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ENVIRONMENTAL FACTORS AFFECTING STRESS AND MORTALITY OF THE HAWAIIAN ANCHOVY IN CAPTIVITY

by

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INTRODUCTION

Stolephorus purpureus (Hawaiian anchovy or nehu) is the predominant baitfish used in the Hawaiian skipjack tuna *(Euthynnus pelamis)* fishery. The nehu is small, readily loses scales, and is susceptible to shock and subsequent osmoregulatory stress and most of its mortality originates from injuries sustained during capture and handling. The capture and holding of nehu in vessel bait wells have been problems for some time, as bait wells currently in use on most tuna vessels are inadequate for optimal survival of nehu. Average baitfish mortalities following capture often reach approximately 25 percent per day or more (Brock and Uchida, 1968).

The baitfish problem is twofold: (1) time spent seeking and capturing bait decreases time spent tuna fishing and (2) high bait well mortality shortens fishing time. These and other operational aspects of the Hawaiian live bait fishery are extensively described by Brock and Uchida (1968), who emphasize the development of a means of reducing baitfish mortality to increase fishing efficiency as a profitable research goal.

The objective of this research is the reduction of nehu mortality. This work is an extension of studies by Brock and Takata (1955) which demonstrated that reduced salinity and increased aeration significantly reduce nehu mortality in bait wells. In addition to aeration and salinity, the effects of pure oxygen, temperature, current, light, tank color, recirculated reduced salinity, buffers, protein skimmer, density, food, disease, and predation were also examined.

Note: All tables have been omitted from this report, although references to them have been retained. Except for Table 24, which summarizes the correlation matrix of 34 laboratory variables, all tables are available on request from the Hawaii Institute of Marine Biology, P.O. Box 1346, Kaneohe, Hawaii 96744. A listing of the tables can be found in the Appendix to this report.

Other studies of nehu include those of Hiatt (1951) on food and feeding habits; Tester (1951, 1955) on distribution of eggs and larvae in Kaneohe Bay; Au (1965) on distribution of eggs and larvae in Pearl Harbor; Yamashita (1951) on larval development; Burdick (1969) on larval feeding; Bachman (1963) on aspects of population dynamics; and Pritchard (1955) on lethal oxygen level. The National Marine Fisheries Service, Honolulu Laboratory also conducted several studies on nehu, the most recent being experimentation with a baitfish-holding facility in Kaneohe Bay ([U.S.] Bureau of Commercial Fisheries, 1969). Many of these studies and others are summarized in a synopsis prepared by Nakamura (1965).

The research program was organized into two phases: (1) a study of factors affecting survival and methods to increase survival and (2) application of the experimental results. The study under the first phase was made (a) during bait capture and transfer to bait wells, (b) in 180-gal experimental laboratory tanks, (c) in 1,000-gal bait wells on an experimental barge, and (d) in vessel bait wells at sea. The experimental results were applied (a) to the designing and testing of an improved, experimental bait well and (b) to providing extension service to the tuna industry. This report encompasses the results of capture, transfer, and laboratory and barge experiments. Results of the remaining factors are given by Baldwin et al. (1972) and Baldwin (1973).

By suggesting small and inexpensive improvements of bait wells on existing vessels and also by aiding in the designing of new vessel bait-holding facilities it is hoped that this research will eventually benefit the tuna industry.

METHODS: BAIT CAPTURE

The equipment and general procedures for capture of bait and for barge and laboratory experiments are given below. Experimental modifications for individual barge and laboratory experiments are described where applicable in the "results" section.

Capture and Transport Equipment

Barge

An experimental barge was designed to test the effects of improved bait-handling techniques on the survival of nehu (Plate 1). Two bait wells with movable gates were provided so that bait could swim from the capture net into the wells with minimum handling. This procedure eliminated the bucketing process which usually resulted in considerable handling injury.

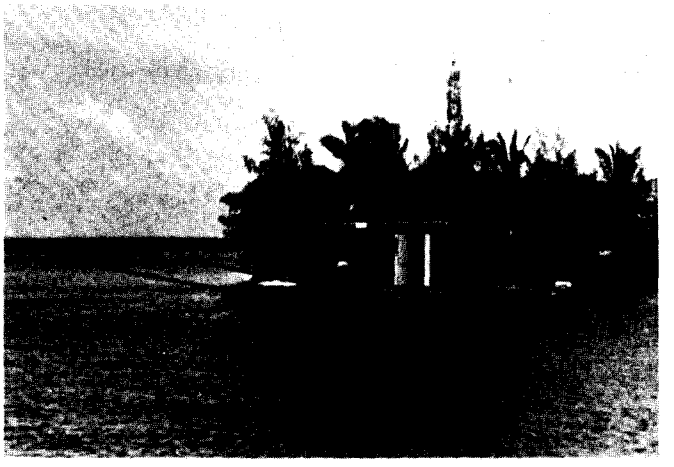


Plate 1. The nehu barge anchored near the west side of Coconut Island, Kaneohe Bay, Oahu. The barge is 11.0 m by 5.5 m (36 x 18 ft) and is equipped with two 3,784-liter (1,000-gal) bait wells; a laboratory containing a desk, sink, refrigerator, and two bunks; and a generator room with two small gasoline generators. The two long poles or "outriggers" at left support the lift net and are used to raise and lower the net during night baiting.

The design of the bait wells was modified throughout the experimental period. These modifications included two current standpipes and a seawater pump (Plate 2), a protein skimmer (Plate 3), an oxygen system, and a green gel-coat, resin-painted interior.

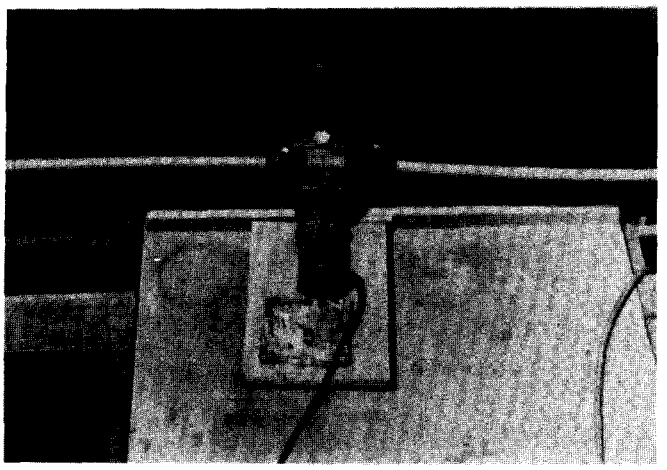


Plate 2. A 1-hp Jaccuzi pump set up on the nehu barge. The seawater intake hose is at top center and extends to about 2.4 m (8 ft) below the surface. The two valves and PVC pipes lead to both bait wells discharging seawater through a perforated current standpipe located in each well.

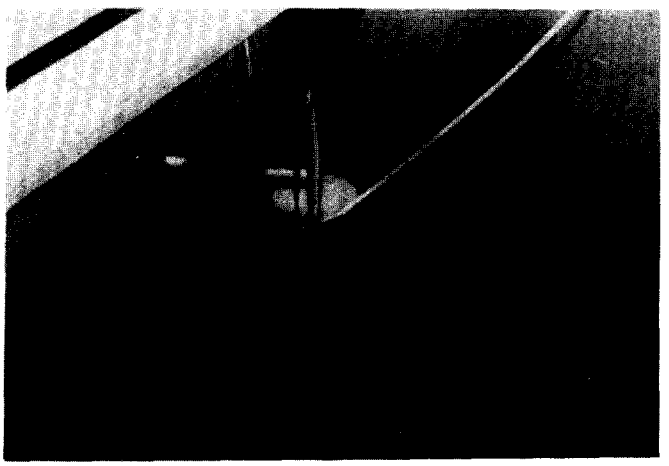


Plate 3. One of the nehu barge 3,784-liter (1,000-gal) bait wells. The removable gate through which the nehu enter the bait well is at the lower left. A large 25.4-cm (10-in) ID protein skimmer is suspended in one corner during an experiment. The horizontal PVC pipe leading to the left from the top of the skimmer discharges the concentrated foam outside the bait well. The hoses are for oxygen, seawater, and compressed air.

Bait receiver

A small barge was designed and built specifically to function as a bait receiver (Plate 4). It was constructed of 1/4-inch marine plywood with ten screened openings to provide adequate water circulation. A removable gate through which the nehu entered from the bait seine was added to each side of the receiver. The main receiver compartment in which nehu were confined measured 1.8 m by 1.2 m with a depth of 0.7 m (70 in x 48 in x 29 in). When submerged to a depth of 0.6 m (22 in) it contained 1,140 liters (302 gal) of seawater excluding the 0.9-m (36-in) bow compartment. Several large weights were placed in the floating bait receiver to sink it to the desired depth. A portable oxygen cylinder and stone were used to maintain oxygen in the receiver during transport of bait (Plates 5 and 6).

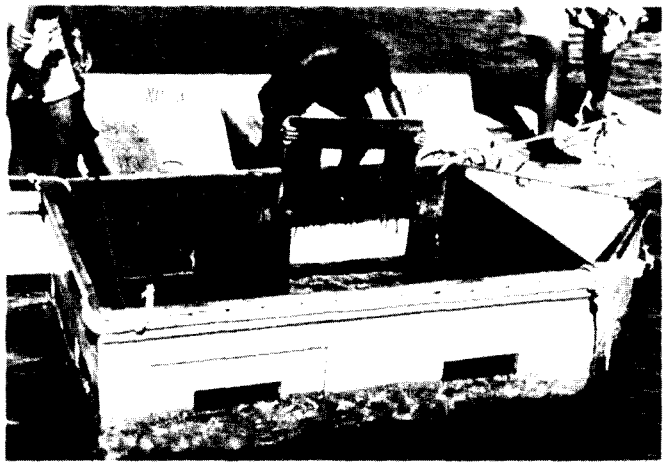


Plate 4. Nehu bait receiver being readied for moving nehu. The gate through which the nehu were forced to swim is being removed. The screened openings allow seawater to circulate in the bait receiver during transport. Two heavy weights are placed on the bottom of the receiver to sink it to a point just above the top of the closed gates.

Plate 5. The portable oxygen unit used during nehu transport from the capture area to the laboratory. The unit consists of a 0.6-m^3 (22-ft^3) oxygen cylinder, pressure regulator and gauges, hose, and oxygen stone.

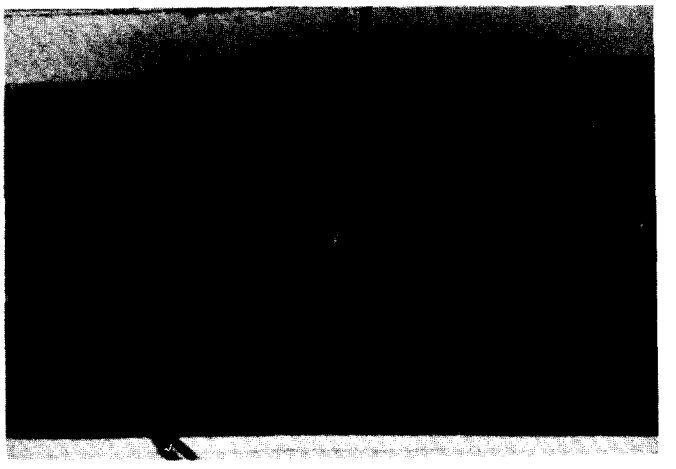


Plate 6. A concentration of nehu enclosed within the plywood bait receiver. The white cloud at center is pure oxygen being released from the oxygen stone placed on the bottom of the receiver.

Seines for day capture

Two bait seines were constructed of 1/8-inch bar mesh for daytime capture of nehu. A 110 m by 6 m (360 ft x 20 ft) deep seine similar to but shorter than commercial seines was constructed. This net was designed for collecting nehu in areas of 1.5 m (5 ft) or more in depth. A small 46-m seine measuring 3.7 m deep at the center and tapering to 1.8 m at the wings (150 ft x 12 ft x 6 ft) was designed for use in shallower areas to reduce injuries to nehu captured in large nets.

Net for night capture

A net was constructed using a 1/8-inch square mesh on the bottom and with 3/16-inch mesh on each side. It was approximately 8.2 m long by 6.1 m wide by 6.1 m deep (27 ft x 20 ft x 20 ft). In a lowered position the net was similar to the net described and illustrated by Brock and Takata (1955).

Night Capture Procedures

Capture area

Nehu were captured at night with lights suspended from the experimental barge anchored about 6.1 m (20 ft) off the southwest shore of Coconut Island, Kaneohe Bay, Oahu (Plate 1). The depth of the water in front of the barge was approximately 9 to 10 m (30 ft).

Capture technique

Around sunset (between the hours of 1730 and 1900) three white floodlights (100 watts each) attached to the barge were switched on and the sides of the net lowered. Another light (250 or 300 watts, white incandescent, suspended from a long pole, was submerged approximately 2.5 m (8 ft) to the center of the net area.

Periodically throughout the night, the water surrounding the center submerged light was examined for nehu. When sufficient nehu were detected, the submerged light was dimmed to 50 or 60 watts to consolidate the fish around the light. The edges of the net were then raised surrounding the school. However, if nehu were not observed during the night, the net was raised at dawn.

Environmental data

From April 1967 to June 1970, 78 net sets were made at night from the barge to obtain bait for experiments. Major environmental factors were measured during all-night capture experiments regardless of capture success. Attempts to obtain bait were usually correlated with need for experimental nehu and not with determining appropriate times for capturing night bait. Data obtained were not temporally systematic. However, data on major environmental variables were recorded, in hope that they might reveal opportune times for night baiting. Records were made of the following variables for the last 67 sets: approximate number of buckets of nehu captured, date, time, moon phase, time of tide, range of tide, weather (rainfall, wave action, wind speed), turbidity, temperature, salinity, and presence of predators.

Loading and transport

According to experimental plan, the captured nehu were made to either (1) swim from the lift net through the gates into the barge wells (Plate 3) or (2) swim from the net into the bait receiver and then were transported to laboratory tanks on the opposite side of Coconut Island. Further details of bait transport over longer distances follow.

Day Capture Procedures

Capture of nehu during daylight hours followed essentially the same method described by June (1951). Since 1951 there has been little change in bait-seining techniques except for more general use of modern outboard motors and improved nets made of synthetic materials.

Capture areas

Nehu were seined primarily in the southern sector of Kaneohe Bay (Figure 1). Other seining areas exist along the northern shores of the bay but these were not utilized because of their extreme distance from Coconut Island.

The most consistent supply of nehu occurred along the southeast shoreline adjacent to the Nuupia-Halekou pond complex and well within a restricted zone extending 457 m (500 yd) offshore from the Mokapu Peninsula (U.S. Coast and Geodetic Survey Chart No. 4143). Normally, commercial aku fishermen are not permitted in this area to seine nehu without clearance from military authorities. This policy no doubt contributed to successful seining. Of the 34 laboratory and barge experiments in which day-captured nehu were used, 30 were conducted with nehu captured within or near this area.

The topography of this area is representative of other nehu-seining areas in Kaneohe Bay. The water is usually murky and the inshore zone frequently exposed during low tides. The bottom contour gradually slopes toward deeper water where there is a steep drop-off caused either by a coral shelf or from bottom dredging. The bottom is soft mud or soft mud and sand mixed with coral rubble. With the exception of one seining

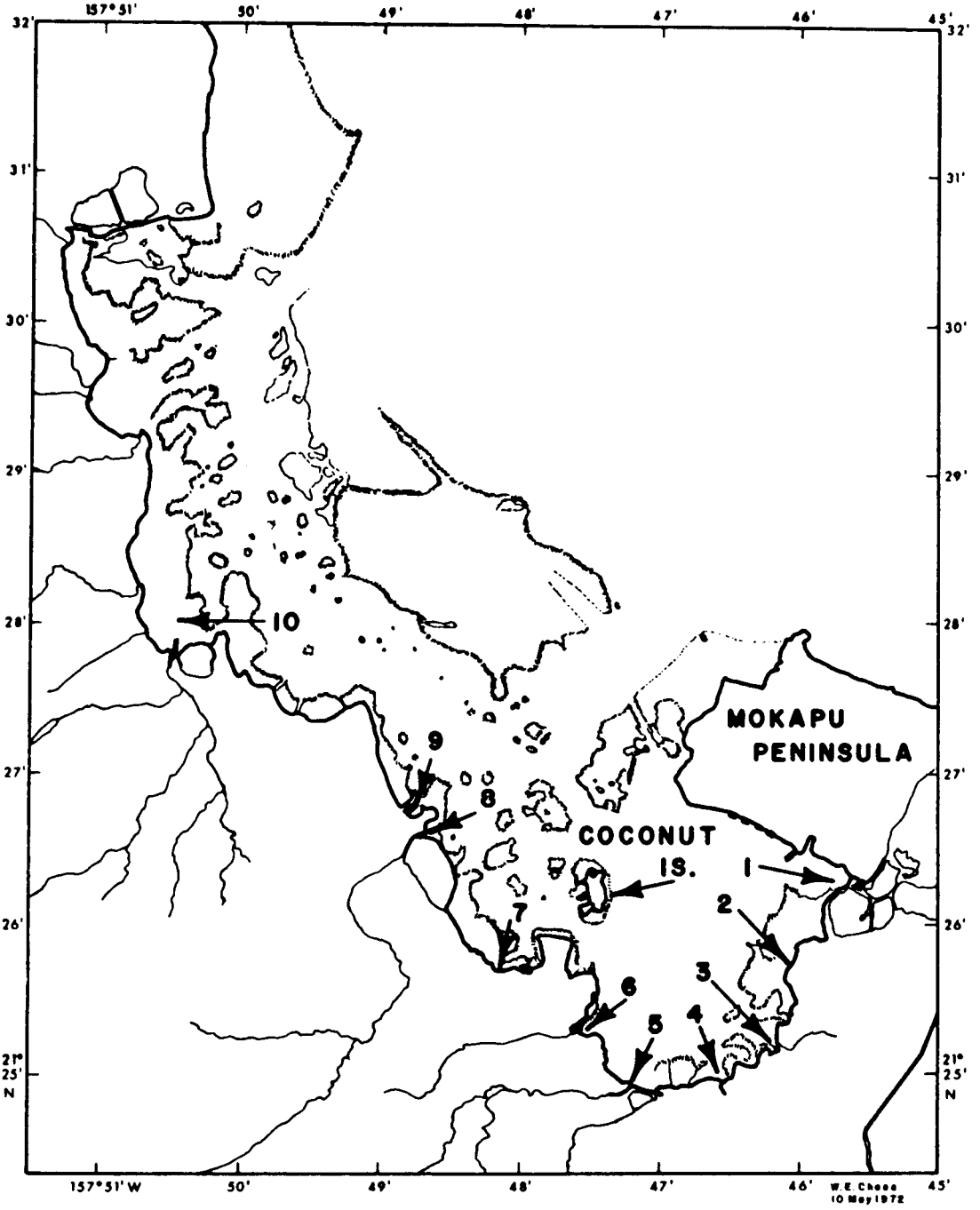


Figure 1. Map of Kaneohe Bay, Oahu showing collection localities for day-captured nehu from 1967 to 1970: (1) Nuupia Pond area, (2) Malae, (3) Keaalu, (4) Kokokahi, (5) Kaneohe and Kawa Streams, (6) Keaahala Stream, (7) Alii Shores, (8) Heeia Stream, (9) Heeia Pier, and (10) Kahaluu.

collection, all successful captures were made in the inshore, mud-bottom habitats having a maximum depth of 1.5 to 1.8 m (5 to 6 ft).

Seining

The bait-seining operation required two skiffs: one for setting the bait seine and another for handling the bait receiver. After the concentration of nehu was located and the net skiff maneuvered into position, one person jumped into the water holding one end of the net. The net skiff and seine were then rapidly circled around the nehu and the ends of the seine were brought together to close the net completely. While one person remained in the water to keep the net closed, others slowly pulled the net in toward the skiff (Plate 7) until a large pocket remained that enclosed the nehu. Setting and closing the 46-m seine required about 10 minutes; the 110-m net took 15 to 20 minutes. It was only occasionally necessary to set the entire length of the larger net.

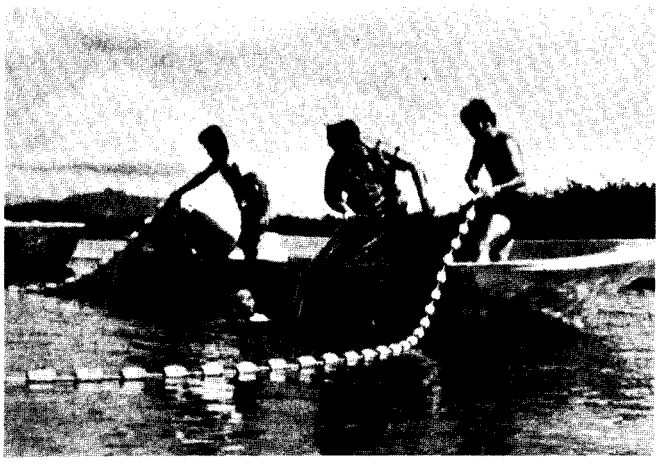


Plate 7. The 46 m by 3.7 m (150 ft by 12 ft) nehu bait seine being closed after setting around a school of nehu. The person in the water at center is keeping the net closed to prevent nehu from escaping.

During the final moments of closing the bait seine, the bait receiver was maneuvered into position with the nearer gate removed and the seine attached onto four hooks around the open gate. By gradually closing the pocket of the seine, nehu were forced to swim into the bait receiver. After a suitable number of nehu had been forced inside, the gate was closed, the net unhooked, and the portable oxygen unit turned on (Plates 5 and 6). Then excess nehu were released and the seine loaded into the net skiff which in turn was maneuvered alongside the bait receiver and secured (Plate 8).

Plate 8. The bait receiver being towed to the laboratory with a collection of nehu. The receiver is secured between the two skiffs and slowly towed through the water at approximately 2 mph while adding pure oxygen.

Transport

The receiver was pulled through the water at a speed of approximately 2 mph to provide water circulation through screened openings while keeping agitation inside to a minimum. On days of strong winds no attempt was made to capture and transport nehu in this manner since injuries and subsequent heavy mortalities increased during these conditions.

Depending upon the collection locality and the distance to Coconut Island, transporting the bait receiver took from 20 to 90 minutes. During this time oxygen was continuously added and dead and critically injured nehu, along with predators and other unwanted fishes or invertebrates, were removed by hand net. Nehu were immediately removed from the bait receiver after reaching the laboratory. Transfer time by bucket from the bait receiver to laboratory tanks usually took less than 5 minutes. Swimming or bucketing nehu into barge bait wells required 5 minutes or less.

METHODS: LABORATORY AND BARGE EXPERIMENTS

Equipment

Barge wells

The two barge bait wells held approximately 1,000 gal of seawater and were gradually modified throughout the experimental period as previously described (Plates 1 to 3). The final design provided either open or closed circulation, pure oxygen, and current. The interior was painted with green gel-coat resin and a protein skimmer was added for the removal of mucus, scales, and organic debris.

Laboratory tanks

Some of the laboratory experimental tanks are shown in Plates 9 and 10. Altogether there were ten experimental tanks; five on each side of the laboratory. The arrangement was designed to provide five simultaneous experimental situations, each with a replicate. The tank design is that of Alderdice et al. (1966); tanks were purchased from Vancouver Plastics, Vancouver, B.C., Canada. The oval tanks have slightly sloping walls and

a center drain and are finished with green-blue, gel-coat resin. They contain approximately 680 liters (180 gal) when the water level is 4 inches below the top.

