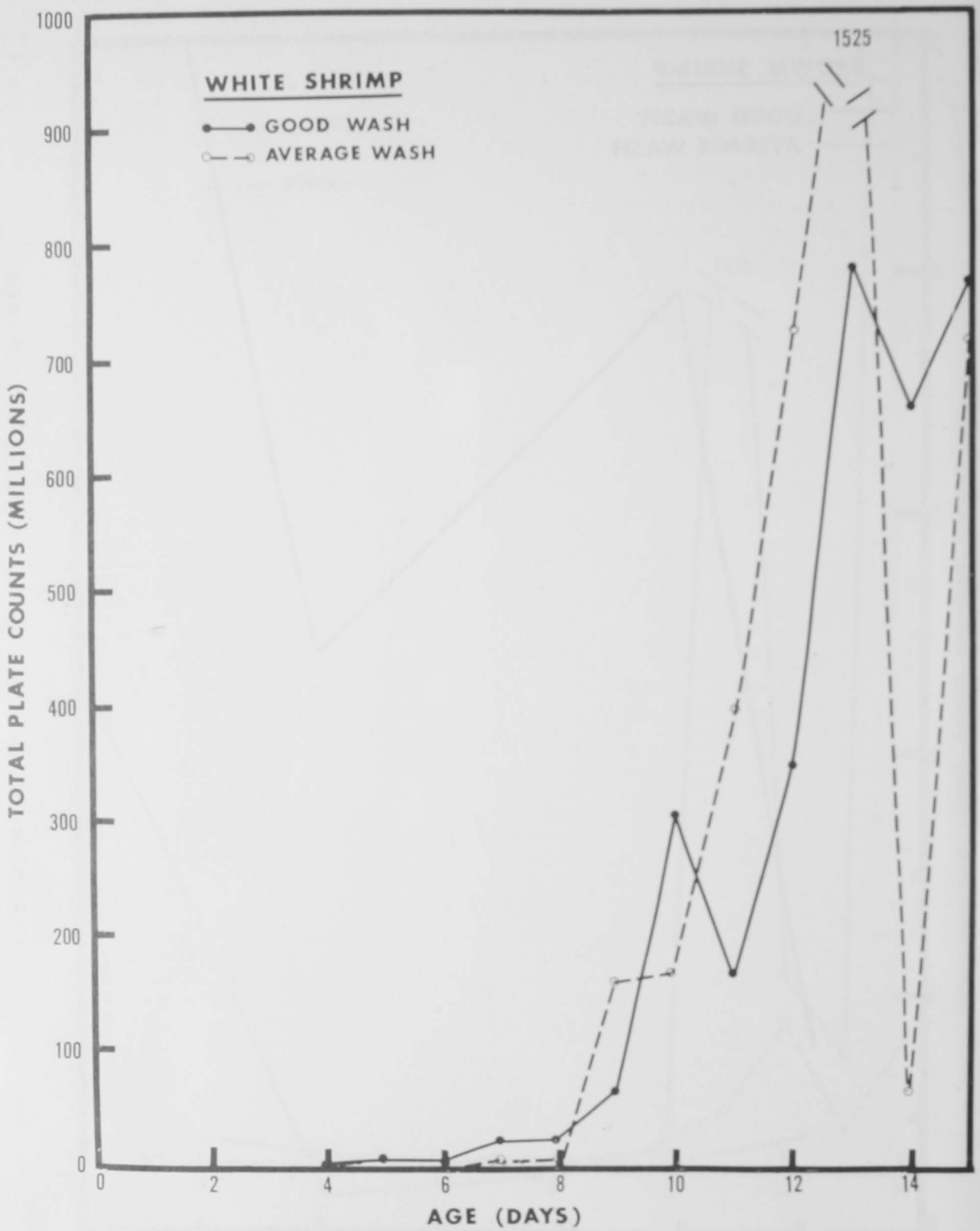


Figure 4.--Total plate counts of homogenates of white shrimp on salt-water media at 25° C.