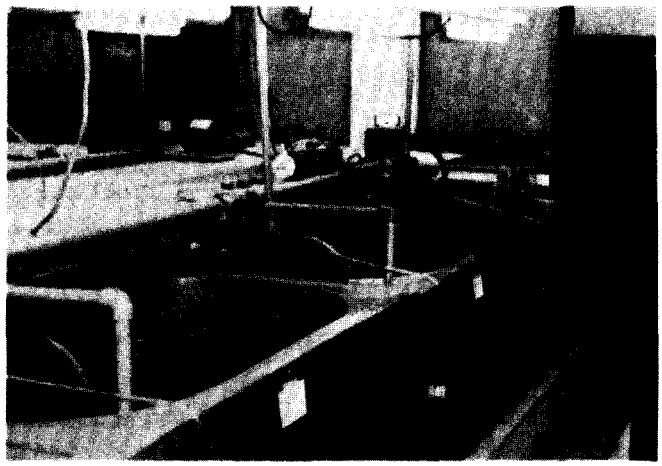


Plate 9. Several of the 680-liter (180-gal) fiberglass tanks as they were set up in the laboratory. Ten of these tanks were arranged in two rows and were provided with seawater, compressed air, pure oxygen, overhead lighting, and current standpipe. For full description of tanks see Alderdice et al. (1966).

Plate 10. The interior of one of the laboratory tanks. The inside was finished with a green gel-coat resin and had round ends, sloping sides, and a double standpipe central drain. The perforated current standpipe is at left.

The tanks were gradually modified throughout the experimental period. They were supplied with a water-inflow valve, current standpipe, air outlet, oxygen outlet, oxygen releaser, and protein skimmer. The oxygen equipment is shown in Plates 11 and 12; the protein skimmer in Plates 13 and 14. The oxygen releaser described by Baldwin (1970) was designed to emit small bubbles of oxygen into seawater with a maximum degree of saturation and minimum oxygen loss; it was adapted for use in laboratory tanks. Water was supplied from Kaneohe Bay, pumped to storage tanks through PVC lines, and fed to the laboratory by gravity through Cuno filters (Plate 15).

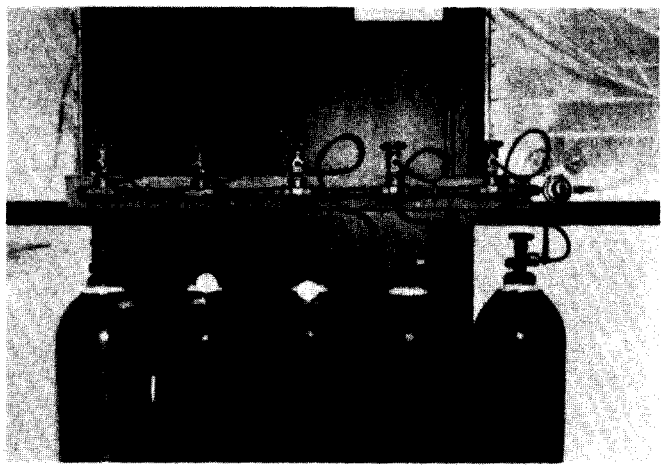


Plate 11. Five 6.8-m^3 (240-ft^3) industrial oxygen cylinders mounted against one wall of the laboratory. Normally, only two cylinders were in use at any given time; the additional three are reserves. The oxygen manifold with the five selector valves is mounted over the tanks with pressure regulator and gages and oxygen delivery hose at right.

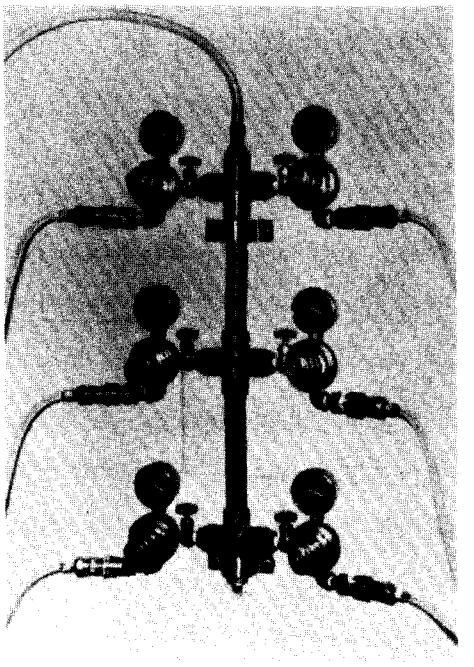


Plate 12. A low pressure PVC oxygen manifold used for metering the oxygen to individual releasers during experiments. The hose at top connects to the large oxygen cylinders (Plate 11). The smaller transparent hoses lead to individual stones and have quick-release, snap-on stainless steel fittings.

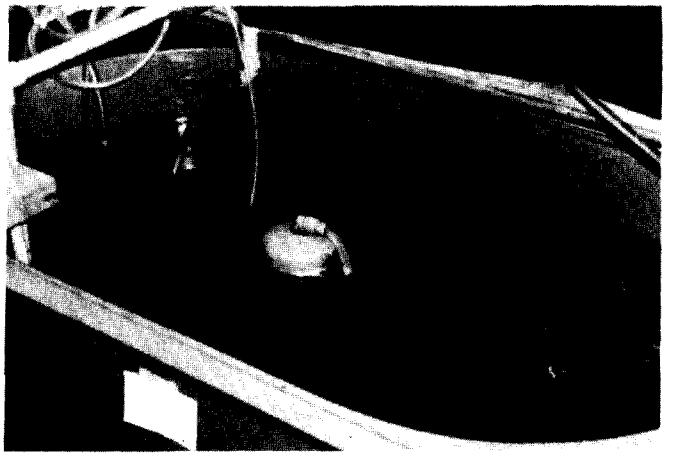


Plate 13. A 7.6-cm (3-in) ID laboratory protein skimmer secured to the center drain standpipe. The hoses at left are for pure oxygen, compressed air, and seawater circulation to the protein skimmer. The current standpipe is at the far left.

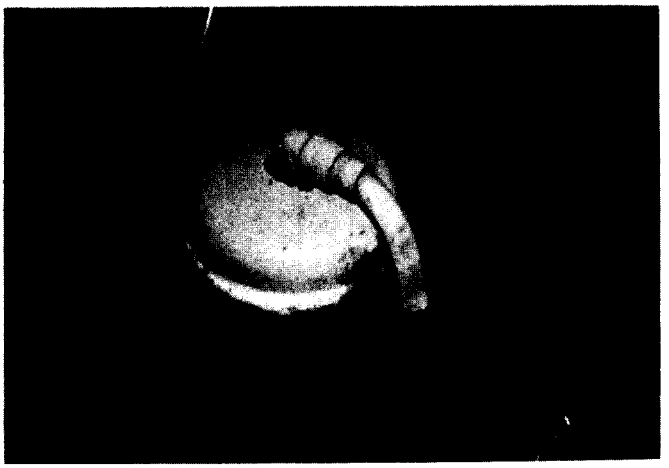


Plate 14. The 7.6-cm (3-in) ID laboratory protein skimmer in operation during an experiment. Seawater is moved through the vertical skimmer while air is bubbled from the bottom. The foam is concentrated at the top of the skimmer and discharged from the laboratory tank through the transparent hose that empties into the center drain.

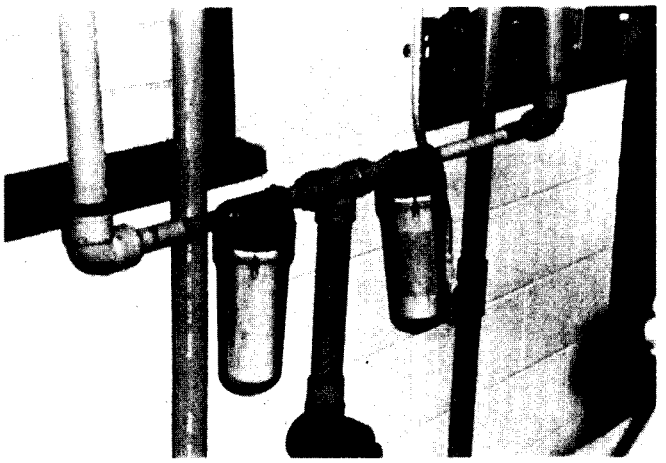


Plate 15. Two Cuno filters used for filtering seawater for laboratory experiments. Seawater passes up the central PVC pipe through the two filters and to the two banks of fiberglass tanks. The filter elements were changed twice daily to ensure a continuous, steady flow of seawater.

Filling Laboratory Tanks and Barge Wells

Number of nehu per bucket

A common unit used by Hawaiian fishermen to measure nehu is the bucket. Obviously, the number and weight of nehu in one bucket vary. For purposes of this discussion, one bucket of bait is defined as containing an average of 2,700 fish and weighing approximately 1,300 to 1,900 gm (3 to 4 lb) with a mean weight of 1,700 gm (3-3/4 lb). Experimental containers were 5 to 6-gal stainless steel buckets filled approximately one-half to three-quarters full with water and fish (Plate 16). Usually, local commercial fishermen concentrate the nehu in buckets to as much as 2,700 to 3,600 gm (6 to 8 lb). Reducing the number of fish per bucket in proportion to the amount of seawater reduces injuries.

Order of filling tanks or wells

Initially, it appeared that the number of nehu per bucket varied as tanks were filled, with later buckets containing fewer fish. A possibility

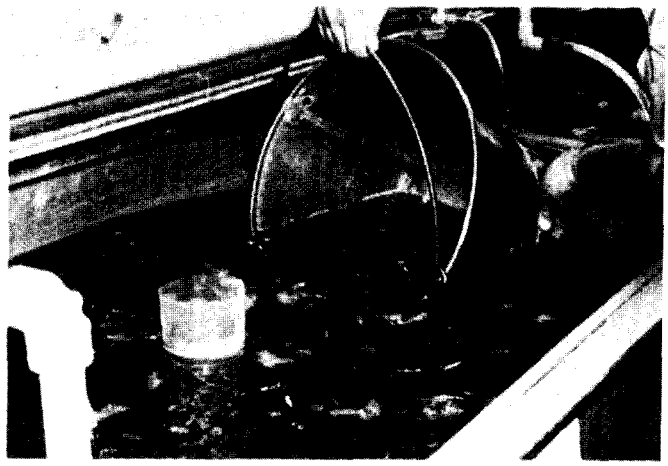


Plate 16. A bucket of nehu being emptied into one of the laboratory tanks at the beginning of an experiment. The screened standpipe at left is a double standpipe drain system in which the water is removed from the bottom instead of the top.

of differences in size or condition of fish as tanks were filled also existed. Subsequently, tanks were filled at random to eliminate any variation possibly influencing mortality rates.

Daily Measurement Procedure

Measurement procedures varied with experimental objectives, but a basic procedure was followed throughout the experimental period. Any modification of the procedure is indicated in the "results" section.

Variables were usually measured three times daily. The fish were first observed and their milling direction and other behavior noted. Oxygen (in ppm) was measured with a YSI Model 50 oxygen meter standardized by the Winkler method (Strickland and Parsons, 1968); salinity (in ppt) was measured indirectly with a Goldberg T/C Refractometer (Brix) calibrated to Copenhagen seawater standards; and temperature (in °C) was measured with a laboratory thermometer. Most measurements were accurate to the nearest 0.1 unit. In some experiments measurements of pH were made with a Coleman Model 37A pH meter to the nearest 0.01 unit. Measurement of CO_2 was made according to the indirect method of Strickland and Parsons (1968) to the nearest 0.001 unit.

Following this procedure, tanks were cleaned and dead fish removed, weighed, and counted. Whenever possible, all fish were counted and weighed to the nearest 0.1 gm. During periods of heavy mortality it was often necessary to weigh 50 dead fish at random, calculate the mean net weight, and divide the figure into the total weight to estimate the number of dead. These estimates have an average maximum error of about 5 percent. When designated by the experimental plan, fish were fed three times daily (at about 0830, 1330, and 1630) on frozen brine shrimp. The amount of

food varied between experiments depending on the size, number, and condition of the fish.

The total experimental period was usually 14 days (from 12 to 16 days), depending upon objectives.

Length-weight data

In some experiments, random samples of 25 live fish were removed to determine standard length and wet and dry weights. Excess water was removed from the dead nehu by quickly blotting with a paper towel. Standard length was measured to ± 0.1 mm and wet weight of fish was determined on a Semi-micro Mettler Balance (H16) to ± 0.5 mg. Samples were placed in a mechanical convection oven (Theco Model 18) and dried at 60°C for 24 hours. Dry weights were then determined by a similar procedure as described for wet weights.

RESULTS AND CONCLUSIONS

In the following section, variables of laboratory and barge experiments are examined separately using one or two successful experiments to illustrate conclusions. Some experiments were unsuccessful due primarily to equipment failures, i.e., power and pump failures, and poor water quality. Data from such experiments were not included in the "analyses" section.

Results of Field Work

Day capture

Three out of four attempts to capture nehu by bait seining were successful. At times it was necessary to make more than one set, but never more than three. The occasional lack of success in capturing nehu

was attributed to the following reasons: (1) adverse weather conditions made nehu concentrations difficult to locate, (2) insufficient numbers of nehu were seen, or (3) the size of nehu was too small. Several "blind sets" made when nehu could not be visually located were always unsuccessful and subsequently discontinued.

Night capture

Of the 78 sets made at night from the barge, 45 sets (58 percent) were unsuccessful and 33 sets (42 percent) were successful, yielding 1 or more buckets of nehu. Since the possibility exists that nehu may have approached the light but left before the net was lifted, an estimate of at least 50 percent success in attracting nehu to night lights seems reasonable.

A factor analysis (Dixon, 1970) was applied to environmental data using an IBM 360 computer. The results are summarized in Tables 1 to 4. However, a limitation is imposed on the results since all negative catches do not necessarily indicate absence of nehu.

Eleven--one dependent and ten independent--variables were evaluated: (1) buckets of nehu captured (dependent variable), (2) month, (3) moon phase, (4) tidal sequence, (5) tidal range, (6) time from sunset of lowest low tide, (7) weather, (8) turbidity, (9) temperature, (10) salinity, and (11) presence of predators. The correlation and factor matrices for these variables can be found in Tables 1 and 2. Percentage of capture success and number of buckets captured were significantly less in spring than the rest of the year (Table 3). Significantly more nehu were captured during the first quarter moon phase (Table 4). The greater number of predators occurring during the last quarter may have reduced capture success during that phase. The influence of tidal factors appears negligible. The influence of

weather upon capture success is not known since most attempts of night capture were made during good weather.

Several organisms other than nehu were also attracted to night lights during night baiting. The most common observed fishes were *Pranesus insularum* and *Etrumeus teres* which are also used as baitfish for skipjack tuna. Predators observed attacking or feeding on nehu under the light include *Caranx mate*, *C. melampygus*, *Gnathanodon speciosus*, *Scomberoides lysan*, *Trachurops crumenopthalmus*, and *Decapterus maruadsi*. A complete list of species observed under night lights, the months of occurrence, relative abundance, and observations of predation are given in Table 34.

Results of Laboratory and Barge Experiments

Types of mortality curves

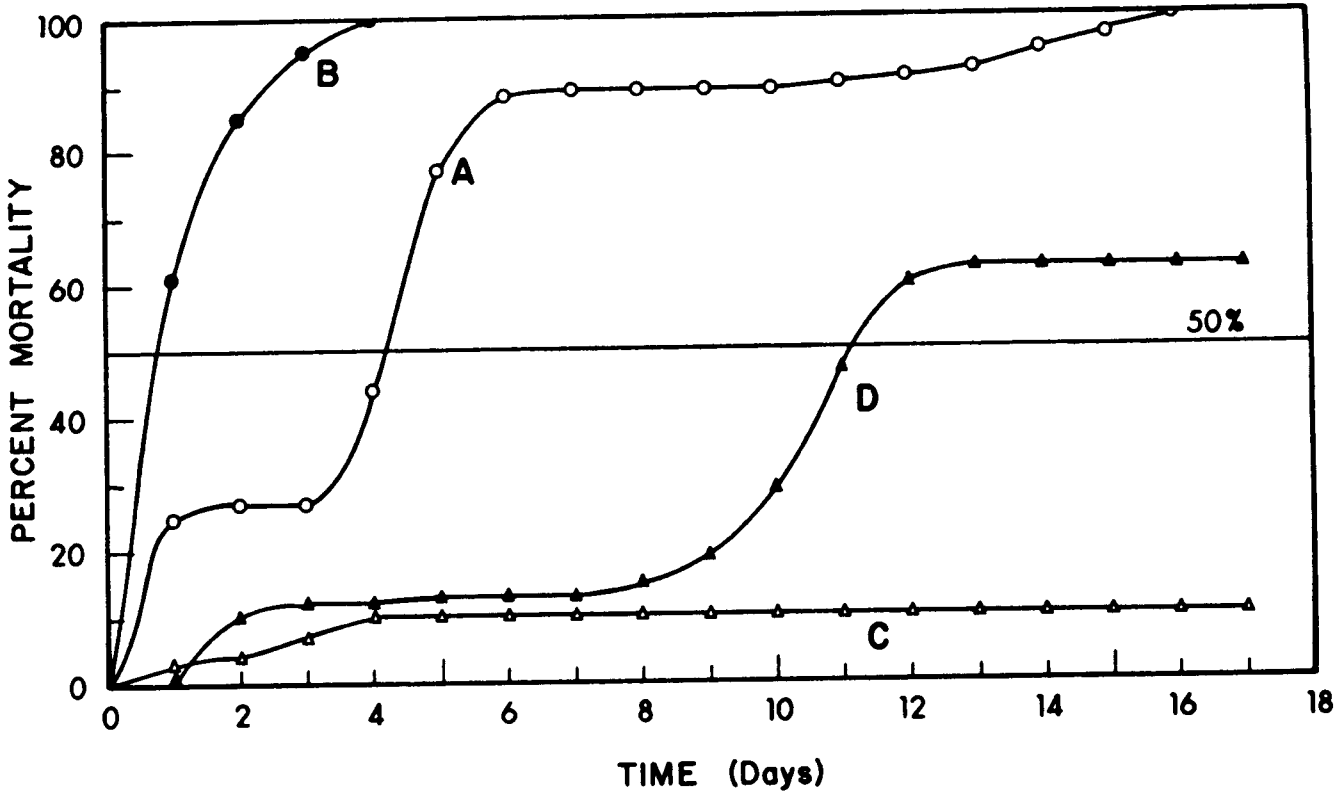
During initial experiments, certain patterns of mortality emerged. To provide a basis for comparison between results of the following experiments, types of mortality are described below. Mortality of nehu in captivity is clearly illustrated by cumulative percentage of mortality curves. The percentage of mortality (Y) is defined as the total number dead by day (X) divided by total number of fish at the beginning of the experiment (X = 0). The cumulative percentage of mortality is shown as a function of time in days (Y = f [X]). The shape of the curve is determined by the interaction of inherent and environmental factors producing stress and mortality in the nehu. The characteristics of the mortality curve used in comparing experiments are: (1) percentage initial mortality (percentage of dead [Y] by end of Day 1 [X = 1]); (2) day on which delayed mortality begins (X = m); (3) time interval over which delayed mortality occurs

(X_n - X_m); (4) day on which delayed mortality ceases (X = n); (5) extent or range of percentage of delayed mortality (Y_n - Y_m); (6) day of 50 percent mortality (Y = 50); and (7) percentage of surviving at asymptotic mortality level after delayed mortality has occurred (Y_∞, or maximum percentage of mortality). Further discussion of the mortality curves is in the "conclusions" section.

Figure 2 typifies commonly observed mortality curves in populations of captive nehu. The curves represent results of environmental conditions ranging from maximum stress (i.e., fishing vessels with unimproved wells) to minimum stress (i.e., optimal environment produced by experimental improvements).

In Figure 2 is a mortality curve (B) typical of a maximum stress environment. Handling of fish was by bucketing. The fish were further injured in bait wells under rough seas where insufficient oxygen was present, fish were disoriented, and 100 percent seawater produced osmoregulatory stress in injured fish. Initial mortality (primarily from injuries) under these conditions was high; more than 50 percent were dead by the end of Day 1 and all fish were dead by the end of Day 4. Mortality increased logarithmically until all fish were dead. There was no time interval over which there was no mortality.

In Figure 2, curve A shows the mortality pattern commonly encountered in early experiments with partial bait-well improvements. Fish were bucketed and sufficient oxygen was present, but 100 percent seawater induced osmoregulatory stress. In such a case, initial mortality is relatively high and for a short period thereafter, no mortality occurs. Subsequently, mortality increases rapidly until an asymptotic level where no further mortality is reached (Days 6 through 10). The early period of



A = partial improvement of tank or well environment
B = maximum stress environment
C = optimal environment
D = further improvement of environment than (A)

Figure 2. Examples of typical mortality curves of *S. purpureus* in captivity.

no mortality (Days 1 through 3) is defined here as the "stage of resistance" (Selye, 1950). The period of increasing mortality (Days 3 through 6) is defined as the "stage of exhaustion" or "delayed mortality" or "stress mortality." The characteristics of the curve vary depending upon environmental conditions and alleviation of stress. For example, addition of current or stronger light intensity may increase the time before onset of delayed mortality. Unless conditions are optimal, however, delayed mortality nearly always occurs. The mortality curve A in Figure 2 is sigmoid from Days 1 through 7 and shows that mortality may again increase exponentially following Day 10 until all fish are dead. This occurs from approximately Days 12 through 14 when fish are not fed and begin dying of starvation. It also occurs when fish gills are infected with protozoan cysts.

Curve D in Figure 2 is a mortality curve resulting from reducing osmoregulatory stress of injured fish by initially placing them in 50 percent seawater. In this example other factors were also optimal. Characterisitcs of this treatment are low initial mortality (less than 1 percent) and a long stage of resistance (Days 2 through 8) before delayed mortality begins (Day 8). The extent of delayed mortality (from Days 8 through 12) is also reduced, with more fish surviving. Occasionally during particularly optimal conditions, even though fish are roughly handled, placing them into 50 percent seawater completely eliminates delayed mortality.

Finally, curve C in Figure 2 shows the effect of careful handling (e.g., by swimming fish into wells) and placing of fish into 50 percent seawater. All other factors were optimal. Little or no mortality occurred throughout the experimental period and no delayed mortality occurred at all.

Fish that were carefully handled, although placed in 100 percent seawater, also suffered much less delayed mortality than usual.

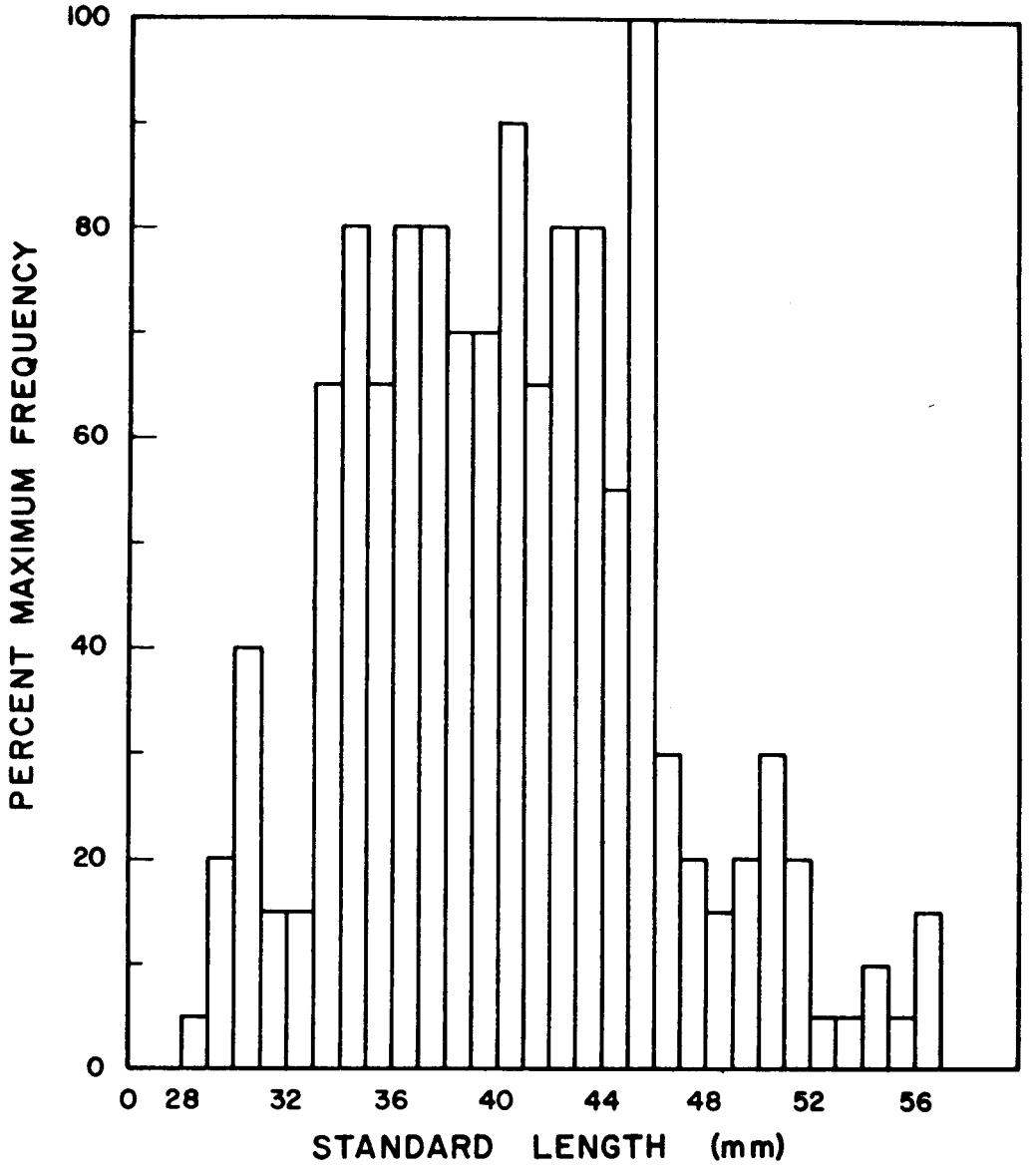
Factors affecting mortality

These factors are divided into inherent and environmental variables.

Inherent variables. Several characteristics inherent to nehu populations probably affect subsequent survival in captivity. It was not possible to assess, for example, genetic variation and previous environmental history completely. The effects of size, sex ratios, disease, and acclimation are partly assessed below.

1. Size. Figures 3, 4, and 5 show the frequency distributions of standard length and wet and dry weights of fish randomly sampled just after capture (10 barge and laboratory experiments; 10 samples of 25 fish each). The range in standard length is approximately 28.0 to 60.0 mm. Most fish in the samples are post-metamorphic juveniles and adults (Plate 17). According to Yamashita (1951) metamorphosis occurs at approximately 30 mm. The frequency distributions approximate a normal distribution with no apparent year classes.

Plate 17. Post-metamorphic juveniles and adult nehu used in most of the experiments. The standard length ranged from 28.0 to 60.0 mm.



Total n = 225
Mean SL = 40.2 mm

Figure 3. Frequency distribution of standard length of post-metamorphic *S. purpureus* based on ten random samples of 25 fish per sample.

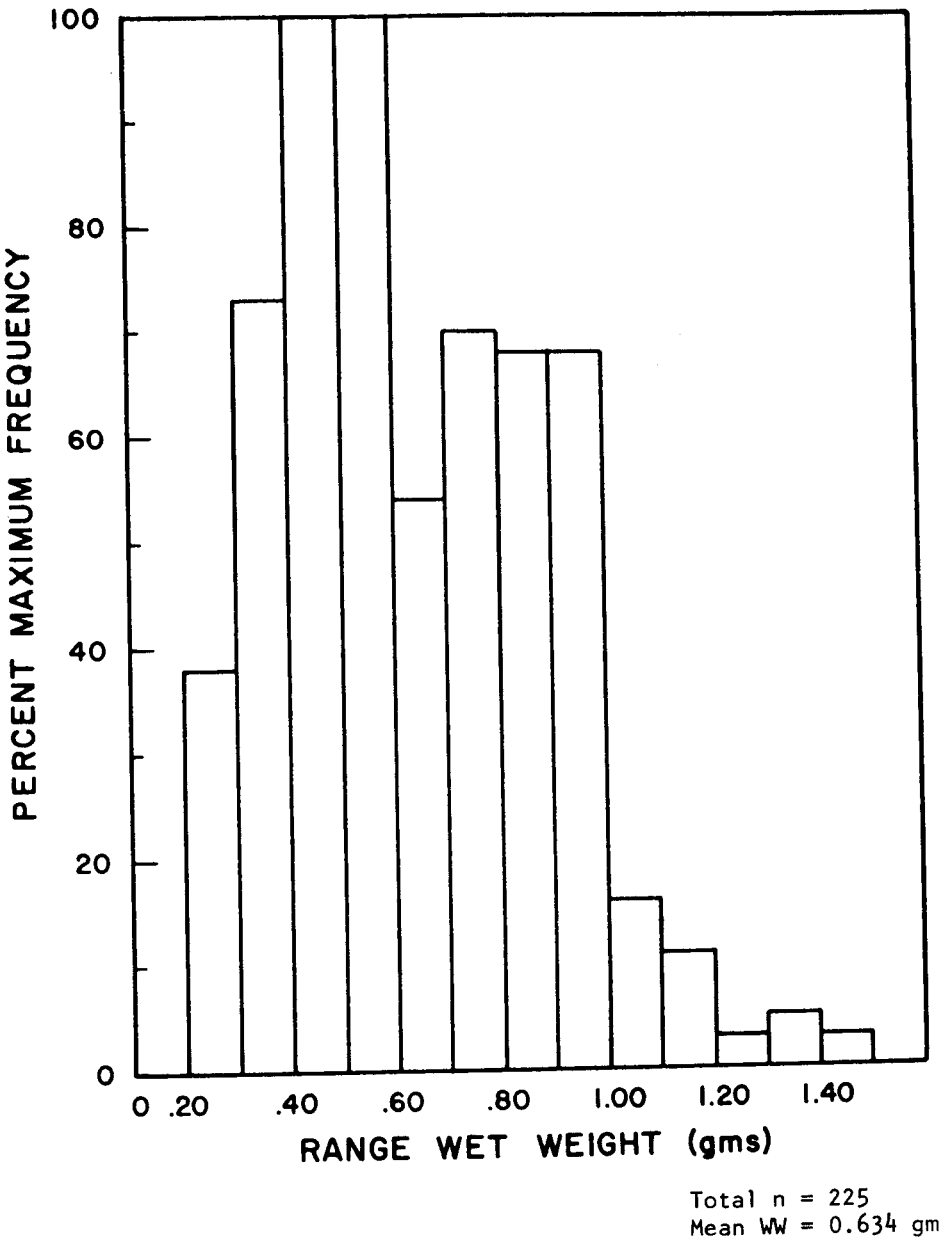


Figure 4. Frequency distribution of wet weight of post-metamorphic *S. purpureus* based on ten random samples of 25 fish per sample.

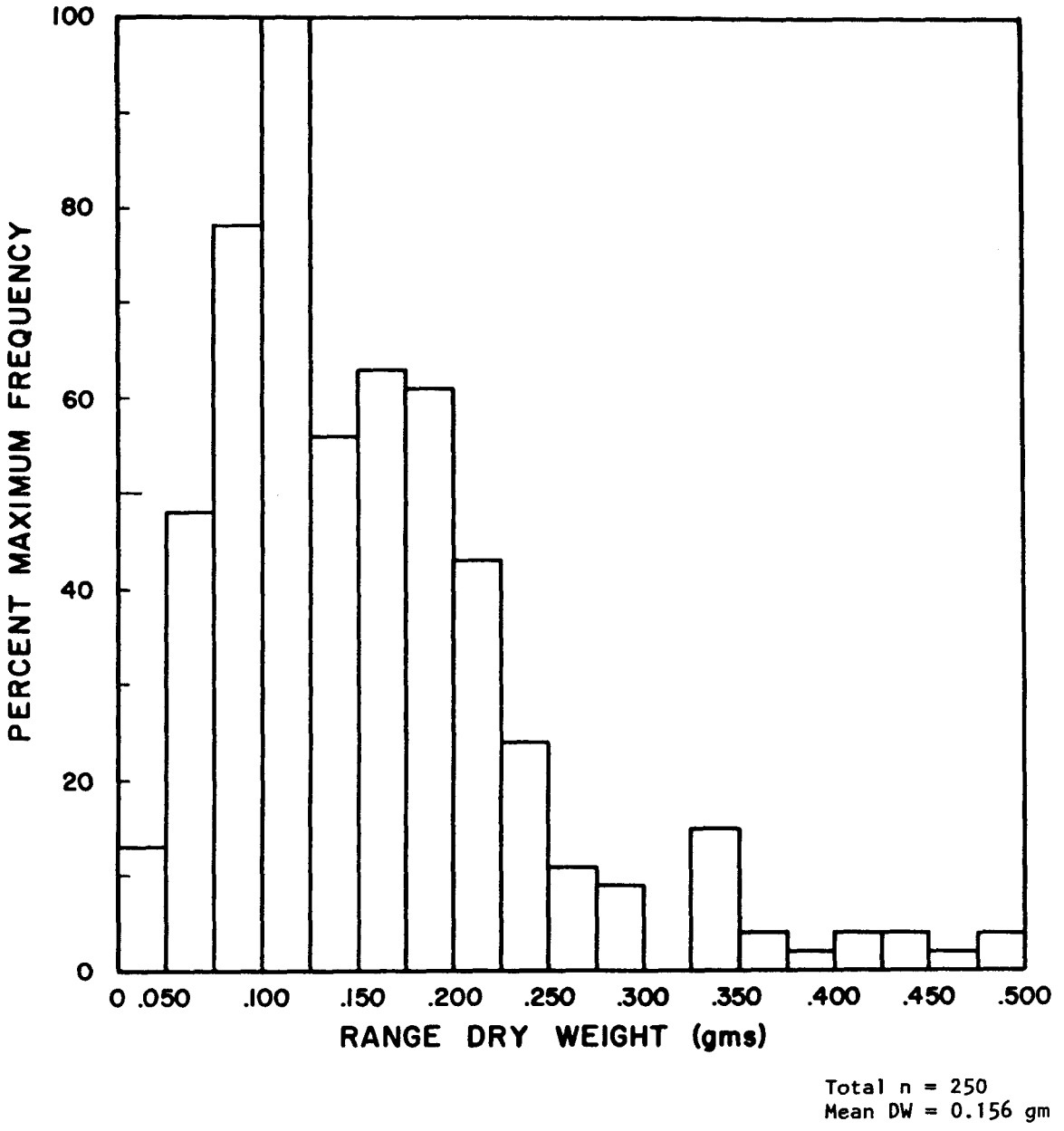


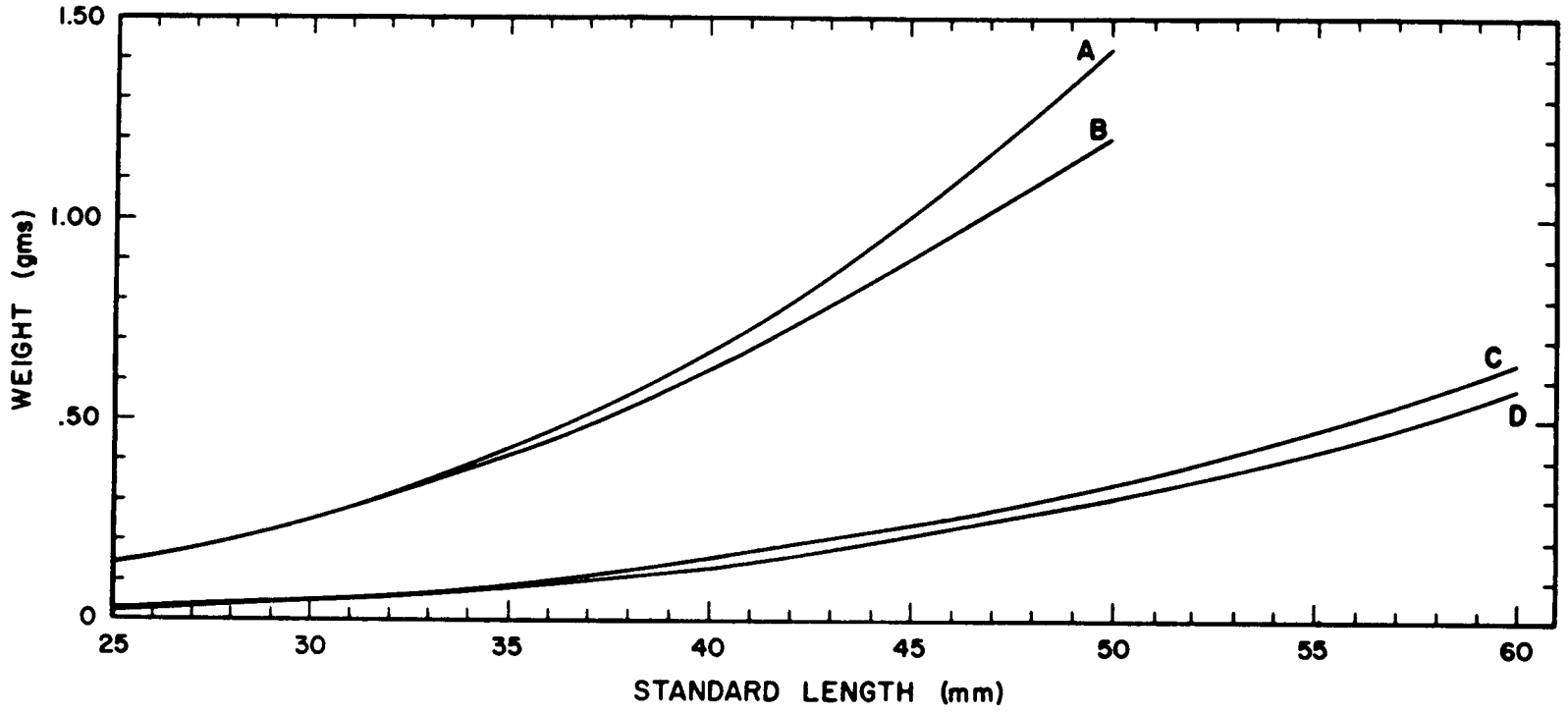
Figure 5. Frequency distribution of dry weight of post-metamorphic *S. purpureus* based on ten random samples of 25 fish per sample.

Size data are summarized in Table 5. Mean standard length, wet weight, and dry weight are 40.2 mm, 0.634 gm, and 0.156 gm, respectively for the total sample. The mean dry weight-wet weight ratio is 0.246 (25 percent dry weight) and the mean percentage of body water is 75 percent. The size of fish in laboratory samples is less than in barge samples. This probably reflects the fact that whereas most laboratory samples were day bait, most barge samples were night bait and larger fish are frequently caught at night.

The calculated regressions of wet and dry weights on standard length are shown in Figure 6. Regressions of wet weight on standard length are described by a power function ($\hat{Y} = aX^b$) where $\underline{b}$ = 3.366 in the laboratory sample (Figure 6, curve B) and $\underline{b}$ = 3.109 in the barge sample (Figure 6, curve A). The difference in $\underline{b}$ is probably due to the different size ranges of the two samples. Regression of dry weight on standard length is also a power function with $\underline{b}$ = 3.584 in the laboratory sample (Figure 6, curve D) and $\underline{b}$ = 3.606 in the barge sample (Figure 6, curve C). The two regressions are similar in this case.

Survival varied with size (also found by Brock and Takata, 1955). Smaller fish died earlier, with more larger fish surviving at the end of the experimental period. This was observed in all experiments. An example is given in Table 6 which compares the mean wet weight of dead fish on Day 1 and the mean wet weight of live fish on Days 1 and 14 for four experiments. Younger fish are probably injured more easily and are more susceptible to stress conditions in captivity.

2. Sex ratios. There is apparently considerable variation in sex ratios as determined from inshore samples of nehu (T.A. Clarke, 1971: personal communication). Of four samples (40 fish per sample) from four different



A = wet weight (gm) vs standard length (mm), barge samples ($\underline{a}$ = 6.53 x 10^{-6}, $\underline{b}$ = 3.109)
B = wet weight (gm) vs standard length (mm), laboratory samples ($\underline{a}$ = 2.72 x 10^{-6}, $\underline{b}$ = 3.366)
C = dry weight (gm) vs standard length (mm), barge samples ($\underline{a}$ = 2.25 x 10^{-7}, $\underline{b}$ = 3.606)
D = dry weight (gm) vs standard length (mm), laboratory samples ($\underline{a}$ = 2.69 x 10^{-7}, $\underline{b}$ = 3.584)

Figure 6. Calculated regression curves of wet and dry weights on standard length of *S. purpureus*.

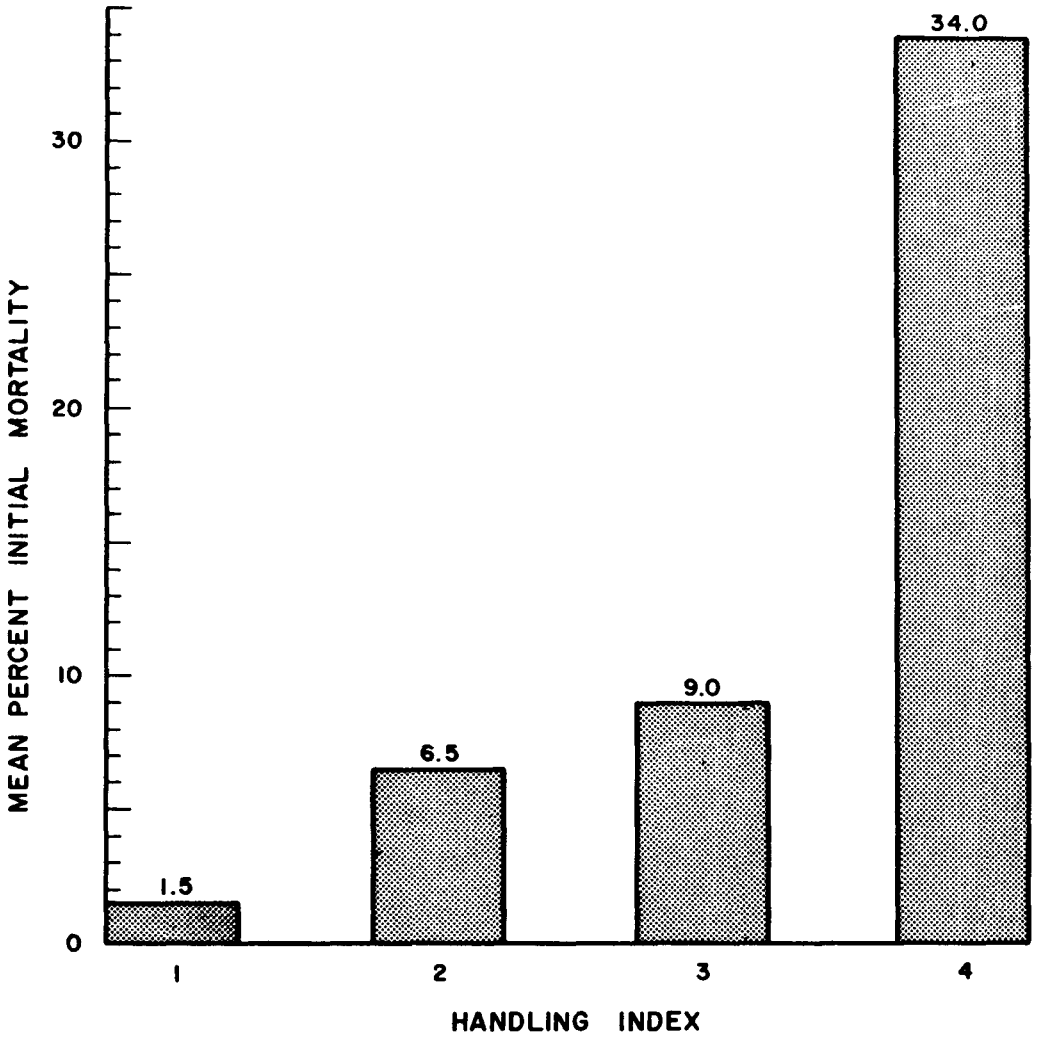
experiments, three were found with a 1:1 sex ratio and the fourth with slightly more males. When the four samples were combined, the sex ratio was approximately 1:1, with 52 percent males and 48 percent females. Differential mortality between sexes was not measured.

3. Disease. The effect of protozoan parasites on mortality in captivity is often considerable. Although gills of fish from the natural populations were infected with protozoan cysts, the summer examinations of fish when first captured indicated that the infection was usually light (one to five cysts per gill chamber). It is possible, however, that cysts may contribute to stress in fish during the first few days after capture. Infections increased over the experimental period and it is possible that definite stress and mortality may eventually occur.

4. Acclimation. In a few experiments, nehu were maintained in barge bait wells for 14 to 20 days before they were transferred to the laboratory. The period of acclimation eliminates delayed mortality from handling. Only a small initial mortality from handling occurred after transfer to the laboratory. The mean percentage of initial mortality in acclimated fish after transfer was only 1.5 percent (range = 0 to 5 percent) as opposed to 9.0 percent (range = 1 to 18 percent) initial mortality in unacclimated fish handled and transferred in the same way.

Environmental variables. Environmental variables affecting survival of nehu in captivity are discussed in the order of importance. Interactions are briefly considered; a detailed discussion of interactions is given with the factor analysis in the "conclusions" section.

1. Handling. The effect of increased handling on initial mortality of nehu is shown in Figure 7, which gives mean percentage of dead by the end of



Handling Index: 1 = night bait, transferred by swimming directly into barge wells
2 = day bait, transport time 50 to 70 min, transferred by swimming into barge wells
3 = night bait, transport time 10 to 20 min, bucketed into laboratory tanks
4 = day bait, transport time 50 to 70 min, bucketed into laboratory tanks

Figure 7. Effect of handling on initial mortality of *S. purpureus* placed in 100 percent seawater during capture.