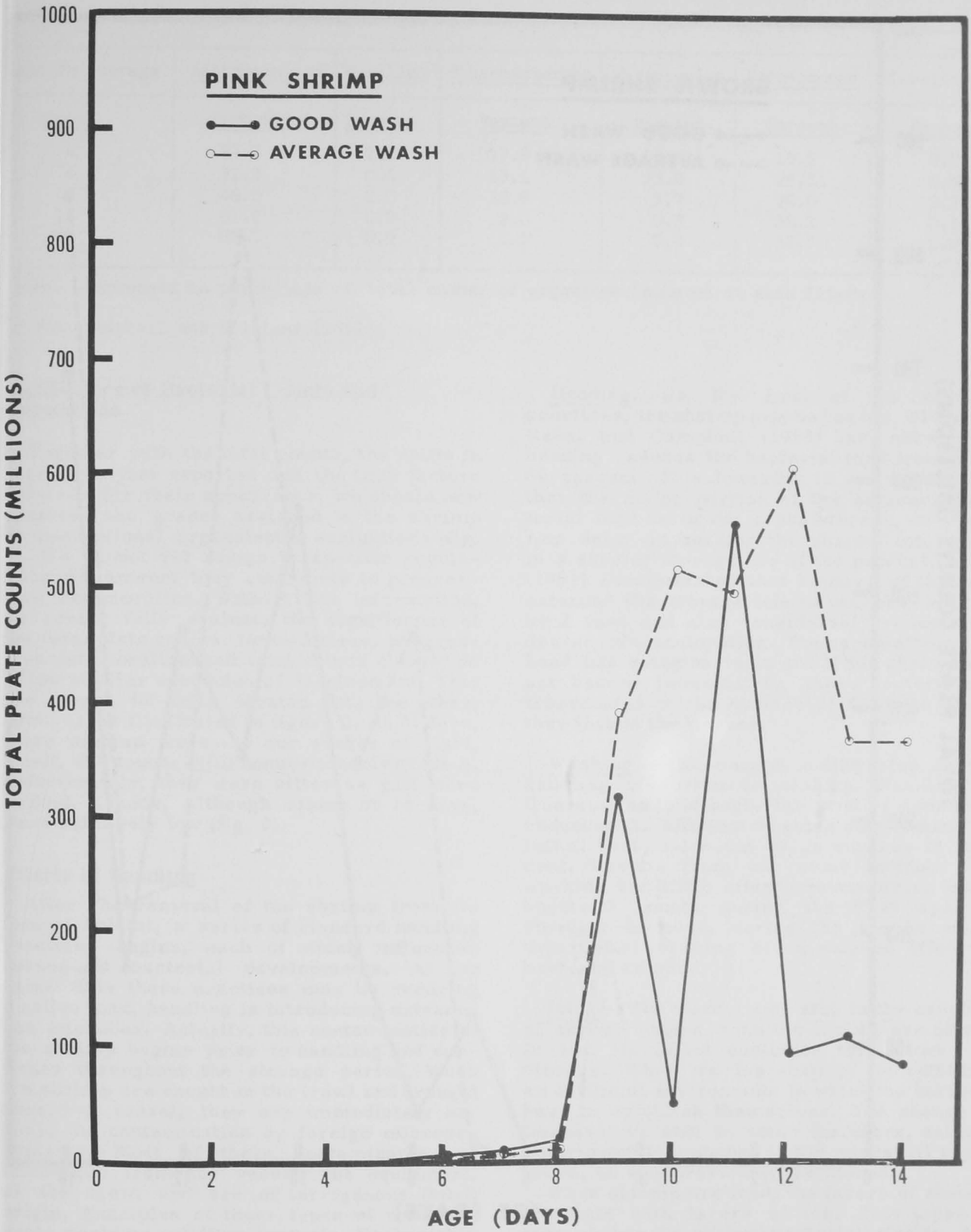


Figure 5.--Total plate counts of homogenates of pink shrimp on salt-water media at 25° C.