Day 1 for all experiments with 100 percent seawater. Most fish died directly from lacerations and contusions suffered during capture and transfer. In barge experiments where fish were captured by night baiting and then transferred by swimming directly into the bait wells with little or no handling (Handling Index [HI] = 1; Figure 7), the mean initial mortality was only 1.5 percent (range = 0 to 3 percent). When fish were captured by day baiting, transferred by swimming into the bait receiver, transported for 50 to 70 minutes, and finally transferred by swimming from the bait receiver into the barge bait wells (HI = 2), the mean initial mortality was 6.5 percent (range = 1 to 16 percent). In laboratory experiments, fish were sometimes captured by night baiting, transferred by swimming from the barge net into the bait receiver, transported for 10 to 20 minutes to the laboratory, and finally bucketed from the bait receiver into the laboratory tanks (HI = 3). In this situation, mean initial mortality was 9.0 percent (range = 1 to 18 percent). If fish were captured by day baiting, transferred by swimming into the bait receiver, transported to the laboratory in 50 to 70 minutes, and finally bucketed from the bait receiver into laboratory tanks (HI = 4), the mean initial mortality would be relatively high (34.0 percent; range = 5 to 63 percent). Clearly, increased handling is deleterious to survival. Furthermore, stress induced by handling is responsible for most succeeding delayed mortality.

2. Salinity. The effect of salinity on survival is related to handling of nehu. The integument of the fish is injured and scales and mucus are lost. The results of this study and those of Maginniss (1970) show that roughly handled nehu placed into 100 percent seawater (34.0 to 36.0 ppt) immediately after capture undergo osmoregulatory stress, loss

of body water and a simultaneous increase in blood serum chloride and osmotic pressure.

With lacerations and loss of mucus and scales, fish lose water by increased exosmosis and possibly also by shock diuresis. Normally, nehu contain approximately 79 to 82 percent body water ($\frac{\text{wet weight - dry weight}}{\text{wet weight}}$ X 100). When they are placed in laboratory tanks containing 100 percent seawater, body water decreases a mean of 3 percent to a minimum range of about 74 to 77 percent. Most body water is lost within 1 to 3 hours after capture; the recovery period varies with the extent of injuries and conditions of the capture environment. In 100 percent seawater, partial recovery usually occurs by the end of Day 1 and complete recovery by Days 10 through 14.

The normal range of serum chloride is approximately 150 to 160 mEq/liter (Maginniss, 1970); the maximum recorded is approximately 180 mEq/liter. The mean percentage of increase in serum chloride is approximately 8 to 10 percent, occurring 1 to 3 hours after capture, the same period as maximum water loss. The recovery pattern of serum chloride is similar to that of body water--partial recovery by the end of Day 1 and complete recovery by Days 10 through 14. At least part of the serum chloride increase may result from fish swallowing seawater to compensate for water loss (Maginniss, 1970). During recovery, fish must excrete chloride through gills by means of active transport.

The normal range of serum osmotic pressure is approximately 340 to 405 mOsm/liter (Maginniss, 1970). The serum osmotic pressure also increases in the first 1 to 3 hours of capture. There are insufficient data to state the mean percentage of increase with certainty; limited data

indicate it is about 10 percent. The recovery pattern is similar to that of body water and serum chloride.

Nehu are hypoosmotic in seawater and must continually compensate osmotically by swallowing more water and eliminating excess salts. This process requires energy. When mucus and scales are lost, osmoregulatory stress is increased greatly, requiring further energy for regulation. That many individuals cannot compensate is indicated by the high rate of delayed stress mortality after the resistance stage. A more comprehensive discussion of osmoregulation of nehu is given by Maginniss (1970).

Data from Maginniss (1970) and results of this study indicate that the most favorable salinity range for decreasing osmoregulatory stress and increasing survival is from 40 to 60 percent seawater (approximately 14.0 to 22.0 ppt). The blood serum osmotic pressure averages about 400 mOsm/liter or approximately 40 percent osmotic pressure of seawater. To minimize osmotic stress while permitting a margin of error if salinity control fails and salinity drops, 50 percent seawater (approximately 17.5 ppt) appears optimal for increasing survival experimentally.

Tests by Brock and Takata (1955) showed that mortality of nehu was greater in 100 percent rather than 50 percent seawater. They concluded that brackish water treatment of a few hours might increase survival. This work was continued in the present study.

In most of the salinity experiments, 50 percent seawater (16.0 to 20.0 ppt; mean of 17.5 ppt) was used to test the effect of reduced salinity on mortality of nehu. Several experiments were conducted varying the length of exposure to reduced salinity. When the percentage of initial mortality of nehu in all experiments with 50 percent seawater (irrespective of exposure time) was compared with the percentage of initial

mortality in 100 percent seawater, a highly significant difference ($P < 0.001$) was found (Table 7). The mean percentage of initial mortality in 100 percent seawater (23.4 percent) was approximately five times that in 50 percent seawater (4.7 percent).

Results of experiments with reduced salinity are summarized in Table 8 and the mortality curves shown in Figures 8 and 9. Both 50 percent and 100 percent seawater are circulated in an open system with supplemental oxygen and current. Additional experiments in a recirculated, closed brackish water system are discussed later.

In laboratory experiment 25B (Table 8; Figure 8), nehu were exposed to varying periods of low salinity. Examination of Figure 8 shows that greatest initial mortality, earliest delayed mortality and 50 percent mortality, and greatest total mortality by Day 12 occurred in 100 percent seawater (Figure 8, curve B). Even brief exposure to 50 percent seawater enhanced survival by decreasing initial mortality and prolonging onset of delayed mortality (Figure 8, curve A). An exposure of 6 hours (Figure 8, curve C) was nearly equivalent to that of 24 hours (Figure 8, curve D) in increasing survival. Since the first 1 to 3 hours after capture is the period of greatest osmoregulatory stress, the results are understandable. It is therefore most important to reduce salinity to facilitate recovery in those first few hours after capture. Although exposure to reduced salinity decreased initial mortality and prolonged the onset of delayed mortality, delayed mortality still occurred, indicating that alleviation of stress was still incomplete.

In barge experiment 18 (Figure 9), mortality was greatly decreased by exposing fish to 50 percent seawater for only 4 hours (Figure 9, curve A). Some delayed mortality occurred, but 50 percent mortality was never reached

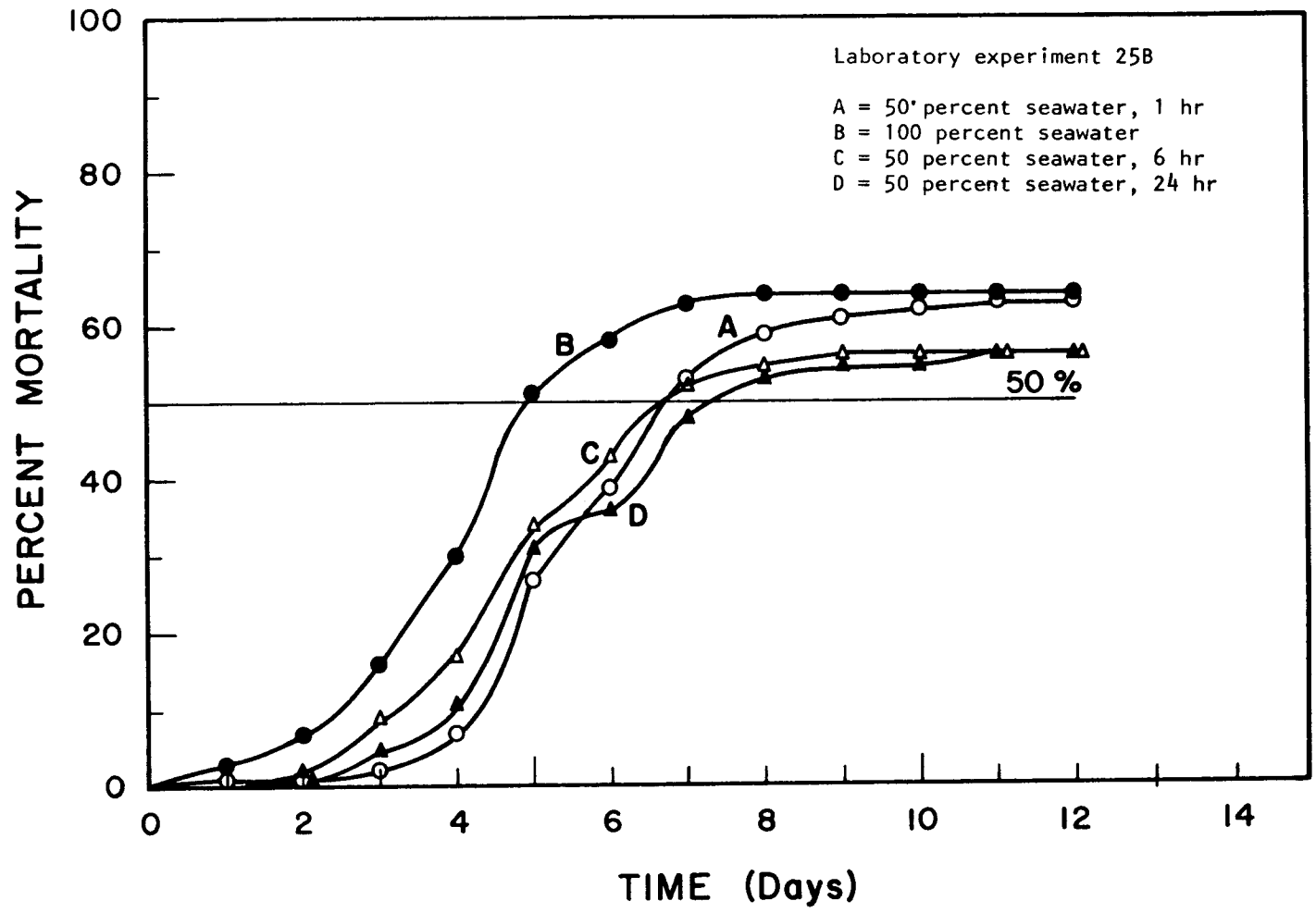


Figure 8. Effect of various exposure times to reduced salinity on mortality of *S. purpureus* in open system.

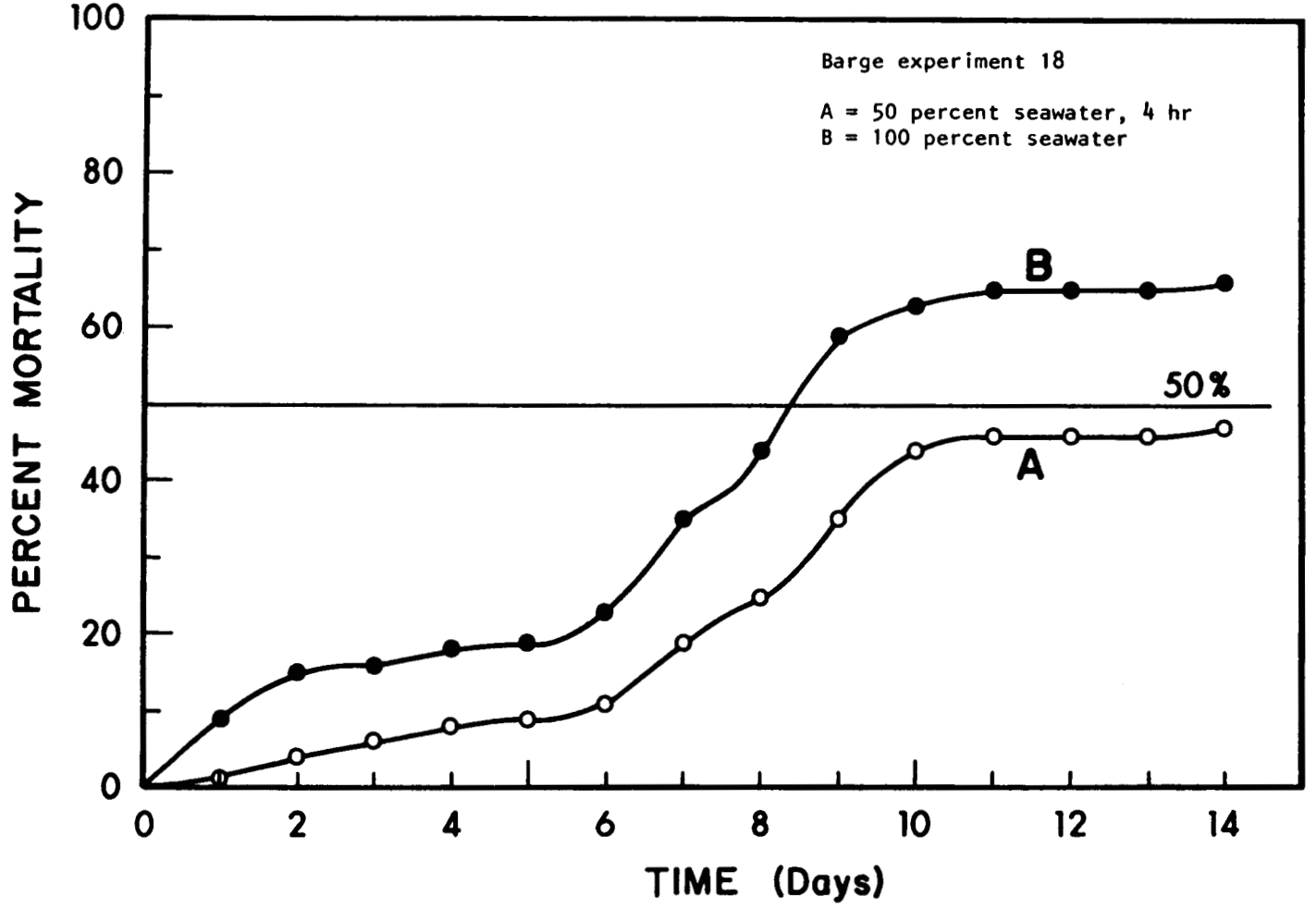


Figure 9. Effect of reduced salinity on mortality of *S. purpureus* in open system.

and 53 percent remained on Day 14. In 100 percent seawater (Figure 9, curve B) 50 percent mortality occurred by Days 8 to 9 and only 34 percent remained on Day 14.

Interaction of salinity with oxygen is reflected in the recovery of body water under different oxygen regimes. Table 9 summarizes results of two experiments (laboratory experiments 12 and 13) where oxygen treatment was varied (supplemental air vs supplemental oxygen). These experiments were conducted in 100 percent seawater where maximum body water loss occurred. There is a significant difference between the two treatments in percentage of body water regained by Day 14 ($P < 0.01$). In tanks with air, mean oxygen ranged from 5.0 to 6.0 ppm; in tanks with oxygen, from 6.5 to 8.0 ppm. Mean percentage of increase in body water in tanks with air was 0.44 percent and full recovery was incomplete by Day 14. In tanks with oxygen, mean percentage of increase in body water was 2.36 percent with nearly complete recovery by Day 14. It appears that high oxygen concentrations facilitate osmoregulation and thereby reduce stress. Several other experiments in which supplemental oxygen was not provided and critically low oxygen levels occurred also show that recovery of body water is incomplete by Day 14. In 100 percent seawater, complete recovery of body water takes place only when oxygen is maintained at saturation.

3. Oxygen. Sufficient oxygen is necessary to sustain the metabolic activity of nehu and is especially important in affecting survival in captivity when stress is increased. Critically low oxygen concentrations frequently occur in unimproved bait wells. No Hawaiian live-bait vessels provide supplemental air or oxygen: oxygen is replaced with seawater entering bung-holes on the bottom of the bait wells. A few vessels have pumping systems which circulate water rapidly through wells. In this case,

the oxygen concentration increases with increased flow rate. Figure 10 shows the oxygen concentration (ppm) in bait wells on the *Buccaneer*. Data show that oxygen falls to critical low levels in wells with no pumping system, especially when the vessel is stationary in Kewalo Basin. When the vessel is under way, oxygen concentration usually rises. Observations on the *Buccaneer* and other vessels lead to the conclusion that oxygen depletion is a major factor contributing to high bait-well mortality, particularly if there are no circulation pumps.

Oxygen consumption of nehu under various environmental conditions is summarized in Table 10. Active oxygen consumption in groups of nehu was measured and the values of oxygen consumption (cc oxygen/gm fish/hr) were determined from the linear regression coefficient b. Often, however, the regression is not linear but curvilinear. Figure 11 demonstrates that oxygen consumption is usually higher in the first 1 to 2 hours, decreasing gradually to a constant linear decrease until an equilibrium level of oxygen concentration is reached (Figure 11, curves B and D). The consumption rates, therefore, are estimates only, assuming a constant rate of consumption. Time in these regressions spanned the period from the beginning of the experiment until an equilibrium level was reached, varying with density of fish and conditions of the capture environment. The level of equilibrium oxygen concentration is considered here as equivalent to the incipient lethal level (Prosser and Brown, 1961) or critical level (Pritchard, 1955).

Table 10 (section A) shows consumption rates of individual nehu as determined by Pritchard (1955). Pritchard did not measure wet weight of fish in his experiments, giving instead the standard lengths of six fish for experiments in August 1955. Using Pritchard's standard lengths and the regression of wet weight on standard length (Figure 6) of this study, the wet weight of

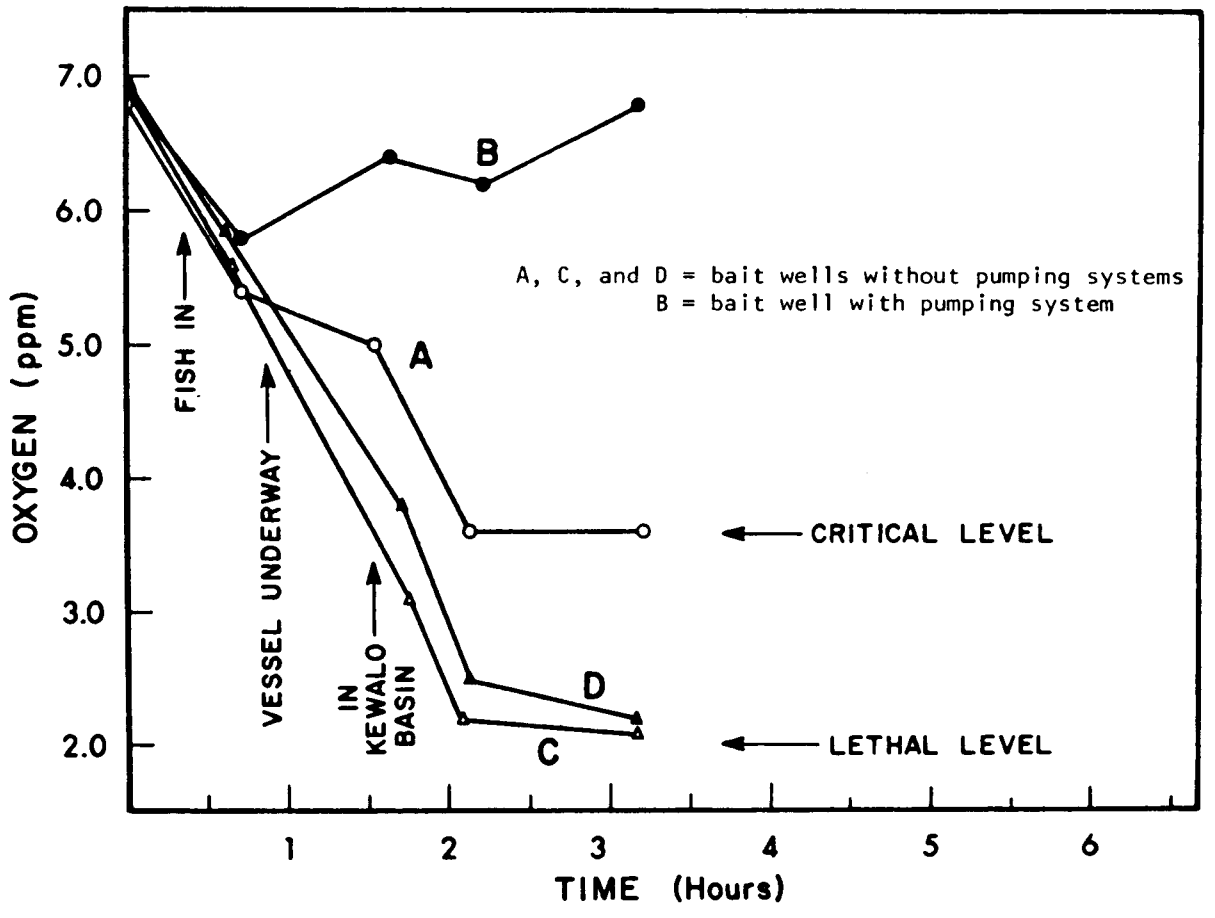
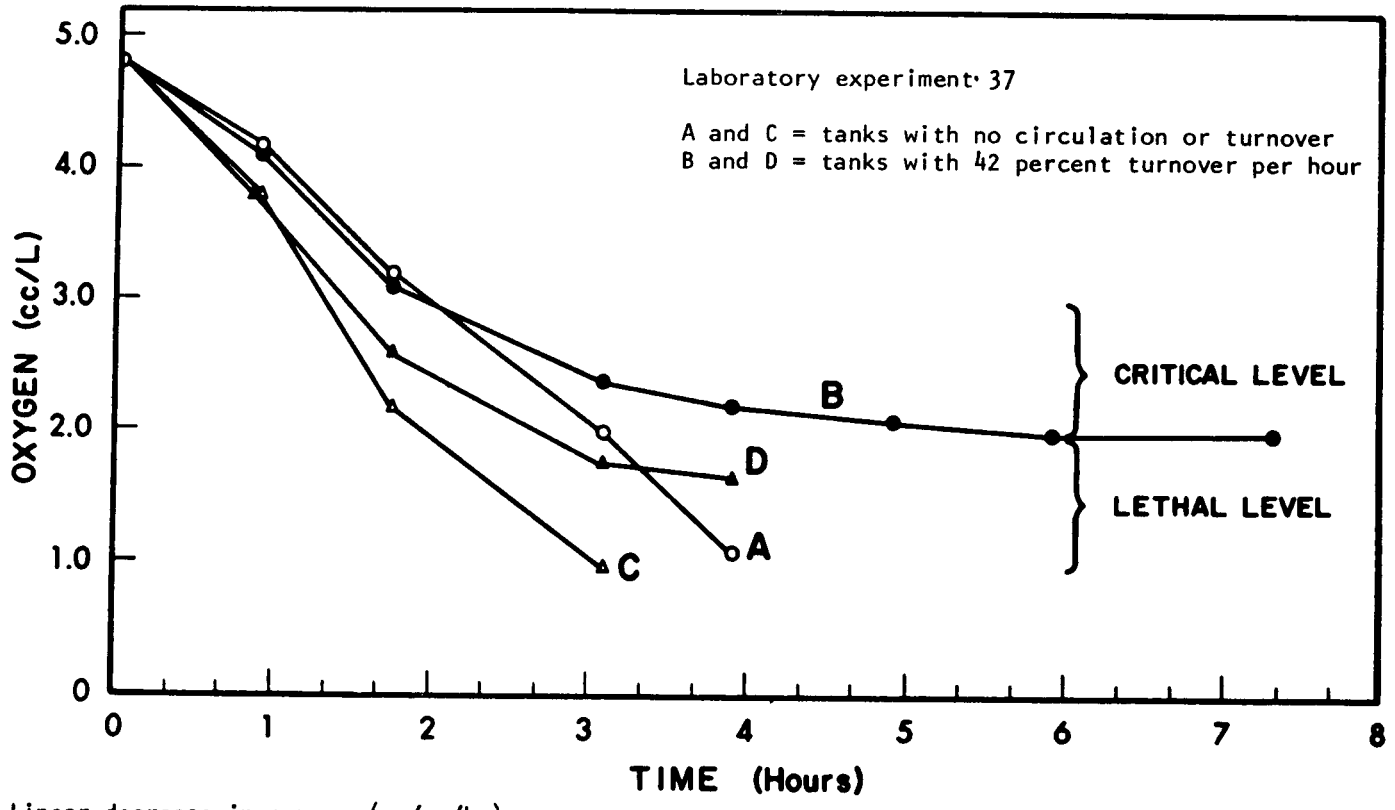


Figure 10. Decrease in oxygen concentration (ppm) in bait wells of the *Buccaneer* from time of *S. purpureus* capture until lethal level is reached in Kewalo Basin.