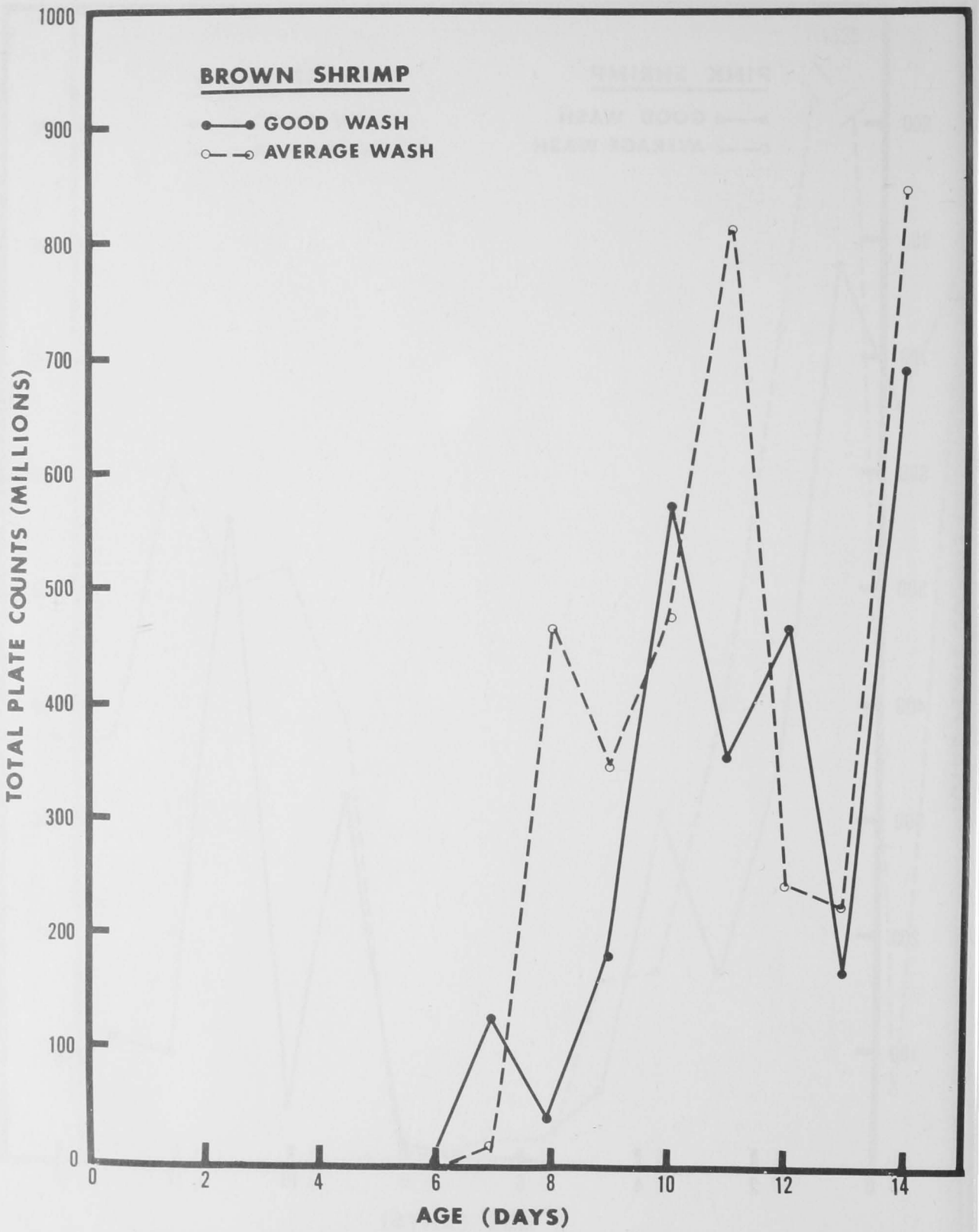


Figure 6.--Total plate counts of homogenates of brown shrimp on salt-water media at 25° C.

Table 1.--The changes in bacterial flora of Gulf of Mexico shrimp during storage in crushed ice

Held in storage	Achromobacter	Bacillus	Flavobacterium	Micrococcus	Pseudomonas	Miscellaneous
Days	Percent	Percent	Percent	Percent	Percent	Percent
0	27.2	2.0	17.8	33.6	19.2	0.2
4	31.3	0.6	13.1	23.0	26.5	0.5
8	46.0	2.0	18.0	5.7	28.0	0.3
12	67.0	0.0	2.0	0.8	30.1	0.1
16	82.0	0.0	1.5	0.0	16.5	0.0

Note: Expressed as percentage of total number of organisms isolated at each interval.

Source: Campbell and Williams (1952).

Significance of Bacterial Counts and Populations

Together with the total counts, the shifts in population just reported and the time factors required for their appearance, we should now consider the grades assigned to the shrimp by conventional organoleptic evaluations (fig. 7). We cannot yet assign to specific populations the amount they contribute to progressive deterioration. Without this information, we cannot fully evaluate the significance of the total plate counts. Nevertheless, progressive deterioration and total counts clearly do follow similar schedules of development. That one cannot be fully equated with the other, however, is illustrated in figure 2. At 21 days, these shrimp were--to our senses of sight, smell, and touch--still unquestionably grade A. Unfortunately, they were bitter as gall when cooked. Counts, although rising at 14 days, were relatively low (fig. 2).