Linear decrease in oxygen (cc/gm/hr):

A - $\underline{b}$ = .601
B - $\underline{b}$ = .572
C - $\underline{b}$ = .883
D - $\underline{b}$ = .617

Figure 11. Decrease in oxygen concentration in laboratory tanks from the time *S. purpureus* is placed in tanks until lethal level is reached.

each length was estimated, and the mean consumption rate for six individual fish calculated to be 0.774 cc oxygen/gm fish/hr. The mean consumption of individual nehu appears to be approximately twice that determined by this study for acclimated groups of fish. The validity of this comparison is questionable, however, since Pritchard gave no description of acclimation.

Table 10 (sections B and C) shows rates of oxygen consumption determined for acclimated groups of fish. Nehu were acclimated for at least two weeks at approximately 25° to 28°C and were fed frozen brine shrimp (3 percent body weight/day) from Days 3 through 15 after capture. After two weeks, mortality ceased. Remaining fish were relatively larger and appeared healthy. In laboratory experiment 1 (Table 10, section B), two tests were conducted on the same group of fish on two successive days. The mean rate was 0.353 cc oxygen/gm fish/hr. In laboratory experiment 2 (Table 10, section C), oxygen consumptions of acclimated fish given different food treatments were compared. These fish were first acclimated then given different foods for 10 days, after which their oxygen consumption was measured. Those fed frozen brine shrimp exhibited the highest consumption rate; those without food, the lowest. Fish given other diets exhibited intermediate rates of consumption. These results substantiate other experiments showing that frozen brine shrimp is the best food for maintaining weight in captivity.

Oxygen consumption of unacclimated fish (measured on Day 1 after capture is higher than that of acclimated fish. Laboratory experiment 37 (Table 10, section D) shows that in tanks with no supplemental air, oxygen, circulation or current, mean oxygen consumption of nehu was 0.742 cc oxygen/gm fish/hr, approximately twice the rate of acclimated fish (0.354 cc oxygen/gm fish/hr.

With only circulation of water (Table 10, section E) mean rate of oxygen loss was 0.591 cc oxygen/gm fish/hr. Obviously, at this flow rate (4.8 liter/min; 1.3 gal/min) the turnover rate of water (42 percent per hr) is insufficient to replace oxygen consumed by the fish.

When air as well as circulation were provided (Table 10, section F), the mean rate of oxygen loss was 0.311 cc oxygen/gm fish/hr. Air and circulation were still insufficient to replace the oxygen lost through respiration.

A horizontal current, in addition to air and circulation (Table 10, section G), did not significantly change the rate of oxygen loss. The mean rate of oxygen loss was 0.292 cc oxygen/gm fish/hr.

Only when tanks were given supplemental oxygen (Table 10, section H) was saturation maintained regardless of oxygen consumption. Other experiments also clearly demonstrate that for average densities of unacclimated fish, supplemental oxygen or substantially increased flow rate is required to maintain saturation. Supplemental air does not usually provide enough oxygen to sustain saturation.

In some experiments oxygen concentration decreased rapidly to a lethal level (Figure 11, curves A and C) at a point when the first fish asphyxiates, loses equilibrium, sinks, and dies. In groups of fish, some individuals reach this level before others. The lethal value given here, therefore, represents the level of oxygen concentration at which the first death occurs. Time to this level depends on density of fish and initial oxygen concentration. Other environmental variables interacting with low oxygen may also raise the oxygen concentration level at which death occurs. For most experiments, the lethal oxygen level was approximately 1.0 to 2.0 cc oxygen/liter (1.3 to 2.7 ppm); the mean value,

1.4 cc oxygen/liter (1.9 ppm). At the equilibrium concentration or critical oxygen level (incipient limiting level of Prosser and Brown, 1961), the oxygen consumption of nehu becomes dependent on the oxygen concentration, which was from 2.0 to 3.0 cc oxygen/liter (2.7 to 4.0 ppm), mean value 2.5 cc oxygen/liter or 3.3 ppm. At the critical level fish decrease their consumption rate. Presumably, the longer the nehu must regulate their consumption, the more they are stressed. After some time (varying between experiments) at the critical level they are unable to decrease consumption further. Then oxygen concentration drops to the lethal level and death occurs. In a few instances, oxygen drops rapidly to the lethal level without any equilibrium period. This takes place when the density of fish is so great that there is insufficient oxygen to sustain fish long enough to regulate and decrease consumption. At lower densities, there is usually a regulation period at the critical level of oxygen concentration before a final decrease to the lethal level.

The mean lethal level of oxygen concentration determined here (1.44 cc oxygen/liter) is less than that determined by Pritchard (1955) of 2.02 cc oxygen/liter. The difference may be due to the fact that the value determined for this report was for groups of fish and his for individual fish.

Results indicate that oxygen concentration should not fall below 3.5 cc oxygen/liter (approximately 5.0 ppm). This concentration is well above the critical level (2.5 cc oxygen/liter; 3.3 ppm). However, since any oxygen concentration below saturation (approximately 4.0 to 5.0 cc oxygen/liter; 6.0 to 7.0 ppm at experimental temperatures and salinities) may create stress, the oxygen concentration should ideally be maintained at saturation by the addition of supplemental oxygen or increased flow rate.

Addition of industrial oxygen may result in high oxygen concentrations

above saturation, particularly if oxygen flow is not carefully regulated. An upper limit of oxygen concentration was determined. The effects of high oxygen (over saturation to 20 ppm) varied, depending upon how fish were exposed. In laboratory experiment 32, fish were placed in high oxygen concentrations immediately after capture (Figure 12). The high oxygen concentration was deleterious. Greatest mortality occurred in tanks with highest mean oxygen concentration (Figure 12, curve C); lowest mortality in tanks with lowest mean oxygen concentration (Figure 12, curve B). In laboratory experiment 33, fish were placed in water at saturation immediately after capture and the oxygen concentration gradually increased to higher values. No significant difference in mortality due to different oxygen concentrations was observed in this case. It is unclear whether mortality in high concentrations is due to tissue damage from excess oxygen in blood vessels or to disorientation of fish by water disturbance. Microscopic examinations revealed no oxygen bubbles in blood vessels of the caudal fin.

These results suggest that fish should initially be placed in water at saturation or just slightly higher. If oxygen decreases during the first 3 hours, oxygen saturation can be maintained by gradually increasing the flow of oxygen until equilibrium is reached, usually by the end of the first day of captivity. For maximum survival oxygen concentration should never exceed 10 ppm.

Survival of nehu in different oxygen environments was contrasted in several laboratory and barge experiments. Of these, laboratory experiment 13 best shows the effect of supplemental oxygen in enhancing survival. Current was also provided in some tanks during this experiment. Figure 13 shows that mortality was highest in tanks provided only with air (Figure 13, curve B), lowest in tanks with supplemental oxygen and current (Figure 13, curve C), and

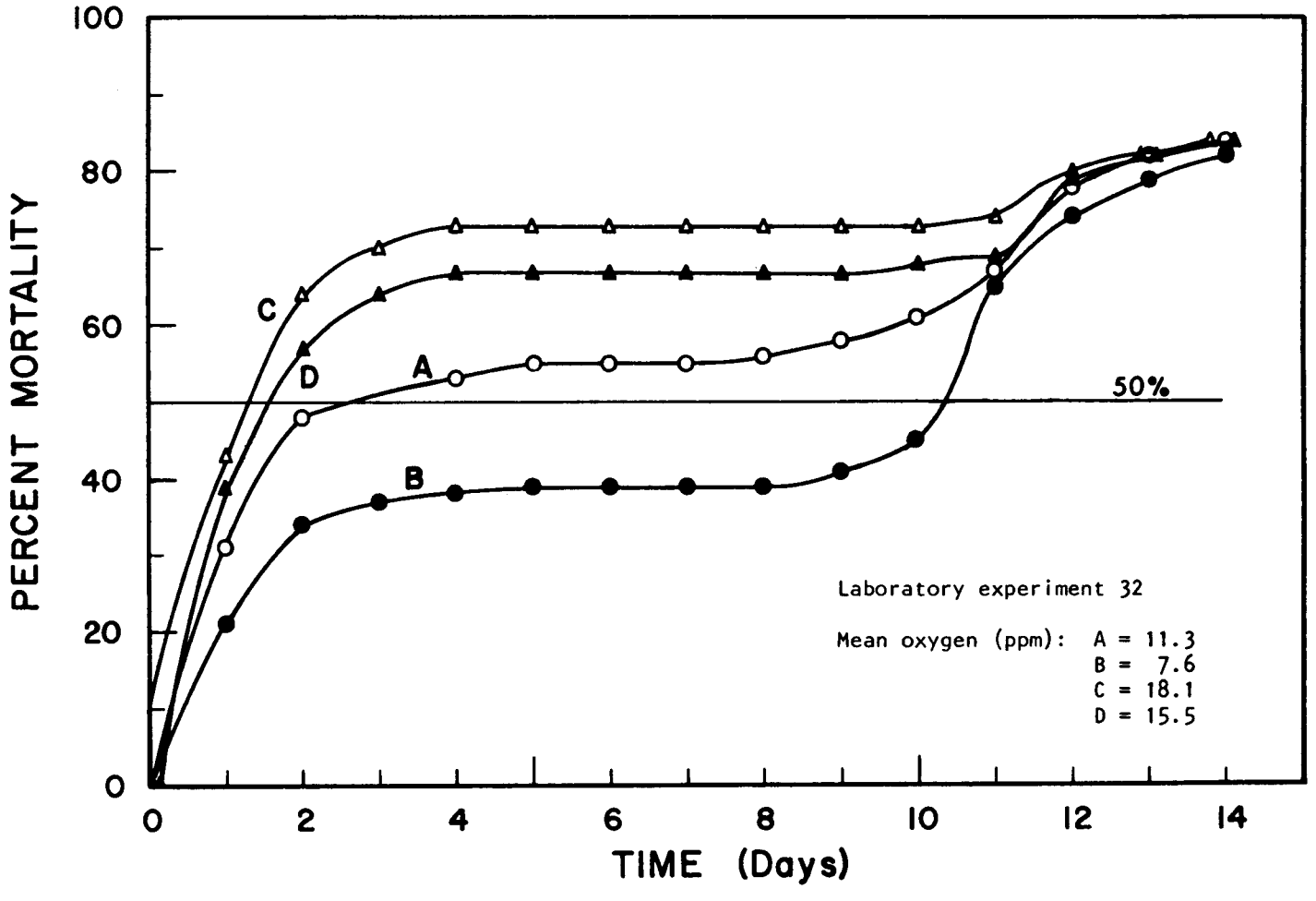


Figure 12. Effect of high oxygen concentrations on mortality of *S. purpureus*.

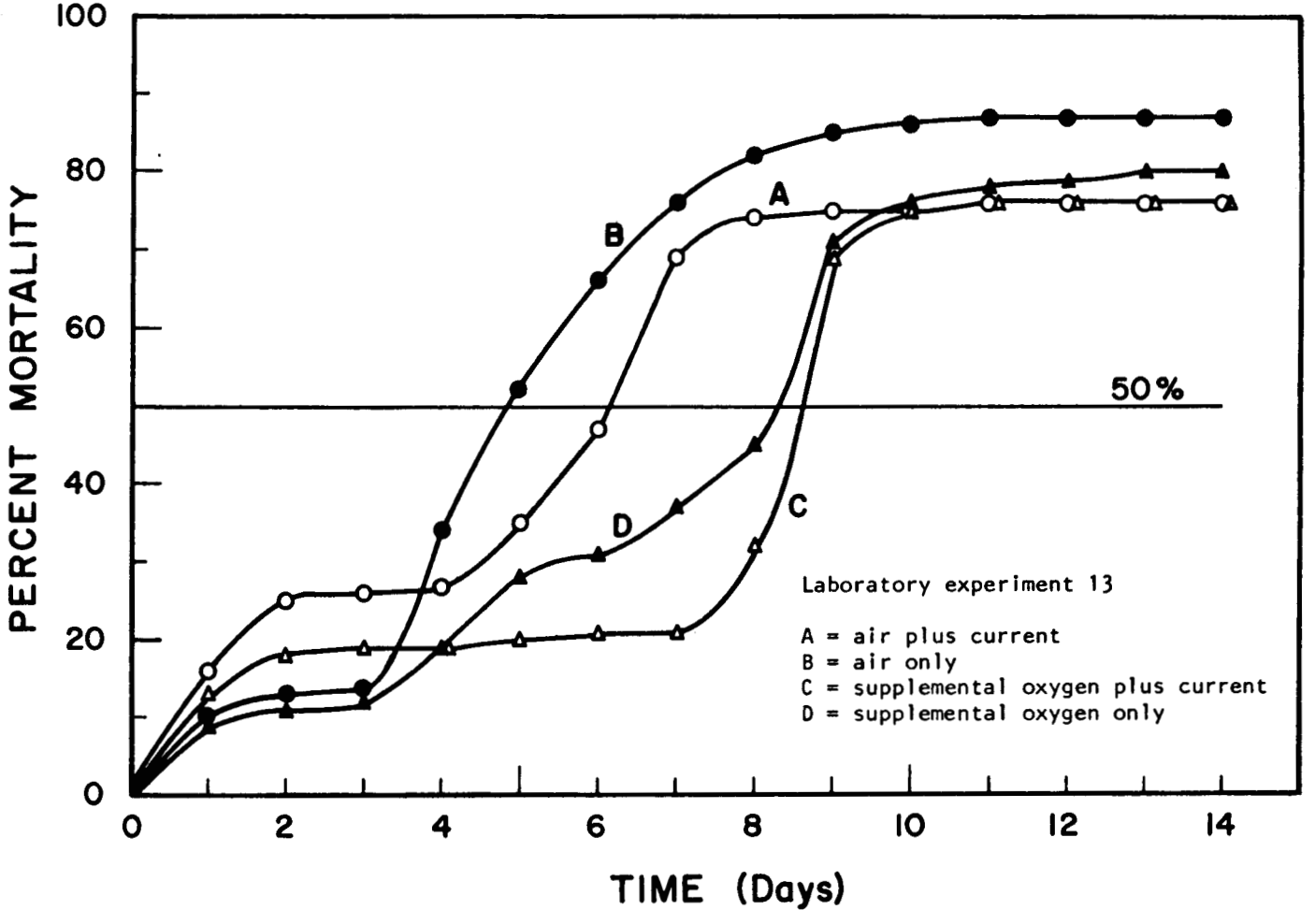


Figure 13. Effect of oxygen and current on mortality of *S. purpureus*.

intermediate in tanks with oxygen only (Figure 13, curve D) or with air and current (Figure 13, curve A). This is consistent with results of other oxygen experiments, although differences in mortality between air and oxygen treatments were often less pronounced than in laboratory experiment 13. Results depend largely upon the density of fish. In some experiments other mortality factors superceded the effect of oxygen, resulting in high mortality regardless of oxygen saturation. Usually, however, the provision of oxygen greatly increased survival. For example, in Figure 13, 50 percent mortality in tanks with oxygen did not occur until Day 8 or 9 as opposed to Day 5 or 6 in tanks with air. Although supplemental oxygen was used to maintain oxygen at saturation level, it did not eliminate delayed mortality, but often prolonged the onset of delayed mortality for several days.

In estimates of total oxygen consumed by a bucket of fish ($\bar{x}$ = 2,700 fish) at different consumption rates and mean wet weights (Table 11), consumption rates range from an estimated minimum (such as in acclimated groups of fish) to average consumption rates of single fish to an estimated maximum (Table 10). The mean wet weight of fish usually ranges from approximately 0.500 to 0.700 gm.

The supplemental oxygen apparatus is shown in Plates 11 and 12. A complete description can be found in Baldwin (1970), together with data showing the amount of oxygen (cc/min) released from the oxygen stone at pressures from 5 to 35 PSI. Table 35 gives line pressures providing sufficient oxygen for 1 to 10 buckets of fish.

The *optimal oxygen environment for nehu requires:*

a. Provision of supplementary oxygen as described.

b. Adjustment of oxygen line pressure to maintain oxygen at saturation concentration for experimental temperature and salinity.

c. Adjustment of oxygen stones and line pressure for the release of minimum-size bubbles to increase efficiency of oxygen dissolution in seawater.

d. Reduction of oxygen flow to minimum pressure necessary for saturation to reduce disorienting turmoil.

e. Oxygen concentration not to exceed a maximum of 10 ppm in 100 percent seawater.

f. Oxygen concentration not to decrease below 3.5 cc/liter (approximately 5.0 ppm).

4. Temperature. The range of temperature in Hawaiian waters is from approximately 20° to 30°C and lethal low or high temperatures normally are not encountered by nehu in captivity. During summer months, however, when at the upper end of this range (26.0° to 30.0°C), higher temperatures appear to interact with other factors to significantly increase stress and mortality.

Experiments on varying temperature were conducted in only laboratory experiment 19. Temperature control equipment ceased functioning after 7 days. Although temperature control was absent after this time, there is an indication that lower temperature prolonged the time to onset of delayed mortality. In Figure 14, the mortality curve (B) for fish treated with 100 percent seawater (34.0 to 36.0 ppt) and maintained at ambient temperature (26.0° to 28.0°C) shows that 50 percent mortality (3 to 4 days) occurred about 1 day earlier than with other treatments (50 percent mortality by Days 4 to 5). Fish subjected to 100 percent seawater but a lower temperature of 21.0° to 23.0°C reached the 50 percent mortality level by Day 5 (Figure 5, curve A). Those in 50 percent seawater (16.0 to 18.0 ppt) at 21.0° to 23.0°C reached the 50 percent mortality level by Days 4 to 5 (Figure 14, curve C), but initial mortality was significantly lower in 50 percent seawater than in either 100 percent seawater treatments.

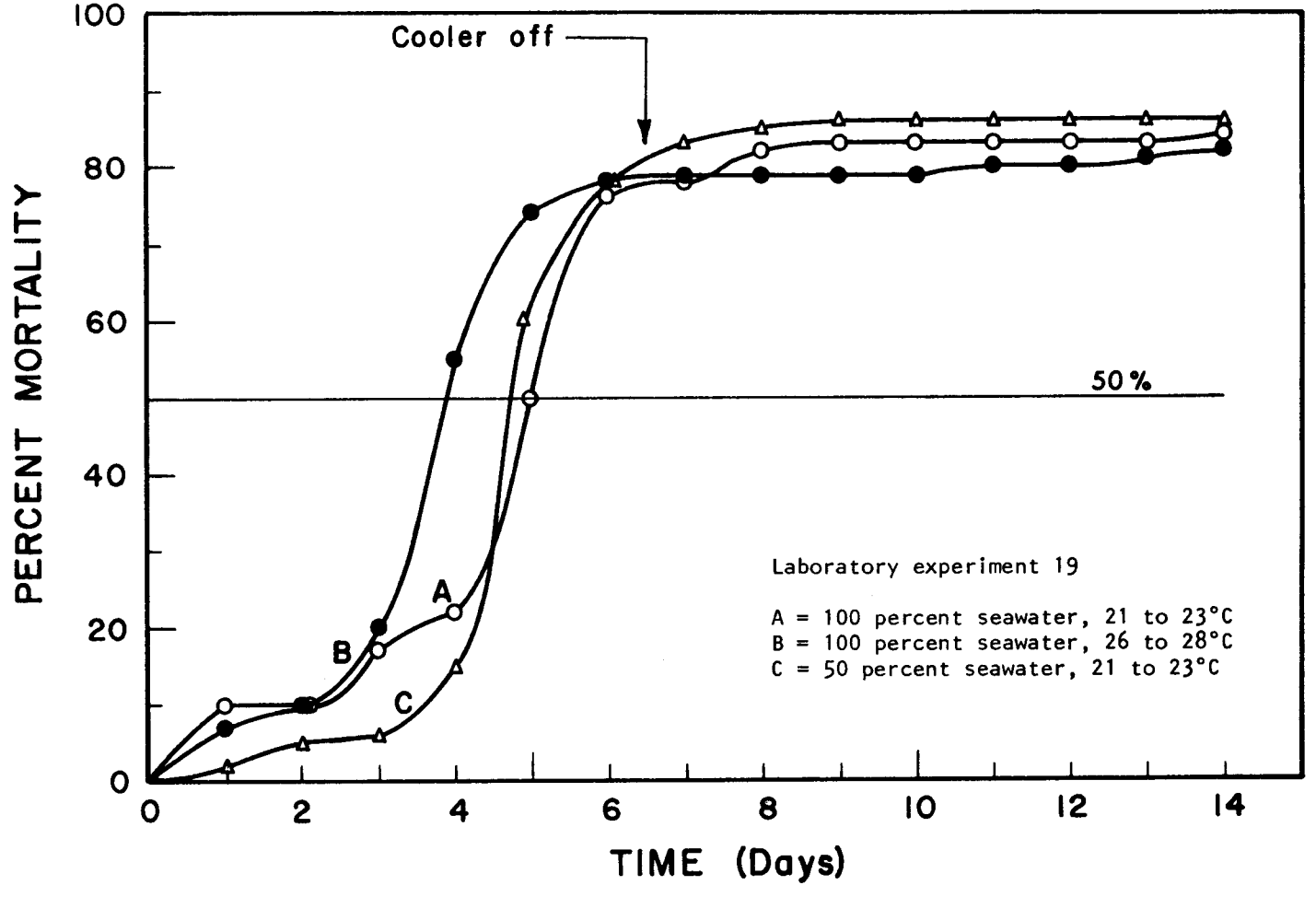


Figure 14. Effect of temperature and salinity on mortality of *S. purpureus*.

An analysis of mortality occurring at various temperatures, when measurements of temperature during all experiments are compiled, shows that temperature affects mortality. Analysis reveals that temperature does not significantly affect initial mortality or percentage of fish remaining, but does significantly affect time to delayed mortality and to the 50 percent mortality level (Table 12). A comparison of days to 50 percent mortality for fish experiencing a maximum temperature of 24.0° to 26.9°C was made with time to 50 percent mortality for fish experiencing a maximum temperature of 27.0° to 29.9°C by means of the Mann-Whitney U-test (Sokal and Rohlf, 1969). The Mann-Whitney statistic (U) was 231, significant at the $P < 0.05$ level. Fish encountering higher maximum temperature reached the 50 percent mortality level significantly earlier (approximately 2 days) than those at lower maximum temperature. A similar analysis was done for the minimum temperature encountered (21.0° to 23.9°C vs 24.0° to 26.9°C). The Mann-Whitney statistic (U) was 236, significant at the $P = 0.025$ level. Fish encountering a lower minimum temperature reached the 50 percent mortality level significantly later (approximately 2 days) than those at higher minimum temperature.

Temperature appears to affect the shape of the mortality curve by increasing stress at higher temperatures and decreasing stress at lower temperatures, probably by influencing respiration and metabolic rate. The optimal range of temperature appears to be approximately from 21.0° to 24.0°C. The influence of temperature on survival may relate to the observation by fishermen that baitfish mortality occurs faster in summer than in winter.

5. Current. In Kaneohe Bay, nehu exhibited negative rheotaxis to currents originating from boat channels or streams emptying into the bay.

They maintained their position or swam against the current. They were also observed to mill in compact circles in shallow water near shore and to orient into currents and mill circularly in tanks and wells.

Swimming speed was determined to ascertain a current speed suitable for orientation while minimizing energy expended in swimming into supplemental current. The mean swimming speed which was determined from 25 trials was 0.17 m/sec (range = 0.15 to 0.20 m/sec, approximately 30 to 40 ft/min). The speed calculated probably represents a maximum since fish measured were large, acclimated, and swimming in a large pond. Newly captured fish confined in small tanks or wells probably swim more slowly. Current speed in excess of 0.13 m/sec (25 ft/min) was deleterious and increased mortality by stressing fish and buffeting them against tank walls. Current speeds were thus set lower than the maximum swimming speed determined above, usually at 0.10 m/sec (20 ft/min), or approximately 40 to 50 percent of the maximum range of swimming speed.

In laboratory tanks, the range of maximum current speed immediately in front of the current standpipe was from 0.05 to 0.20 m/sec (approximately 10 to 40 ft/min) with a mean of 0.10 m/sec (approximately 20 ft/min). Current speed varied from front to back and from top to bottom and was slower at the back and bottom of the tank. The range of current speed in barge wells was 0.08 to 0.13 m/sec (15 to 25 ft/min) with a mean of 0.10 m/sec (approximately 20 ft/min).

The effect of current on survival of nehu in captivity was tested in several experiments. In laboratory experiment 12 (Figure 15) mortality of fish in the tank given air only (Figure 15, curve B) occurred significantly earlier than in the tank with air and current (Figure 15, curve A). In the tank with current, 50 percent mortality was reached about 2 days later.

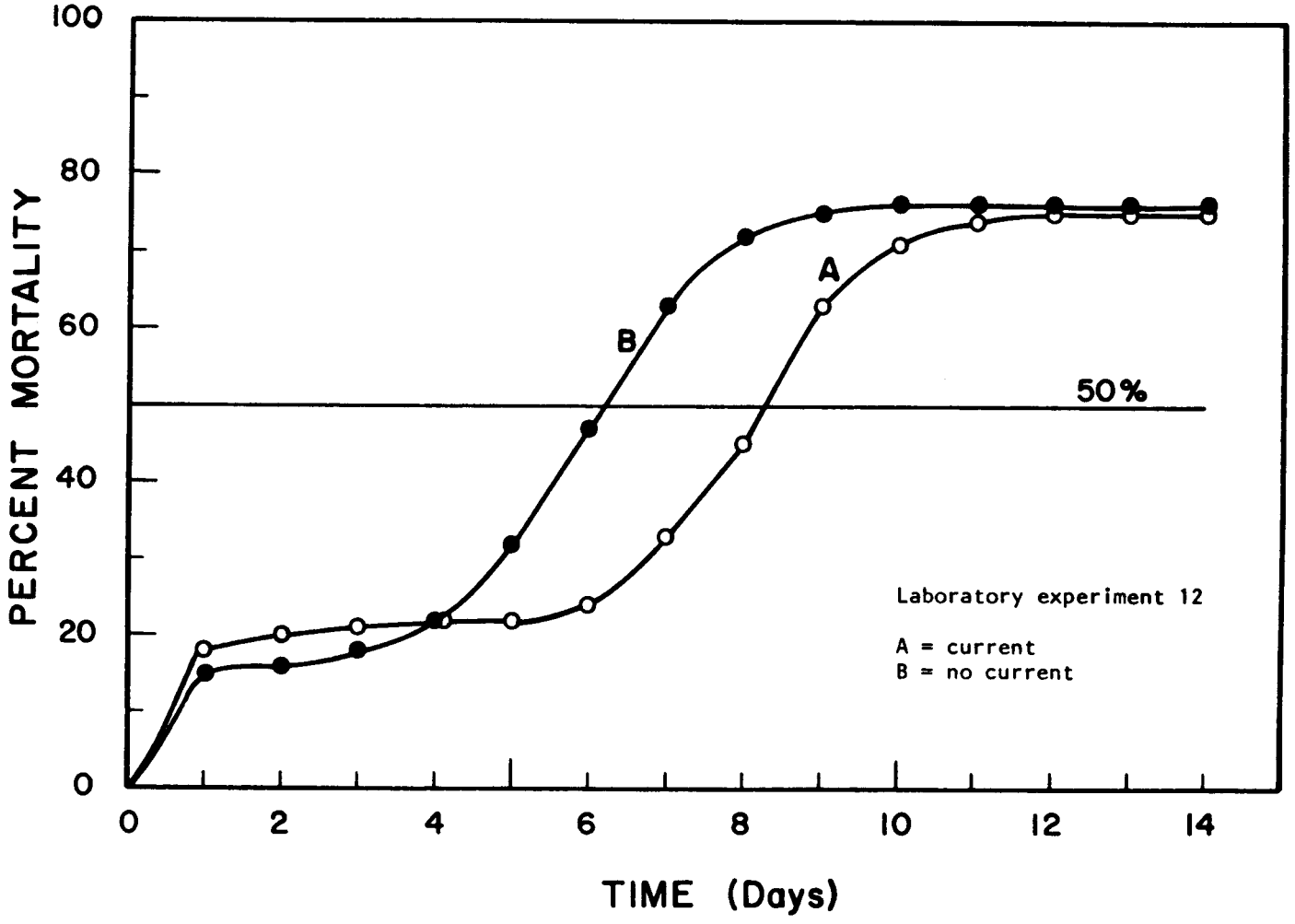


Figure 15. Effect of current on mortality of *S. purpureus*.

In laboratory experiment 13, various oxygen and current treatments were contrasted (Figure 13). Highest mortality occurred among fish given only air; lowest mortality occurred among fish given oxygen and current. Nehu given oxygen but not current survived nearly as well as those given oxygen and current, but delayed mortality began earlier (Figure 13, curve D). Fish given air and current underwent less mortality than those given air only, but mortality was higher than in fish given oxygen or oxygen and current. Provision of current altered survival primarily by postponing the onset of delayed mortality and the 50 percent mortality level. Current did not appear to decrease initial mortality or increase the number surviving by Day 14. Occasionally, initial mortality was higher when current was present, probably because weaker or injured fish were more quickly eliminated.

It was found best to maintain current throughout the experimental period. In laboratory experiment 15, the pump providing current was shut off on Day 6 in one tank (Figure 16, curve B); in another tank, current was left on for the entire 14 days (Figure 16, curve A). Mortality immediately increased in the tank where the current was shut off. Most observations showed that if current were shut off before the asymptotic mortality level were reached, mortality proportionately increased. Shutting off the current seemed to have little or no effect after asymptotic mortality was reached.

Milling behavior of nehu is influenced by current, oxygen concentration, and light. When no directional current is provided, nehu tend to move randomly during the first few days of captivity. The duration of random movement varies with oxygen concentration and severity of other environmental stress factors. If oxygen is maintained at saturation, random movement ceases and circular milling begins by the end of the first day.

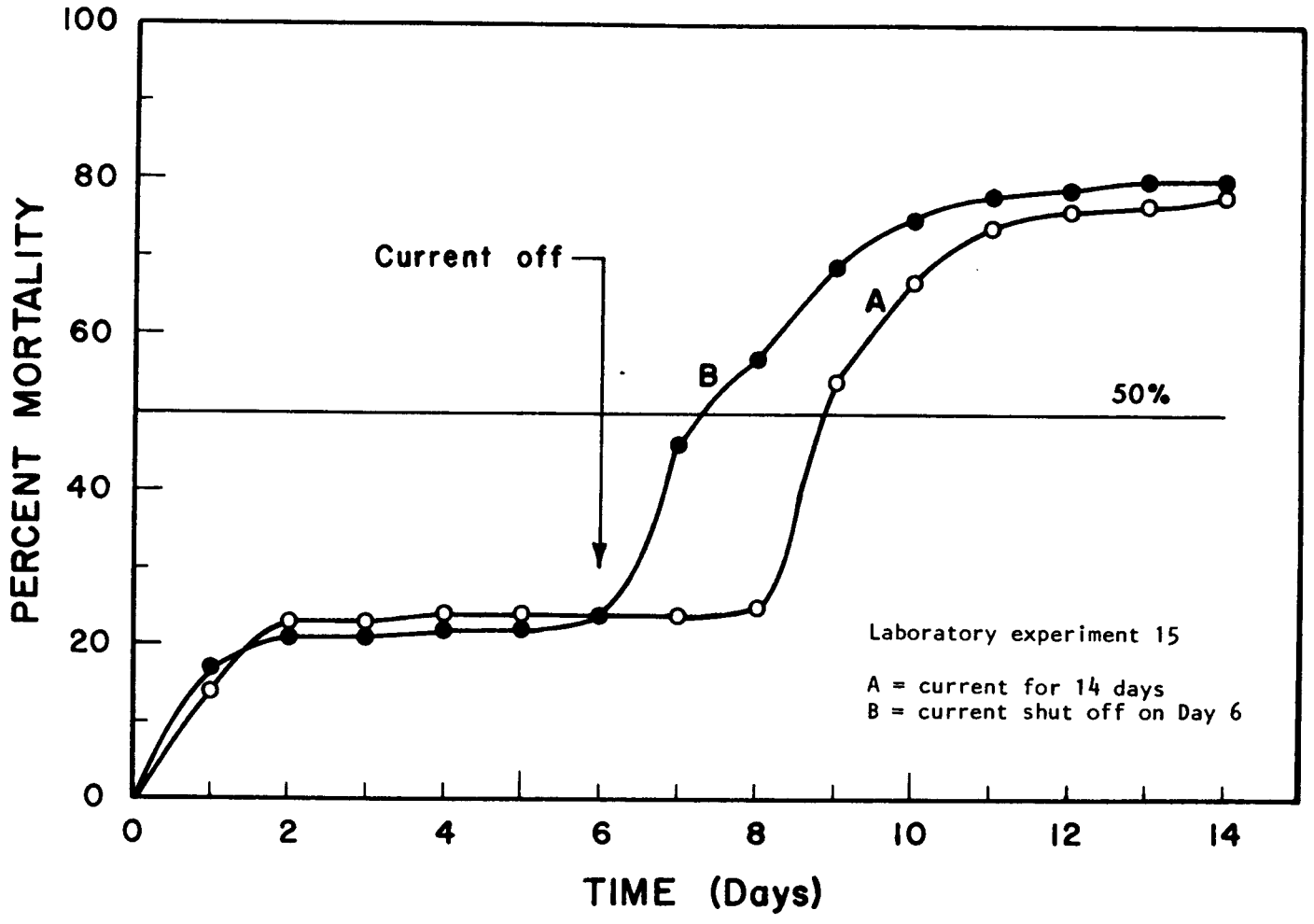


Figure 16. Effect of shutting off current on mortality of *S. purpureus*.

When directional current is added, fish immediately orient themselves to the current. Depending upon current strength and size and condition of fish, fish orient themselves to the current, maintaining a relatively stationary position for one or two days after capture. Thereafter, they begin milling in a complete circle. In most experiments the current was counterclockwise with the fish consequently swimming clockwise.

If the oxygen concentration fell below approximately 5.0 ppm or if other stress factors were severe, fish would mill more slowly at first and spread out to a less compact mill. As stress increases they move more randomly, eventually breaking up the mill. If severe stress were to continue, they would move randomly even if current were present. The mechanism by which current affects survival is not known, however postponement of delayed stress mortality may be due to one or all of the following reasons.

Current may provide a stimulus for immediate orientation when fish are placed in the unfamiliar and restricted capture situation. It probably requires some time for fish to learn visual cues and begin normal milling in small tanks. In the meantime they move randomly, often injuring themselves. This disorientation may also result in excessive energy demand. If current is provided, fish mill immediately, reducing such stress.

Nehu may need to swim at a certain speed in a uniform direction to maintain a sufficient flow of water past the gills. Provision of current and the instigation of milling increase the rate of oxygen availability to the gills, possibly increasing the efficiency of oxygen utilization.

Current appears to reduce the dissolved carbon dioxide concentration, possibly by increasing water turbulence.

6. Light and tank color. Nehu exhibit positive phototactic behavior. For example, during night baiting they are attracted to submerged lights

or will jump from unlighted tanks toward lights some distance away. Therefore, to keep fish milling normally at night a light must be provided over the tank. In early experiments light from windows at one end of the laboratory caused fish to aggregate at the end nearest the window. This difference in light intensity within the tanks interfered with normal milling and induced higher mortality. Therefore, several experiments varying color and intensity of light were conducted.

A major difficulty encountered in experiments measuring light intensity was the lack of available equipment to measure the effect of actual radiant energy on nehu. Attempts were made to equalize light intensity with a Gossen 7.67 to 510 foot-candle meter held approximately 1.25 cm above the water surface. Foot-candle measurements were converted to luminous units of lumen per square meter. It was realized, however, that the use of luminous units is inadequate for measuring the effect of light on organisms other than humans (Tyler, 1971).

Artificial fluorescent lights suspended 17.5 cm above the surface with variation in spectral outputs were also used. Different colors were found to vary characteristics of light penetration through the water column. The specifications of lights used in the experiments were: (1) G.E. F20T12-CW, 20 watts, cool white and (2) Westinghouse F20T12/G, 20 watts, green. The results of the experiments are considered only in terms of the relative effect of light color and intensity on mortality.

Loukashkin and Grant (1965) studied the behavior and reactions of the northern anchovy, *Engraulis mordax*, to different wave lengths of light and found that they prefer a particular wave length of green. It was observed that *S. purpureus* also appeared to prefer the same type of

fluorescent green light. However, because measurements of intensity were inadequate, results were inconclusive.

A few experiments attempting to test the effect of green vs white light on survival were conducted. Results are somewhat confusing but generally indicate that green light may enhance survival if of sufficient intensity, particularly if no current is supplied to provide orientation. Results of laboratory experiment 16 are shown in Figure 17. Green lights were suspended over two tanks and white lights over two other tanks, all equalized to approximately 1,080 lumens/sq m (100 footcandles) at the surface. In tanks with white lights, fish did not mill circularly until Days 7 to 8. In all observations of milling which were made three times daily for 12 days, fish showed a mean of 65 percent random movement (no directional milling). Delayed mortality in these tanks began on Day 3; 50 percent mortality was reached by Days 3 to 4. In tanks with green lights fish began milling circularly immediately on Day 1. Observations for 12 days elicited a mean of 38 percent random movement, much less than in tanks with white lights. Delayed mortality in tanks with green lights did not begin until Day 4; 50 percent mortality was not reached until Days 5 to 6. In summary, there was about two days difference between the light treatments in the onset of delayed mortality. Green lights appear to promote normal orientation and milling, decrease stress, and increase survival.

Laboratory experiment 17 duplicated laboratory experiment 16, using the same lights and intensities, but adding current to the tanks. Fish under both light treatments began circular milling immediately on Day 1, probably because of the current. Figure 18 shows that there was no significant difference in mortality between tanks with green and white

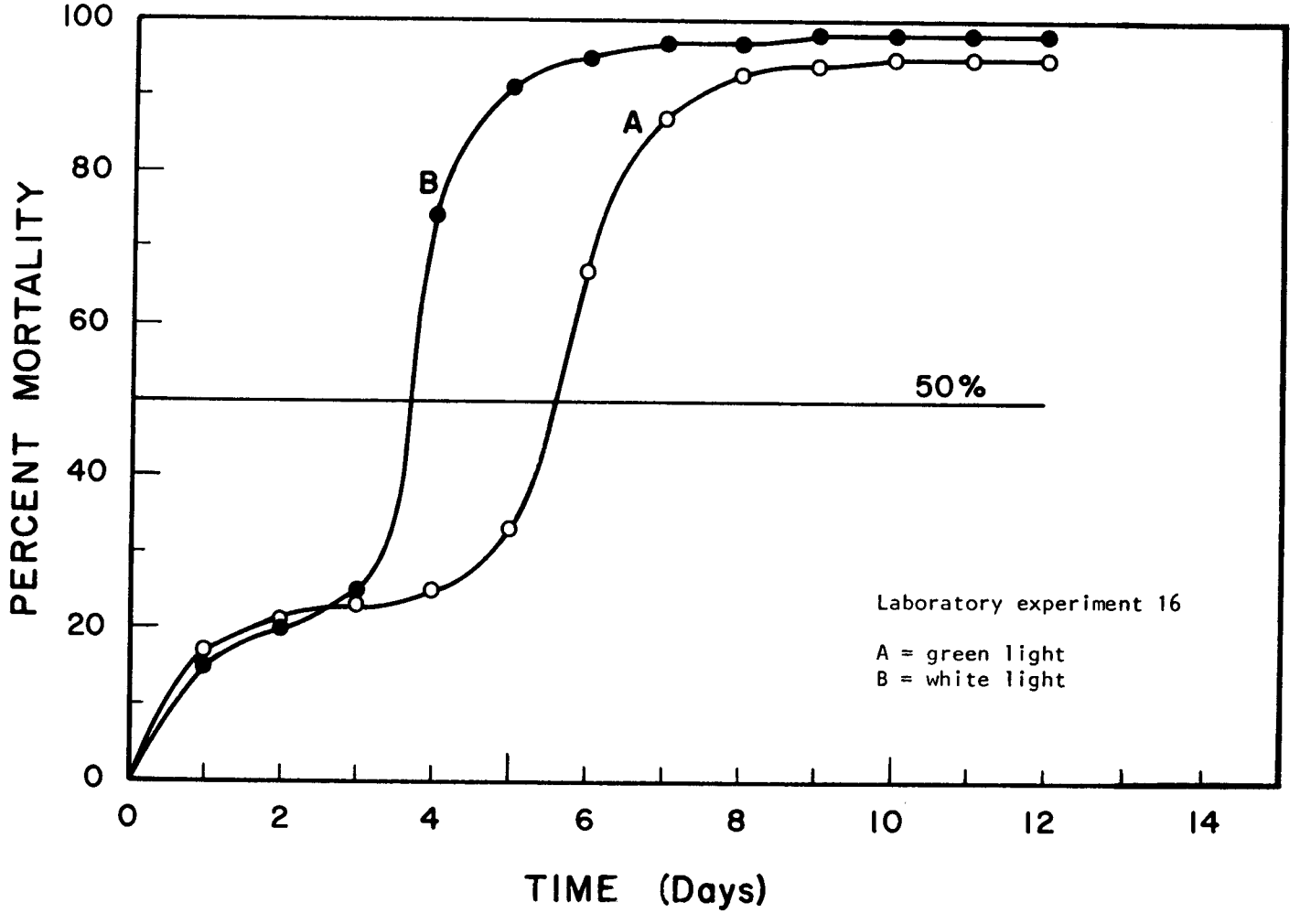


Figure 17. Effect of light color on mortality of *S. purpureus* in tank without current.

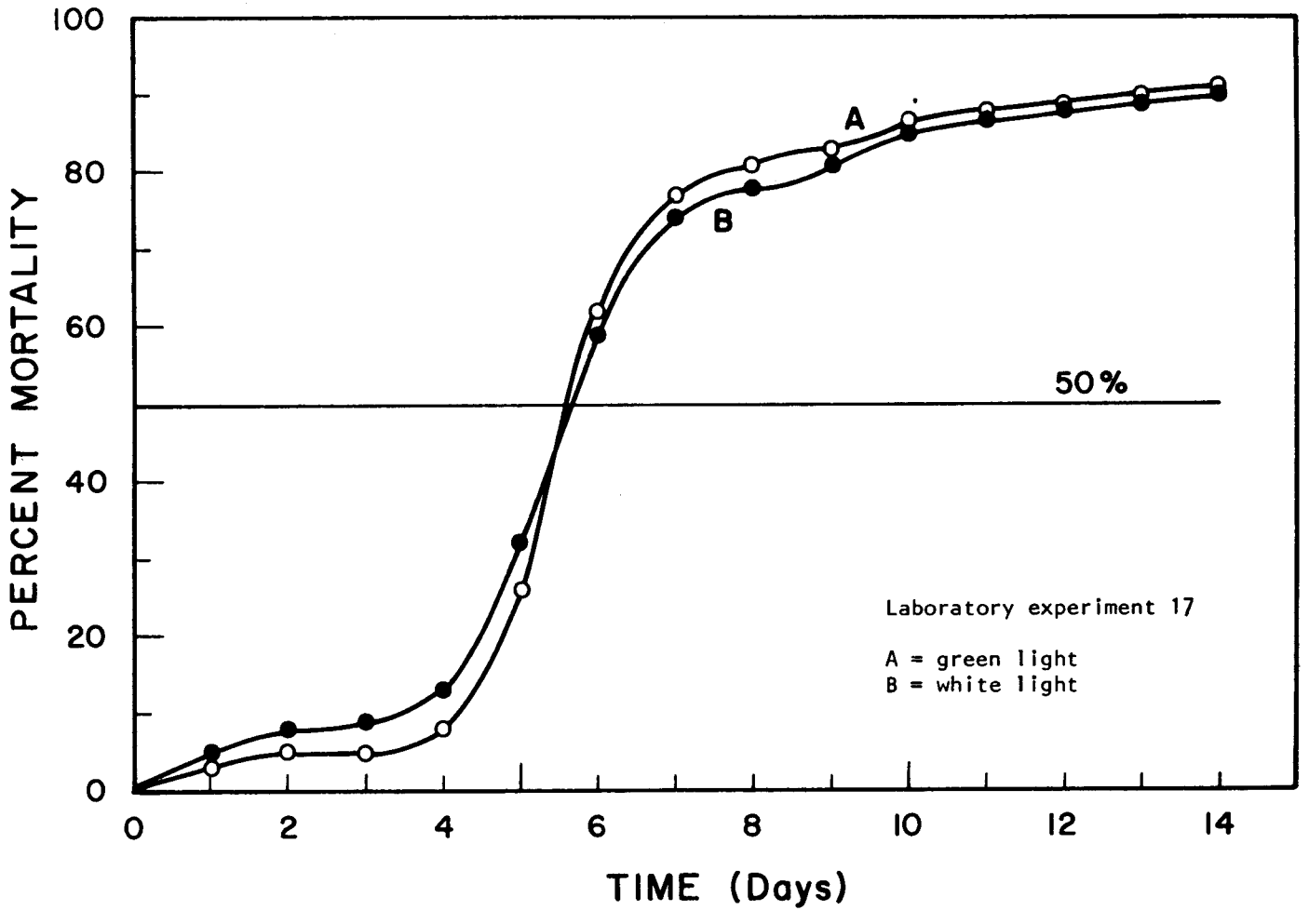


Figure 18. Effect of light color on mortality of *S. purpureus* in tank with current.

lights. A comparison of the two experiments indicates that light color significantly affects mortality only when there is no current for orientation. When current is present, it supercedes the effect of the light environment on orientation.