Effects of Handling

After the removal of the shrimp from its natural habitat, a series of standard handling practices begins, each of which influences subsequent bacterial developments. At the same time these practices may be reducing a native load, handling is introducing extraneous microbes. Actually, this contamination of the shrimp begins prior to handling and continues throughout the storage period. When the shrimp are caught in the trawl and brought aboard a vessel, they are immediately exposed to contamination by foreign microorganisms. Most of these contaminants are introduced from the vessel, the equipment, or the crew, and are of terrigenous (land) origin. Examples of these types of contaminants would be coliform bacteria, Escherichia coli, Streptococci, Staphylococci, Salmonella, Proteus, and many others that one seldom hears about because they have little or no public health significance.

Heading.--As the first of the handling practices, the shrimp may be headed. Williams, Rees, and Campbell (1952) have shown that heading reduces the bacterial load from 50 to 80 percent. This lowering is due to the fact that the major portion of the shrimp's bacterial load is in the cephalothorax, or head. Any delay in heading the shrimp can result in a shorter storage life of the product. Lantz (1951) demonstrated that removal of the head extended the storage life of uncooked shrimp by 2 days and also considerably reduced the degree of discoloration. The cephalothorax or head has many bacteria and if the shrimp are not headed immediately, these bacteria are transmitted to the surface of the tails where they invade the tissues.

Washing.--The second major step in the handling of shrimp is washing. Washing influences the total bacterial count by somewhat reducing it. Efficient washing may reduce the initial bacterial count by as much as 75 percent. Results from our study indicate that washing had little effect in maintaining lower bacterial counts during the first week of storage; however, during the second week, this initial washing had a marked effect on bacterial counts.

Icing.--The third major step in the handling of shrimp begins when the shrimp are placed in ice. Its effect continues throughout iced storage. When we ice shrimp, we establish an artificial environment in which the bacteria have to establish themselves. The change in temperature and, in some instances, salinity contributes to a change in the microbial population, as was previously mentioned.

When shrimp are iced, the layers of shrimp alternate with layers of ice. This layering may change the shrimp bacteriologically. Accordingly, the thickness of the layers should be held at a minimum, and ice should be scattered in with the shrimp. The added ice within the layers of shrimp facilitates the

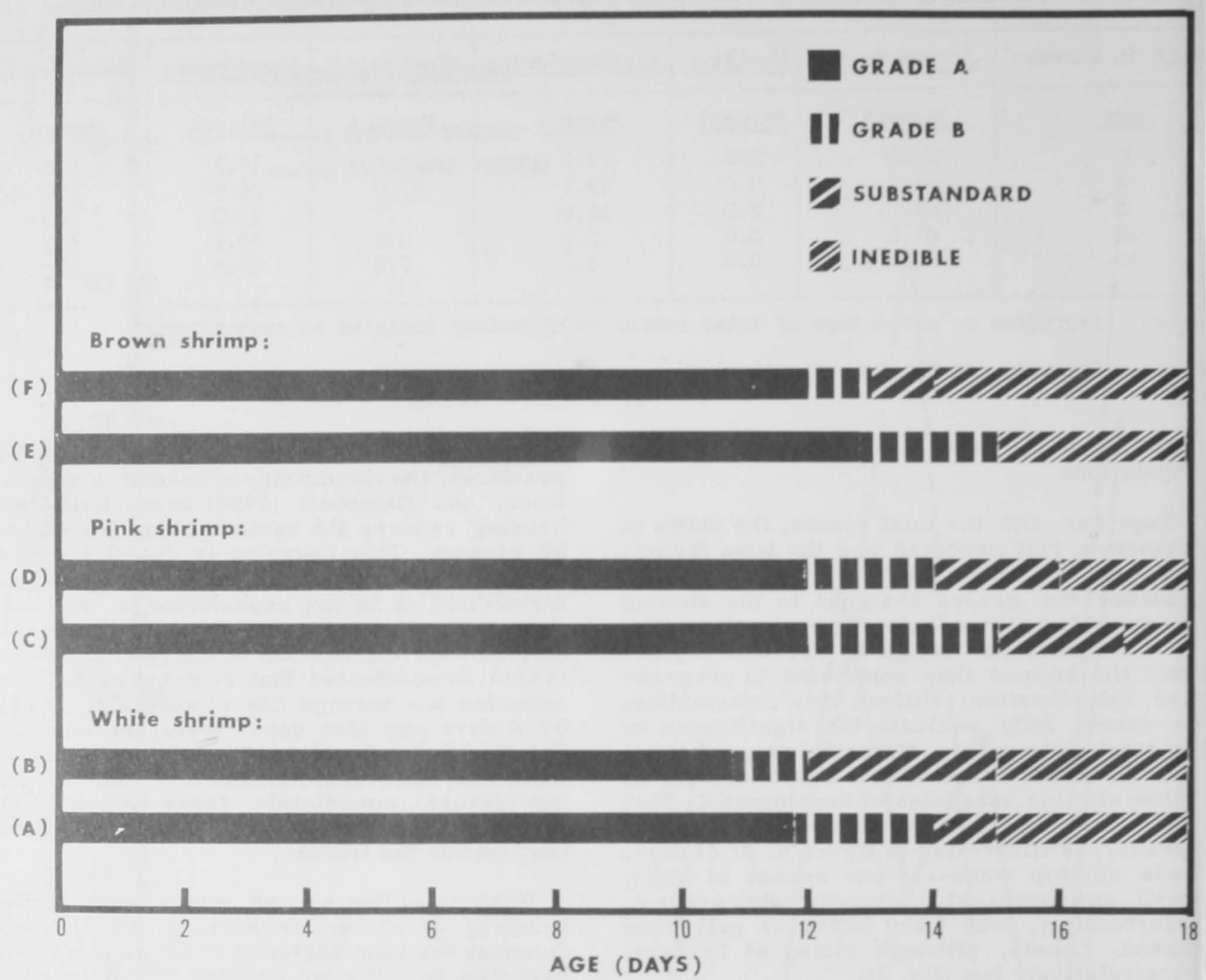


Figure 7.--Organoleptic grade patterns obtained during the iced storage of (A) white shrimp, good wash; (B) white shrimp, average wash; (C) pink shrimp, good wash; (D) pink shrimp, average wash; (E) brown shrimp, good wash; and (F) brown shrimp, average wash.

washing effect that normally takes place. It also reduces the density of packing. With fish, dense packing results in "bilgy" fish, owing to the action of anaerobic microorganisms. We do not know whether this same action could cause "bilgy" shrimp. We have not investigated the anaerobic flora of shrimp, but we believe that it would be worth looking into.

Layering.--Bacterial counts vary in the different layers of shrimp. Green (1949) found that the bacterial counts of shrimp in the uppermost layers of a bin or box increased about twofold, whereas the counts in the bottom layer increased a thousandfold. This effect is produced by a steady washing that takes place as a result of melting ice. Consequently, the shrimp in the lower layer are immersed in, or at least exposed to, the washings from the upper layers that produce this enormous increase in bacterial counts. Logically, "melt water" should be prevented from percolating through more than two or three layers of shrimp. This restriction should reduce the buildup in counts in the lower layers of shrimp; we can offer no economically feasible way of preventing the percolation. We suggest, however, that mixing shrimp with ice, instead of layering, and using an ice-shrimp ratio of 2:1, instead of the all-too-common 1:1, should improve the spacing, hence the washing, of shrimp and minimize shrimp-to-shrimp contact and the accumulation of bacteria in the lower layers.

We consider the lower layer of ice to be exceedingly important. This layer should be thick enough to prevent the shrimp from coming directly into contact with the bottom

of the bin or box. We think the importance of preventing this shrimp-to-box or shrimp-to-bin contact can be illustrated by the color picture (fig. 8a). This figure shows the relative number and morphological types of bacteria taken with a swab from the bottom of a shrimp box from an area no larger than a pencil eraser. Possibly a false bottom in vessel holds may facilitate an easier passage of melt water, thereby preventing this water from accumulating. Such a bottom could be inexpensively constructed to fit into present vessel holds. This bottom should contain numerous small holes for unrestricted passage of water and be constructed in such a manner as to be easily removed for complete scrubbing.

Boxes, Bins, Holds.--Owing to the porous nature of the material that is usually used in the construction of shrimp boxes and bins, bacteria and molds are afforded an opportunity to cling to, and even to penetrate, these surfaces. As a result, a well-established microbial population exists in the box or bin even prior to the introduction of a new catch of shrimp. This population probably consists largely of cold-adapted microorganisms. Figure 8b shows microbes within the thin sections of wood removed from ordinary commercial boxes.

Figures 8a-b show that more care must be paid boxes, bins, and holds, since these surfaces are obviously major sources of contamination. The added care should be periodic scrubbing of these surfaces. If no acceptable sanitizing agents are available, scrubbing with drinking water might help. Ordinary household bleach and water would help even more to reduce the contamination. The surface of the wood should also be smoothed to make it easier to clean. It may be economically feasible to coat with plastic the holds and boxes because plastic has the advantage of sealing the surfaces against the penetration by molds and bacteria.