Following preliminary tests, new lights (Westinghouse F30T12/CW, cool white, 30 watts) were installed over each tank in an attempt to increase light intensity and uniformity. Variation occurred between tanks because of the irregular placement of ceiling lights. This variable is included in the general factor analysis of the concluding section. The correlation matrix of this analysis (Table 23) shows that increased light intensity significantly increases the time to onset of delayed mortality, time to 50 percent mortality, and percentage of survival. Increased variation of light intensity over the tank decreases percentage of survival, while higher and more uniform light intensity decreases mortality. The light intensity ranged from approximately 750 to 1,260 lumens/sq m (70 to 117 footcandles), averaging approximately 1,080 lumens/sq m (100 footcandles) at the surface. Measurements of light intensity taken again after 2 years 8 months show a substantial decrease in intensity of approximately 35 percent, indicating that lights should have been changed more frequently to maintain maximum possible intensity.

The effect of background color of the tanks was also tested. Visual environment was found to significantly affect orientation behavior, milling, and survival of nehu.

In all laboratory tanks the background color was green. On the barge, however, tanks in initial experiments were white. Since observations indicate that green was preferred by nehu, barge experiment 14 on contrasting tank colors was conducted (Figure 19). Experimental conditions were

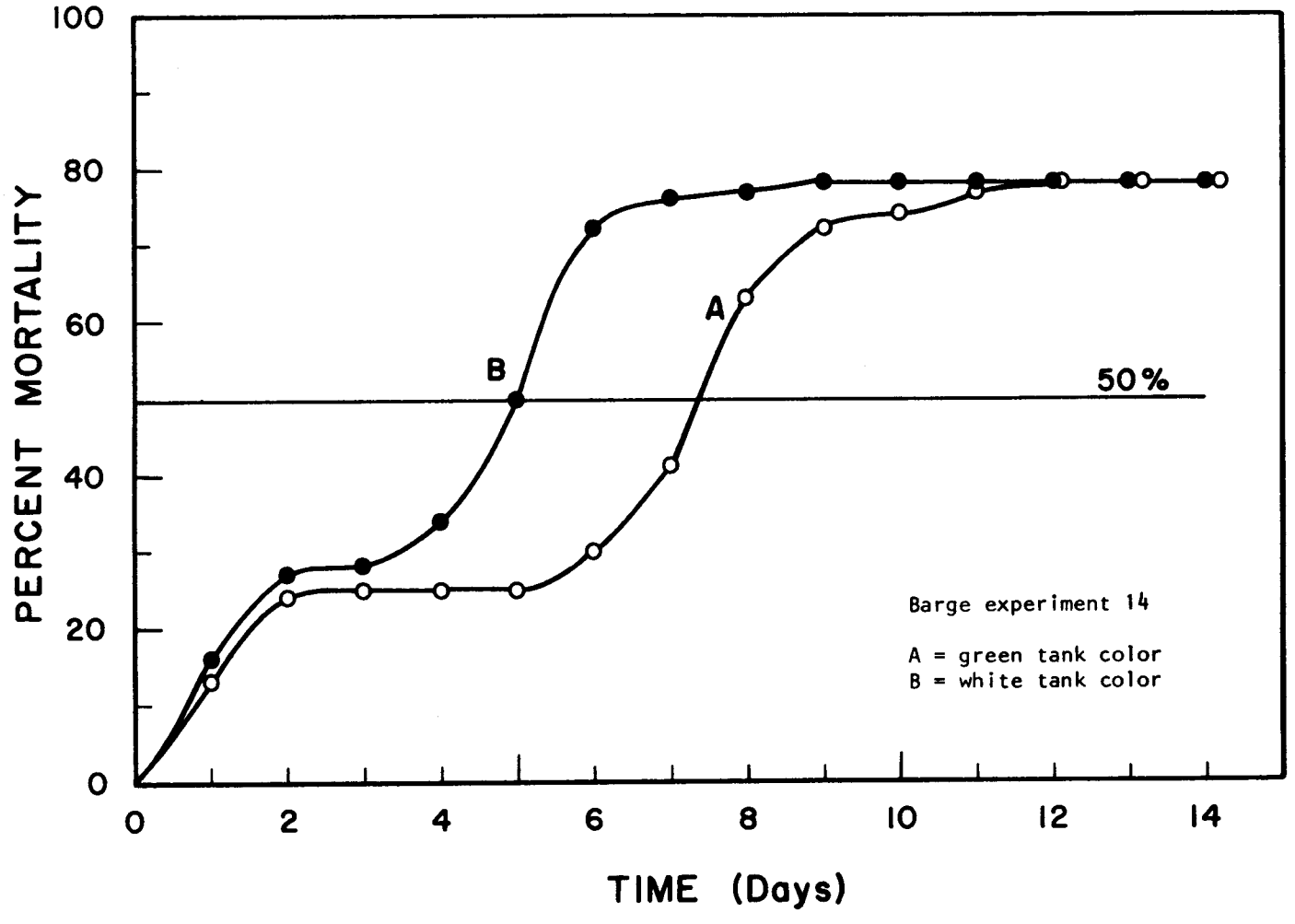


Figure 19. Effect of tank color on mortality of *S. purpureus*.

the same in both tanks, both being provided with oxygen and current. One tank was coated with white gel-coat resin, the other with green gel-coat resin (approximate color was Turquoise Blue, #25, #75 in *Webster's New International Dictionary of the English Language*, 2nd ed., 1961). Nehu in the white tank (Figure 19, curve B) underwent delayed mortality by Day 4 with 50 percent mortality being reached by Day 5. Fish in the green tank (Figure 19, curve A) did not undergo delayed mortality until about Day 6 and 50 percent mortality was reached by Day 7 to Day 8. Thus a significant difference of approximately 2-1/2 days in the onset of delayed mortality occurred between white and green tanks. A noticeable difference in milling behavior was also observed: fish in the green tank were more spread out and milled in a wider radius than those in the white tank.

Other experiments showed that nehu experience difficulty when orienting in black tanks; they move randomly and frequently hit the sides. The background color is therefore apparently important to normal orientation of fish. The closer the visual environment is to the range of maximum visual acuity, the sooner the fish probably orient to the capture environment.

Light experiments are incomplete. A means of measuring light in terms of radiant energy would be particularly desirable. A range of wave lengths of green light at various intensities should be tested to determine the optimal wave length and intensity of light for nehu. Also, in experiments, light was not varied on a diurnal basis. Lowering intensity gradually for the night and increasing again in the morning might enhance survival by affecting energy utilization and stress of fish.

Information so far indicates that the best visual environment for maximum survival is as follows: white or green light should be uniformly distributed over the tank at as high an intensity as is feasible--at least

1,080 lumens/sq m (100 footcandles) at the surface, optimally higher. At least some light should be provided at night, particularly if there is no current to provide orientation and especially during the first few days of capture when fish are adapting to the new environment. Light should not be turned on and off since it induces a startle reaction. The background color of the tank should be a light green-blue; dark colors should be avoided. Obvious visual cues on the sides of the tank may be used to assist in orientation.

7. Circulation--open system. In experiments with a continuous open system, an attempt was made to obtain the maximum turnover rate possible. In the laboratory, flow rate was limited by the line pressure of a gravity flow seawater system. On the barge, inflow was from Jabsco (barge experiments 1 to 10) or Jacuzzi (barge experiments 11 to 30) pumps. The objective was to maintain the highest flow and turnover rates possible without exceeding the optimal current speed (0.10 m/sec; 20 ft/min), which was controlled by valves in the current standpipe system.

The flow rate necessary to provide sufficient oxygen without addition of supplemental oxygen (assuming inflow water is at saturation) was calculated for an average bucket of fish (approximately 2,700 fish, weighing approximately 1,700 gm). The fish are assumed to be unacclimated, with an estimated mean rate of oxygen consumption of 1,270 cc per bucket of fish per hr $[2{,}700 \frac{\text{fish}}{\text{bucket}} \times 0.634 \frac{\text{gm}}{\text{fish}} \times 0.742 \frac{\text{cc/hr}}{\text{gm}}]$. Calculated flow rates in liters per hour are summarized in Table 36. Since the amount of oxygen at saturation varies with different temperatures and salinities, flow rates necessary to provide sufficient oxygen were calculated for the range of temperatures and salinities encountered under most experimental conditions (temperature, 20° to 30°C; salinity, 10 to 36 ppt).

The estimated flow rates (range = 205 to 285 liters/hr) are assumed optimal for the first day after capture when oxygen consumption is greatest. If the number of fish is more or less than one bucket, flow rate can be correspondingly increased or decreased. Further, after fish are acclimated (approximately 10 to 14 days) flow rate can be decreased. If supplemental oxygen is provided, flow rate can be decreased, but the possible accumulation of toxic metabolites must be considered if water exchange is too slow.

For most laboratory experiments the water inflow valve was not fully open and higher flow rates were possible if there were sufficient line pressure in the seawater system. The flow rate in most laboratory experiments ranged from 0.07 to 0.11 liter/sec (1 to 2 gal/min), with a mean flow of 0.08 liter/sec (1.3 gal/min). The percentage of the turnover rate for the 180-gal tanks ranged from 37 to 58 percent per hour, the mean turnover rate being 42 percent per hour. The presence of Cuno filters (Cuno Engineering Corporation, Filter No. P110) in the inflow line (Plate 15) decreased flow rate very slightly when filters were clean. When filters were clogged, the flow rate greatly decreased, so they were regularly replaced. For most experiments, filters were not used.

Table 36 shows that the flow rate necessary to supply 1,270 cc oxygen/hr to an average bucket of fish ranged from 205 to 285 liters/hr (54 to 75 gal/hr). The average flow rate in the laboratory (288 liters/hr or 76 gal/hr) therefore was adequate to supply sufficient oxygen for low to average densities of fish. If stress were excessive and densities higher, however, the flow rate would have been insufficient to provide necessary oxygen. Also, for many experiments line pressure was low, resulting in reduced flow rates. Under most experimental conditions supplemental oxygen was added to ensure oxygen saturation.

In initial barge experiments (1 to 10), circulation inflow was provided by Jabsco pumps, one pump per 1,000-gal well. The flow rate ranged from 0.28 to 0.36 liter/sec (4 to 6 gal/min), with a mean flow rate of 0.30 liter/sec (4.8 gal/min). The percentage of turnover of the 1,000-gal well ranged from 27 to 34 percent per hour, with a mean of 28 percent per hour. It required a flow rate of 205 to 285 liters/hr (over the experimental temperature-salinity range) to provide 1,270 cc oxygen/hr for one bucket of fish. The number of buckets in barge wells ranged from five to ten. The flow rate required for sufficient oxygen was from 1,025 to 1,425 liters/hr for 5 buckets and from 2,050 to 2,850 liters/hr for 10 buckets. The Jabsco pump with a flow rate of 1,010 to 1,300 liters/hr was sufficient to provide oxygen for approximately 5 buckets of average-sized fish, but insufficient for 10. Thus, densities greater than 5 buckets per 1,000 gal required supplemental oxygen.

A 1 hp Jacuzzi pump (Plate 2) was used in later barge experiments (11 to 30). The flow rate to each well ranged from approximately 0.57 to 0.64 liter/sec (9 to 10 gal/min) with a mean of 0.60 liter/sec (9.5 gal/min). The turnover rate of each well was from 54 to 60 percent per hour, with a mean of 57 percent per hour or slightly more than one complete turnover in 2 hours, and is sufficient to provide oxygen for respiration for about 10 buckets of average-sized fish.

Optimal flow rate will vary with temperature, salinity, availability of supplemental air or oxygen, size of fish, and severity of stress and must be determined anew for each experiment. Eventually, however, the maximum flow rate possible should be selected while simultaneously maintaining an optimal current speed.

8. Circulation--closed system with reduced salinity. Previously described experiments show that reduced salinity (40 to 50 percent seawater; 16.0 to 18.0 ppt) is the most significant factor in decreasing stress and subsequent mortality of nehu in captivity. Constant open circulation of 50 percent seawater renders highest survival, but fishing vessels do not carry sufficient fresh water for this to be feasible at sea. If, however, 50 percent seawater is recirculated for at least 3 hours after capture (period of severest osmoregulatory stress), survival is greatly increased. This treatment might be possible on fishing vessels when in port.

Experiments were done to find the maximum time fish could be exposed to recirculated 50 percent seawater before accumulation of metabolites and other stress factors induced excessive mortality. Factors determining the length of this period are density, accumulation of scales, mucus and other organic material, carbon dioxide with decrease in pH, and ammonia. In all experiments with closed systems, oxygen was kept at saturation and current was provided.

In preliminary experiments 50 percent seawater was recirculated in a closed system until fish began dying. After a few hours water became murky and brown, attaining a strong "fishy" odor. Fish initially showed stress by decreasing milling speed and spreading out from the normal mill. They eventually lost orientation to current and began darting or jumping randomly. Finally, they sank to the bottom and died. Figure 20 illustrates results of laboratory experiment 30 in which 50 percent seawater was recirculated for about 9 hours before the system was opened to a continuous flow of 100 percent seawater. There was no supplemental treatment of buffering or filtering. A bucket of nehu (approximately 2,781 fish; 1,427 gm) was placed in the 180-gal laboratory tank (1,550

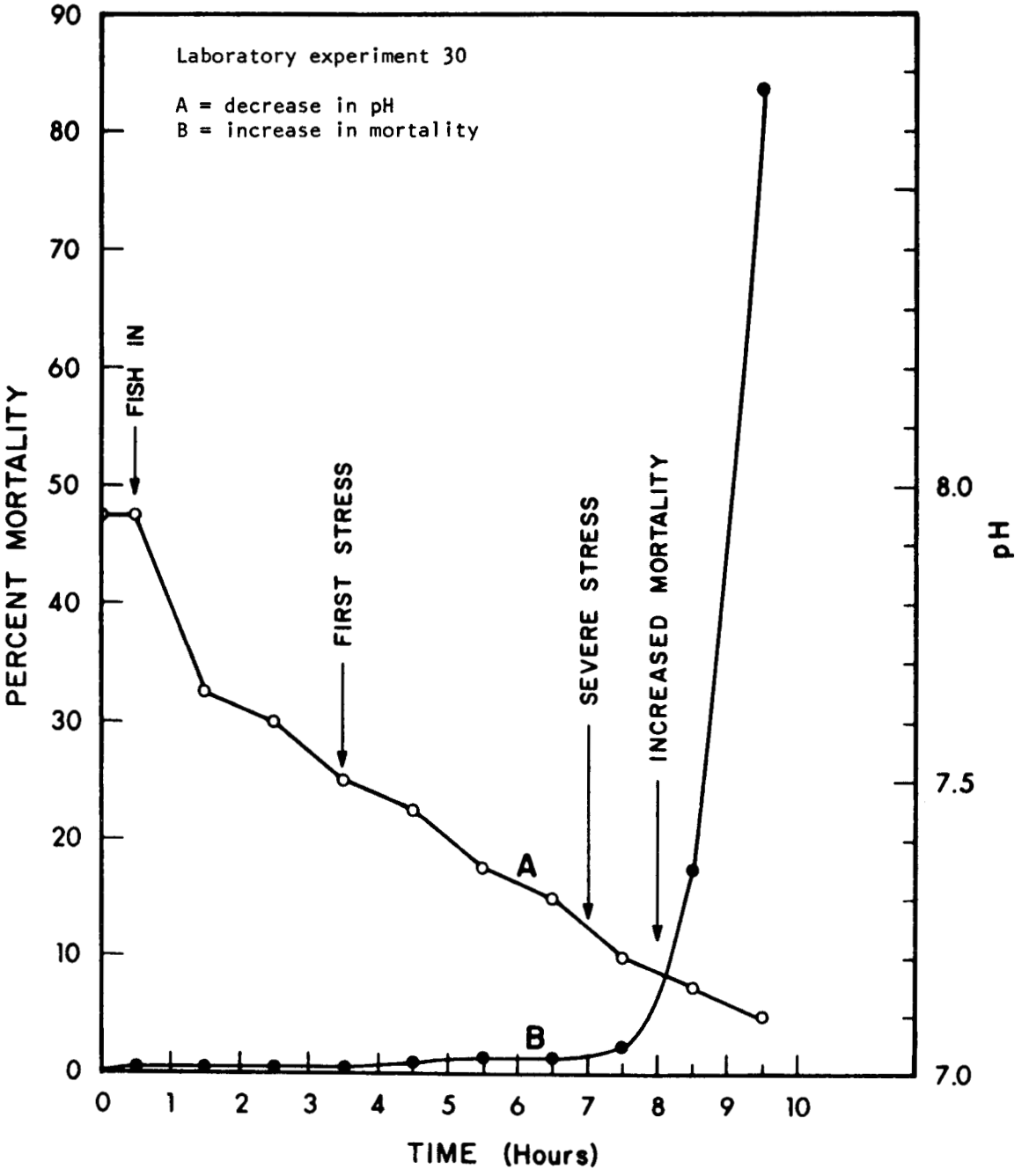


Figure 20. Effect of reduced salinity on mortality of *S. purpureus* and reduction of pH in closed system recirculated for 9-1/2 hours.

fish per 100 gal; 793 gm per 100 gal). Figure 20 shows hourly decrease in pH Figure 20, curve A) and increase in mortality (Figure 20, curve B). The first symptom of stress, exemplified by alteration of normal milling pattern, was observed at pH 7.50. Obvious stress, indicated by erratic and random swimming, was observed at pH 7.25, about 6-1/2 hours after the experiment began. Mortality did not increase until after 7 to 8 hours at pH 7.20 to 7.15; thereafter, mortality occurred rapidly. After 9 hours, the system was opened to 100 percent seawater. By that time pH had decreased to a minimum of 7.10. It is obvious from the daily mortality curve for this experiment (Figure 21) that the system was closed too long. Death was probably primarily due to accumulation of carbon dioxide and reduction of pH. Other factors probably contributing to mortality were accumulation of ammonia and suspended organic matter.

Barge experiment 18 was conducted under similar conditions as laboratory experiment 30 above, except that the system was closed for a shorter period. The density of fish was equivalent to that in the above experiment (725 gm per 100 gal). In this experiment, 50 percent seawater was recirculated for only 4 hours, or until pH decreased to 7.55; then the system was opened to 100 percent seawater. The daily mortality curves of Figure 22 show that mortality was significantly less in the tank with 4 hours of recirculated 50 percent seawater (Figure 22, curve A), than in the control tank with 100 percent seawater in an open system (Figure 22, curve B). In the recirculated tank, only 11 percent of the fish were dead by Day 6 and 46 percent by Day 11. The 50 percent mortality level was not reached by Day 14.

Additional experiments demonstrate that even brief exposure to recirculated 50 percent seawater (3 to 4 hours) substantially increases survival

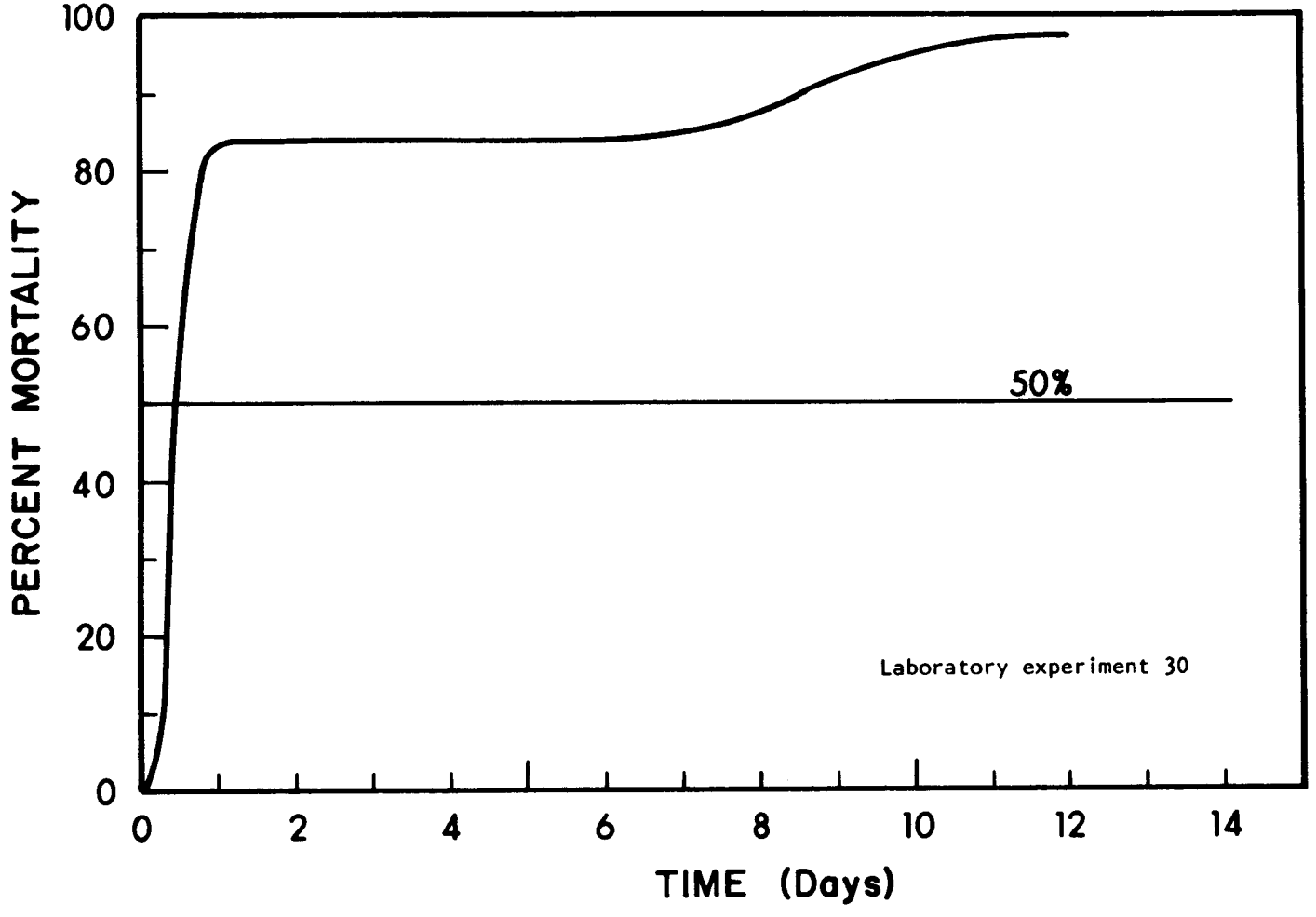


Figure 21. Effect of reduced salinity on mortality of *S. purpureus* in closed system recirculated for 9-1/2 hours on Day 1.