Treated ice.--Fieger, Bailey, and Novak (1956) have reported on the effectiveness of several specially treated ices that inhibit bacteria. They found the following combinations to be no more effective than regular commercial ice: CTC (chlortetracycline) (1 p.p.m.) in ice; acid ice, pH 5.0; tannic acid (500 p.p.m.) in ice; and sodium bisulfite (100 p.p.m.) in ice. CTC (10 p.p.m.) in ice, however, did reduce the bacterial numbers and delayed spoilage from 2 to 4 days.

While testing effectiveness of sodium bisulfite against "black spot," Camber, Vance, and Alexander (1957) noticed how this chemical, in a dip form, affected the bacterial load. Using this dip in a concentration of 1.25 percent for 1 minute immediately after heading did not reduce the bacterial counts. If the time were lengthened to 15 minutes, however, the count was reduced. Use of a 10-percent dip for 1 minute immediately after heading and washing resulted in a lower bacterial count after 14 days of iced storage. If the shrimp were dipped in either 1.25 percent or 10 percent solutions 7 hours after being headed and washed, the dipping did not lower the bacterial counts.

We have mentioned only a few of the publications dealing with additives. Although many of the additives have not proved to be effective in extending storage life and though interest in this type of research has greatly declined because of the generally poor results, we feel that this subject still should receive attention. Many possibilities exist for developing new additives or for applying old additives under different conditions, subject to Food and Drug Administration approval, to help prolong the iced storage of shrimp.

Public health.--Williams and Rees (1952) never found any coliform or enterococci groups in the native bacterial population of the intestine of shrimp. These two groups of bacteria are usually considered to be indicators of feces contamination. So, when they can be isolated and identified from shrimp, they indicate mishandling and unsanitary practices, which in turn imply that human disease-producing microbes may have also been introduced. The search for these indicators is worthwhile, since the consumers' health must be guarded. Usually, however, the indicator or disease-producing organisms constitute such a minor part of the microbial population that they probably have a relatively small effect on keeping quality.

Most of the current reports deal with indicators or pathogens, but these reports have a bearing primarily on people's health, not necessarily on the keeping quality of shrimp. If indicators are present, our catch may infect our comsumers; but organoleptically our shrimp may still taste good. If we have no indicators but do have numerous other forms present, the consumer may reject our product for organoleptic reasons, despite the fact that it is not a hazard to his health. The two are only indirectly related. Spoilage bacteria, as distinct from possible disease germs, have hardly been touched upon.

HISTOLOGICAL FINDINGS

At the beginning of our work, we studied the deterioration of shrimps by noting the changes in their tissues. We wanted to dramatize the effects produced by both intrinsic, or autolytic factors, and extrinsic, or bacterial factors. Although our results do not indicate which of these factors cause the greater damage, we can show the overall damage that results from the combination of them.

Figure 8a.--A typical microscopic field (magnification 980x) on a stained smear of slime from a wooden shrimp box in actual commercial use. Numerous bacteria are shown as chains of rods, single red rods, fine filaments, dark spheres, or ovals.

Figure 8b.--A stained preparation of thin wood shavings from a shrimp box in actual use (x980). Mold filaments with spores and yeastlike forms are seen attached to the wood (upper left-hand corner and along left border).

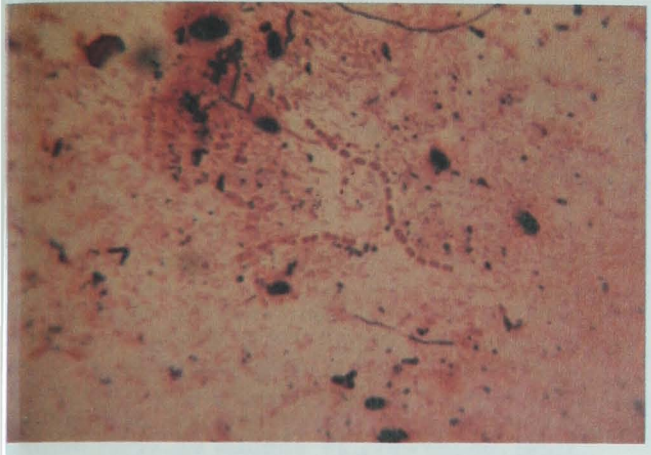
Figure 8c.--In a thin cross section of a shrimp stored 1 day in ice, the microscopic field (x450) shows the tissues that lie just under the shell. Note the compact, unbroken continuity of all tissues, from the epithelium (outer pink layer) through the connective tissue (blue) to the muscle (orange-brown). No deterioration is seen.

Figure 8d.--In a thin cross section of a shrimp stored 1 day in ice, the microscopic field (x450) shows the gut region. The villi (fingerlike projections of tissue into the lumen, or gut cavity, which serve to increase the amount of surface exposed) are well organized, with intact epithelium (thin, pink, outer layer) covering bluish cells.

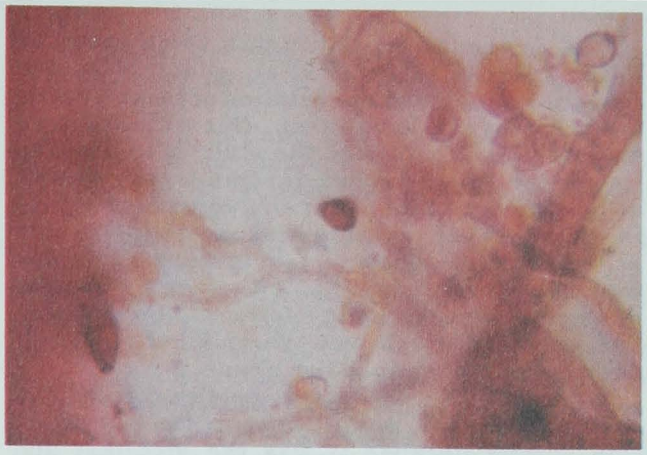
Figure 8e.--In this photograph of a section of a shrimp stored 7 days in ice (x980), the outer surface is again shown. Note how the epithelium (outer layer) has begun to show small discontinuities and slight separation from connective tissue (blue), which has also begun to show deterioration as a broadening of the band. Compaction of muscle tissue (orange-brown) is also reduced. Slight softening of the shrimp is a reflection of these tissue changes. Compare 8e and 8c, keeping in mind the difference in magnification.

Figure 8f.--In the shrimp stored 14 days in ice (x450), only shreds of epithelium remain. Connective tissues (blue) show actual separations between the fibers. At this point, tissue deterioration is so extensive that the shrimp is quite soft.

Figure 8g.--In the region of the gut of a shrimp stored 14 days in ice, this photograph (x450) shows the complete destruction of the system of villi (compare with 8d above). Muscle tissue (red) is now seen to front directly upon the now featureless lumen.

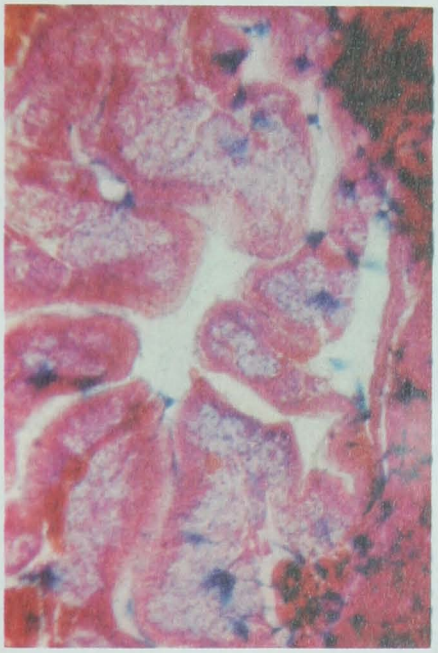


8a

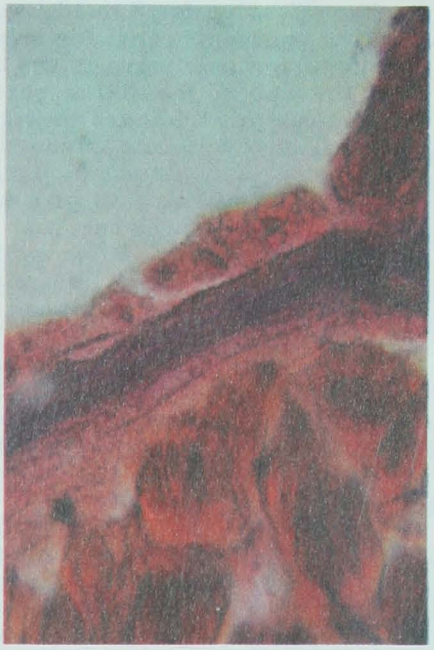


8b

8c

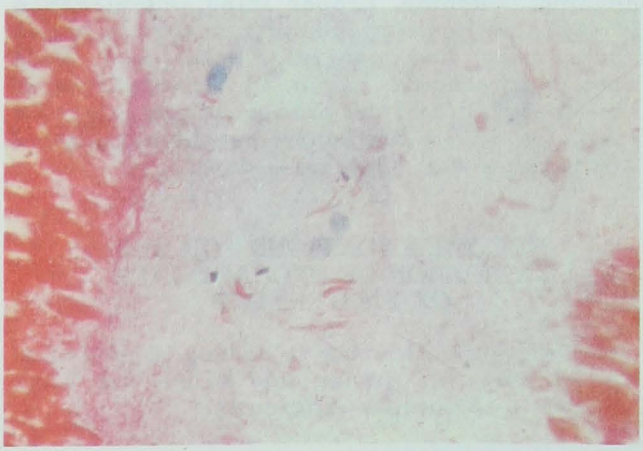


8d



8e

8f



8g

The methods we used in this study show the differences in the epithelial, connective, and muscle tissues and the relation of these tissues to each other in the shrimp. Our methods were directed largely toward the study of connective tissue for two reasons: (1) this tissue serves as a support so its deterioration would result in the loss of overall tissue integrity and (2) we know that some terrigenous and some marine microorganisms can attack by special enzymes such specific elements as collagen and elastin, which are parts of the overall composition of connective tissue.

We obtained the tissue sections from the sixth abdominal somite segment. We chose this somite because it was the smallest and therefore the easiest to embed and section.

After the removal of the shell, a section of tissue about one-fourth of an inch long was removed from the somite and placed in Zenkers fixative for 8 to 12 hours. This fixing solution prevents any further post-mortem changes from occurring in the tissue.

At the end of the fixing period, the tissue was washed for 24 hours in water, dehydrated by ethyl alcohol, and cleaned in xylol. The tissue was then placed in paraffin at 52° C. (125.6° F.) according to commonly used procedures, and the tissue and paraffin were allowed to solidify. After solidification, the tissue was sectioned or sliced at about 1/1,200th of an inch and placed on glass slides and stained. Either Mallory's Triple Connective or Mallory's and Heidenhain's stain was used. We photographed selected slides with a 35-mm. camera mounted on a microscope.

Figures 8c-g show the progressive degradation that takes place in white shrimp tissue during prolonged iced storage.

Slides photographed were of white shrimp, but no marked differences in tissue among the three species were noticed, nor were marked differences found in the appearance of the tissue of shrimp collected aseptically and shrimp handled commercially.

The extension of the storage life of iced shrimp is not the result of a single act. Each step in the operation--for example, prompt heading, washing, proper icing, maintenance of sufficient melting ice, proper care of boxes and holds, and general vessel and plant sanitation--should result in an extension of the storage life of the product. Each extension might be only a few hours, but the cumulative effect could well be several days.

RECOMMENDATIONS FOR THE EXTENSION OF STORAGE LIFE OF ICED SHRIMP

1. Raise hold flooring to reduce pocketing of shrimp and juices and allow the bilge pump to operate efficiently.
2. Use a scrub brush to wash the hold and equipment and rinse the thoroughly cleaned surfaces with water containing 200 p.p.m. of chlorine.
3. Seal penboards and cover sheathing with polyethylene, if possible. Use polyethylene for sheathing so that it can be taken off occasionally to allow the wood to breathe.
4. During the trawling, clean the hose, equipment, deck, and crew's hands to reduce contamination by land bacteria. A rinse of water containing 200 p.p.m. of chlorine would also be very beneficial. If impractical underway, it should be done before sailing.
5. Keep the catch shaded, perhaps with a tarp.
6. Sort, head, wash, and ice shrimp quickly.
7. Wash the shrimp adequately with a hose before they are iced.
8. Use enough ice on bottom, sides, and top of container so that shrimp will not contact anything except ice.
9. Mix one part of shrimp with two parts of ice rather than layer the shrimp and ice.
10. Cap the top and ice the sides of the hold if necessary to prevent the shrimp from coming into contact with the boat.
11. Maintain a temperature slightly higher than 0° C. (32° F.) so that the ice will melt.
12. Wash shrimp at the dock. (Fluming will not reduce the bacterial load on the shrimp unless the water is rushed past them and drained out.)

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