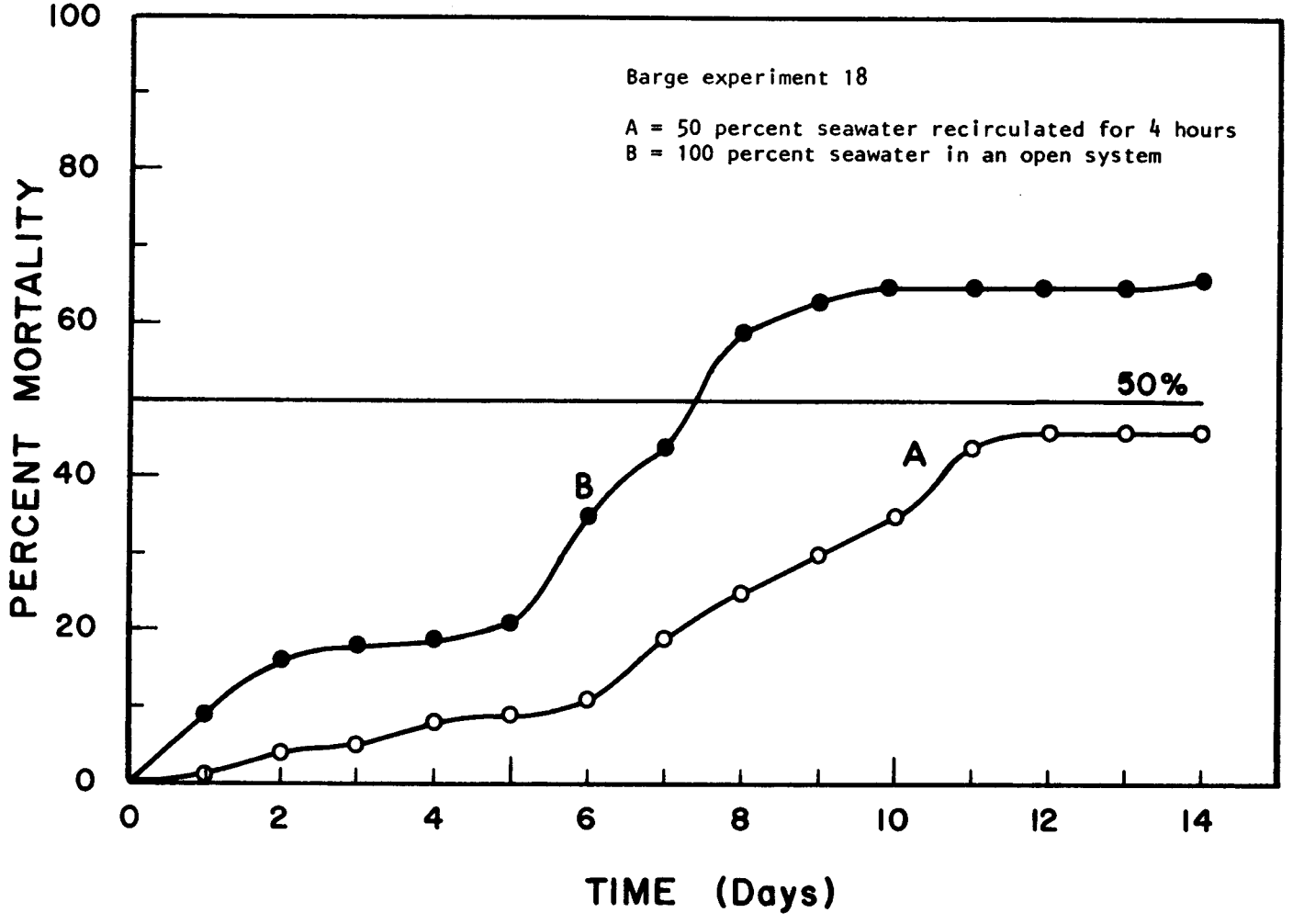


Figure 22. Effect of reduced salinity on mortality of *S. purpureus* in closed system recirculated for 4 hours.

by reducing osmoregulatory stress during the critical first few hours after capture, as long as pH does not fall below 7.5. To maintain 3 or more hours of exposure to recirculating 50 percent seawater, the density of fish must be controlled since the decrease in pH is related to density: if too many fish are crowded into tanks or bait wells, carbon dioxide accumulates and pH decreases too rapidly.

Table 13 summarizes data for experiments with recirculated 50 percent seawater (no supplemental treatment of buffering or filtering). An example of pH decrease with time is shown in Figure 20 (curve A). Results show that pH usually decreases rapidly during the first 2 hours after capture, then decreases linearly. Data show a significant fit to curvilinear regression ($\hat{Y} = 1/a+bX$) with a range in $\underline{b}$ = 3.250 to 11.003 ($X\ 10^{-5}$). The coefficient for linear regression ($\underline{b}$) in Table 13 indicates a rate of pH decrease to 7.5 of approximately 0.001917 to 0.006611 pH points per minute (0.11 to 0.40 pH points/hr).

The time for pH to decrease to 7.5 ranged from about 80 to 200 minutes (approximately 1-1/3 to 3-1/3 hours), depending on density in gram wet weight per 100 gal (Figure 23). The linear regression coefficient ($\underline{b}$) is 0.13636 minute per gram wet weight. Figure 23 shows that to recirculate 50 percent seawater in a closed system for 3 hours without excess stress and mortality due to pH decrease and without supplemental buffering treatment, the concentration of nehu should be about 750 gm wet weight of fish per 100 gal of seawater.

In experiments with open systems circulating 100 and 50 percent seawater continuously, pH occasionally decreased to a limiting value of 7.5 when the density of fish was great or flow rate too slow. This occurred on Day 1, when density exceeded 1,000 gm wet weight per 100 gal.

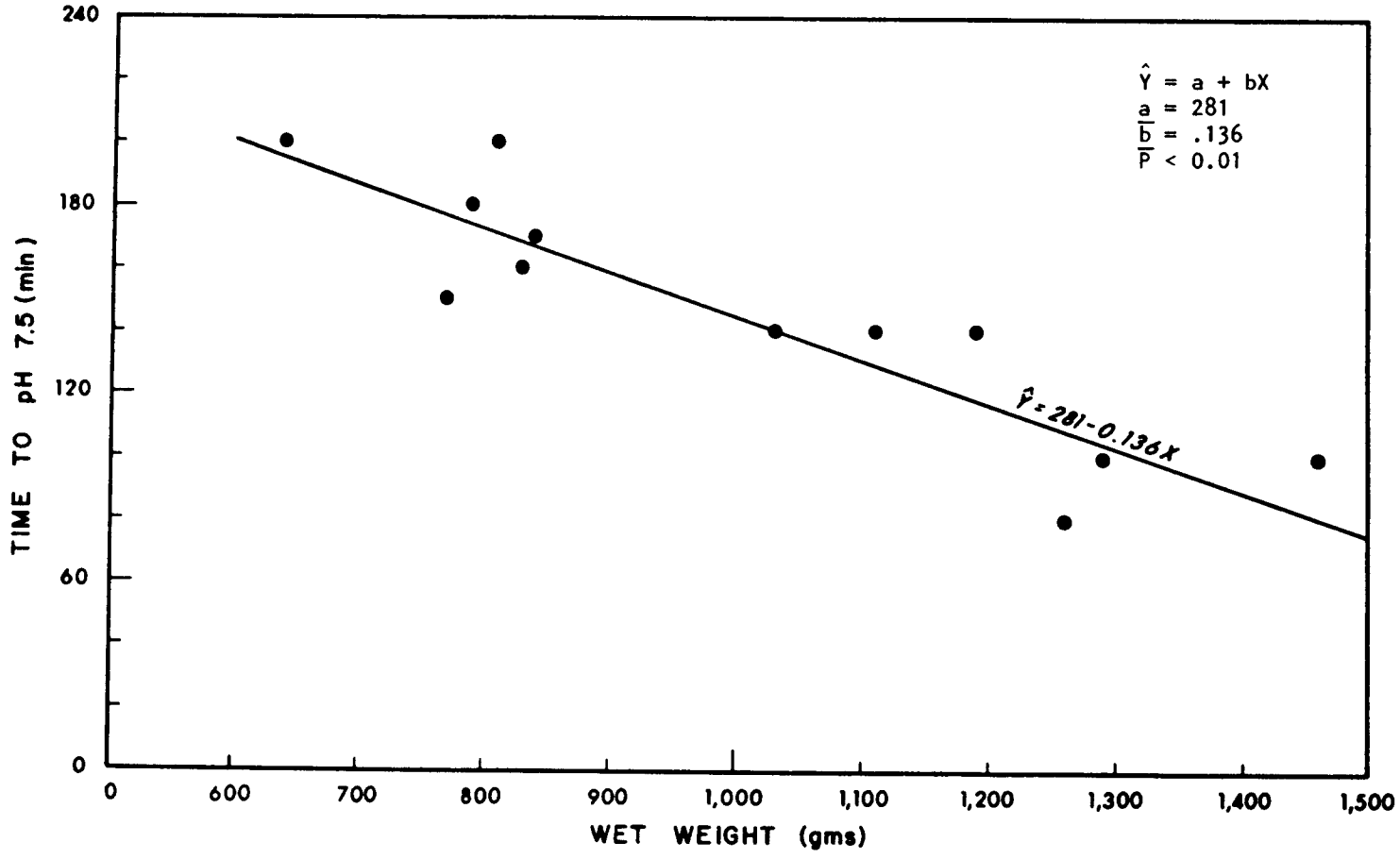


Figure 23. Time for pH to decrease to critical level of 7.5 for different densities of *S. purpureus* in closed system.

To avoid this stress, density in an open system should not exceed 1,000 gm per 100 gal or the flow rate should be increased, at least for the first day after capture.

In closed systems or recirculating 50 percent seawater, mucus, scales, feces, and other organic matter accumulate and probably impede respiration. To test the effect of the removal of such material on survival, a protein skimmer was added (Plates 13 and 14). The protein skimmers used were enlarged and modified from the original German design (Sander Brand, 500, made in Germany). Water was pumped through the skimmer, pulling water from the top to the bottom, while air was simultaneously fed into the bottom. Organic material adhered to rising air bubbles to create a foam at the surface which was trapped in the top section of the skimmer and removed.

The effect of adding a protein skimmer to reduce mortality was significant. Figure 24 shows the results of laboratory experiment 32 in which one tank had a protein skimmer and in the other, none. Fifty percent seawater was recirculated through both tanks for approximately 5 hours when pH was just above 7.5, then the system was opened to 100 percent seawater. Mortality in both tanks was low because of reduced salinity, but in the tank with no protein skimmer (Figure 24, curve B), 50 percent mortality occurred by Days 11 and 12, while in the tank with the protein skimmer (Figure 24, curve A), mortality was significantly less throughout the entire experiment and the 50 percent mortality level was never reached.

In barge experiment 19 (Figure 25), a significant difference in mortality was apparent between fish in the tank with a protein skimmer (Figure 25, curve A) and fish in the tank with no protein skimmer (Figure 25, curve B). Fifty percent seawater was recirculated in both tanks for 4 hours 40 minutes

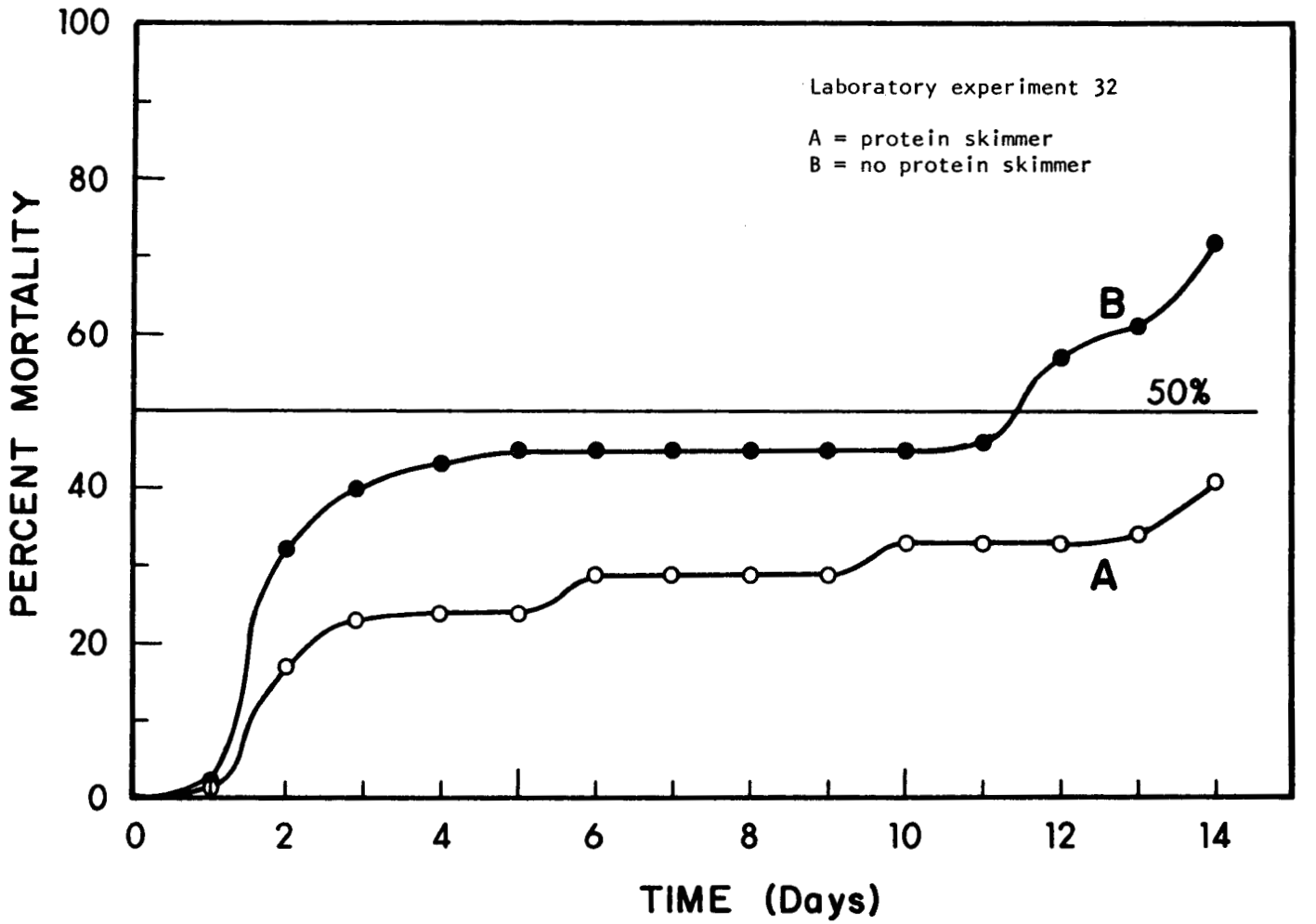


Figure 24. Effect of protein skimmer and reduced salinity on mortality of *S. purpureus* in closed system recirculated for 5 hours.

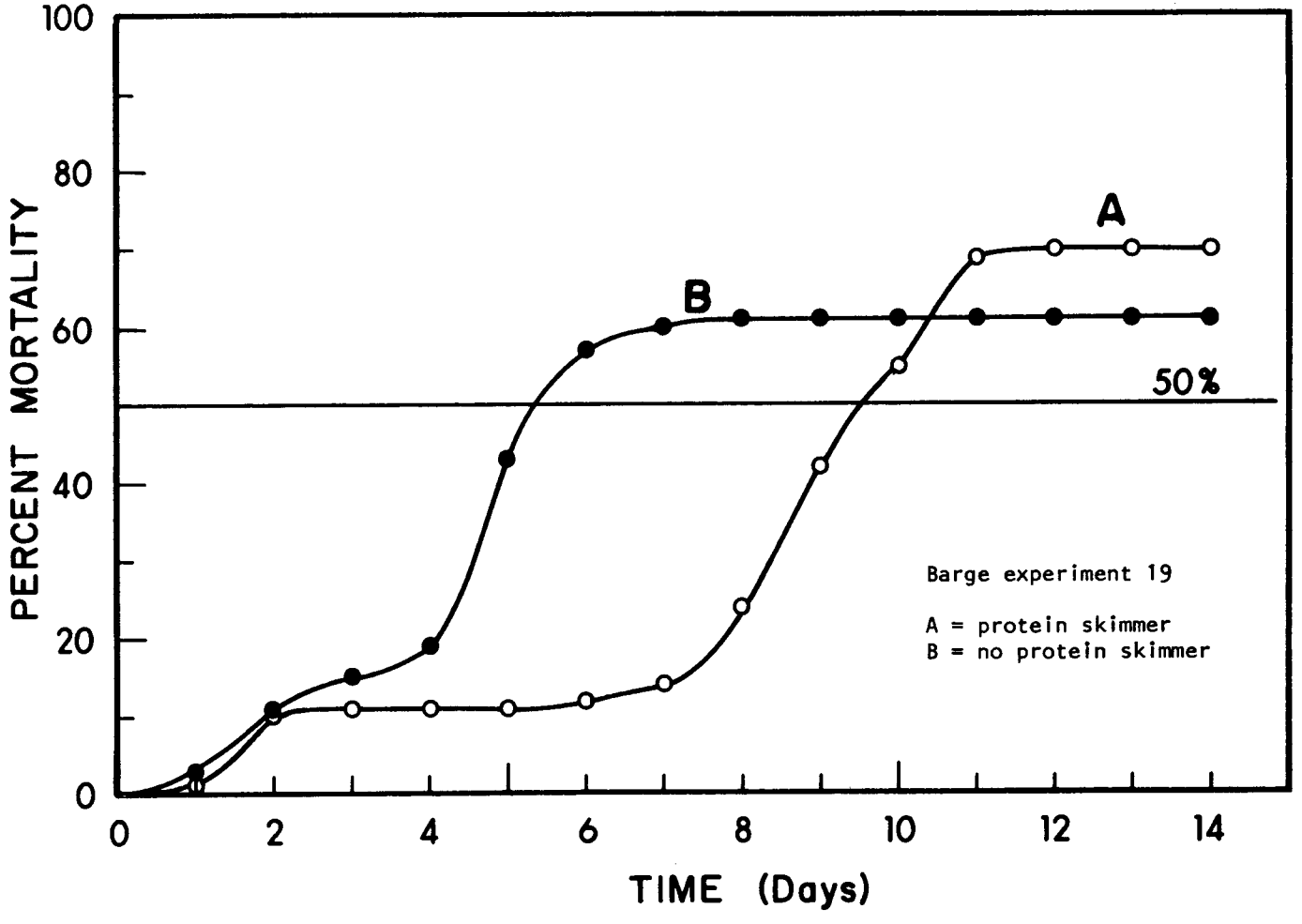


Figure 25. Effect of protein skimmer and reduced salinity on mortality of *S. purpureus* in closed system recirculated for 4 hours 40 minutes.

or until pH was just above 7.5. In the tank without the protein skimmer, 50 percent mortality was reached by Days 5 to 6, while in the tank with the protein skimmer 50 percent mortality was not reached until Days 9 to 10, a difference of approximately 4 days. The pH drops rapidly even when a protein skimmer is present, indicating that it has little effect in decreasing the accumulation of carbon dioxide.

Experiments were then conducted to determine if controlling pH by means of buffers would allow an increase in recirculation time. An amine buffer--Trizma 8.3 (McFarland and Norris, 1961)--was used, Several concentrations were tested: 2 gm/gal of 50 percent seawater was found sufficient to maintain pH of 8.0 to 8.3 for 24 hours at mean densities. In laboratory experiment 34, Trizma buffer was added to one tank (Figure 26, curve A) and not to another (Figure 26, curve B). Both tanks were given supplemental oxygen and current, but no protein skimmer. In the tank with no buffer, pH dropped rapidly to 7.5 in only 1 hour 20 minutes; the system was then opened to 100 percent seawater (Figure 26, curve B). The tank with buffer showed almost no decrease in pH after 9 hours of recirculation when the system was opened to 100 percent seawater (Figure 16, curve A). Mortality in both tanks was relatively high, but mortality curves showed some difference. The tank with buffer reached 50 percent mortality by Days 6 to 7, approximately 1 day after the tank with no buffer (50 percent mortality by Days 5 to 6). Since other toxic substances (e.g., ammonia) accumulated during the recirculation period, it is probable that survival in the buffered tank would have been greater if the recirculation period had been decreased.

In another experiment both buffer and a protein skimmer were tested. Laboratory experiment 36 (Figure 27) showed that although buffer and skimmer were present and pH did not decrease significantly, mortality is still high

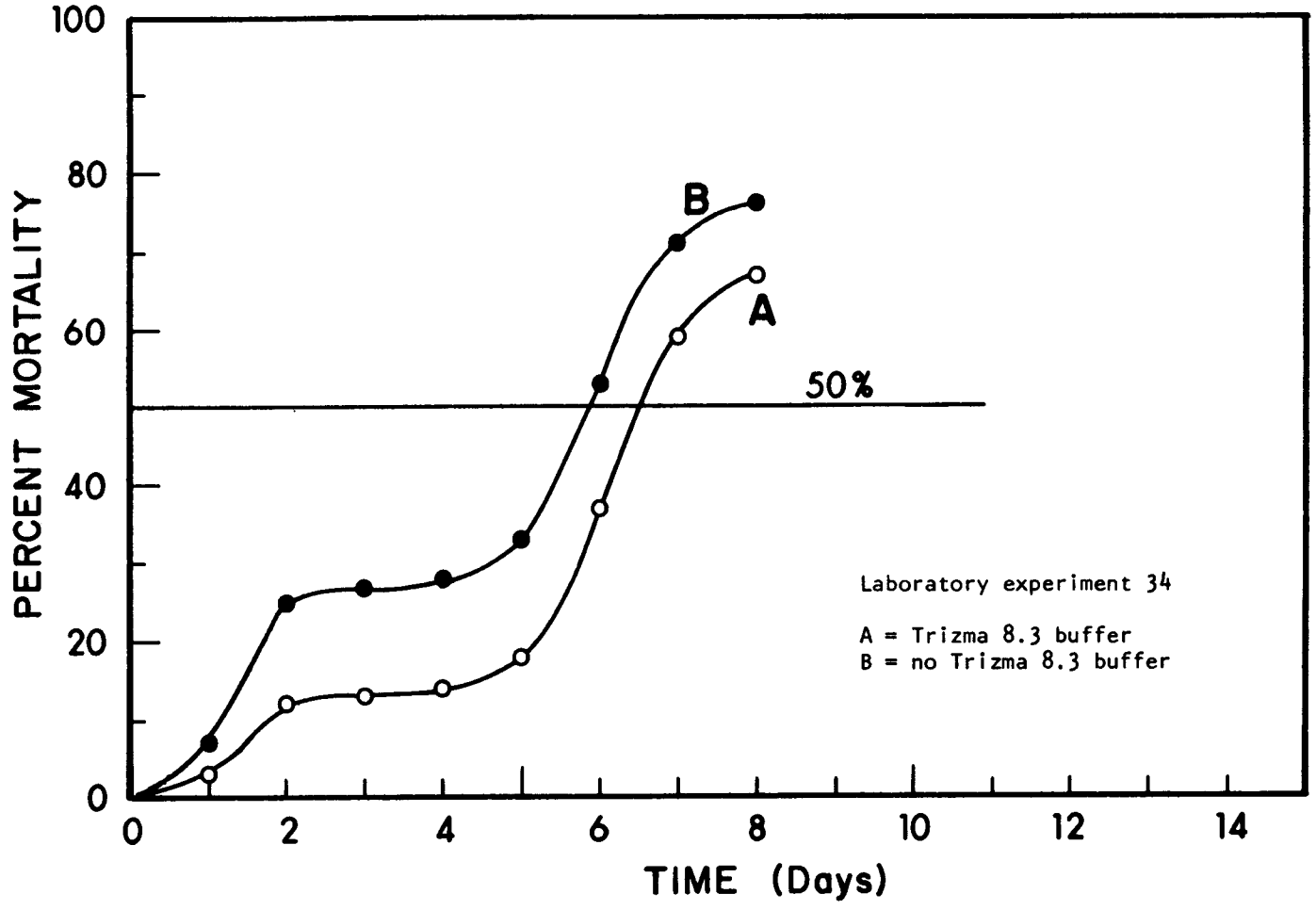


Figure 26. Effect of Trizma 8.3 buffer and reduced salinity on mortality of *S. purpureus* in closed system recirculated for 9 hours.

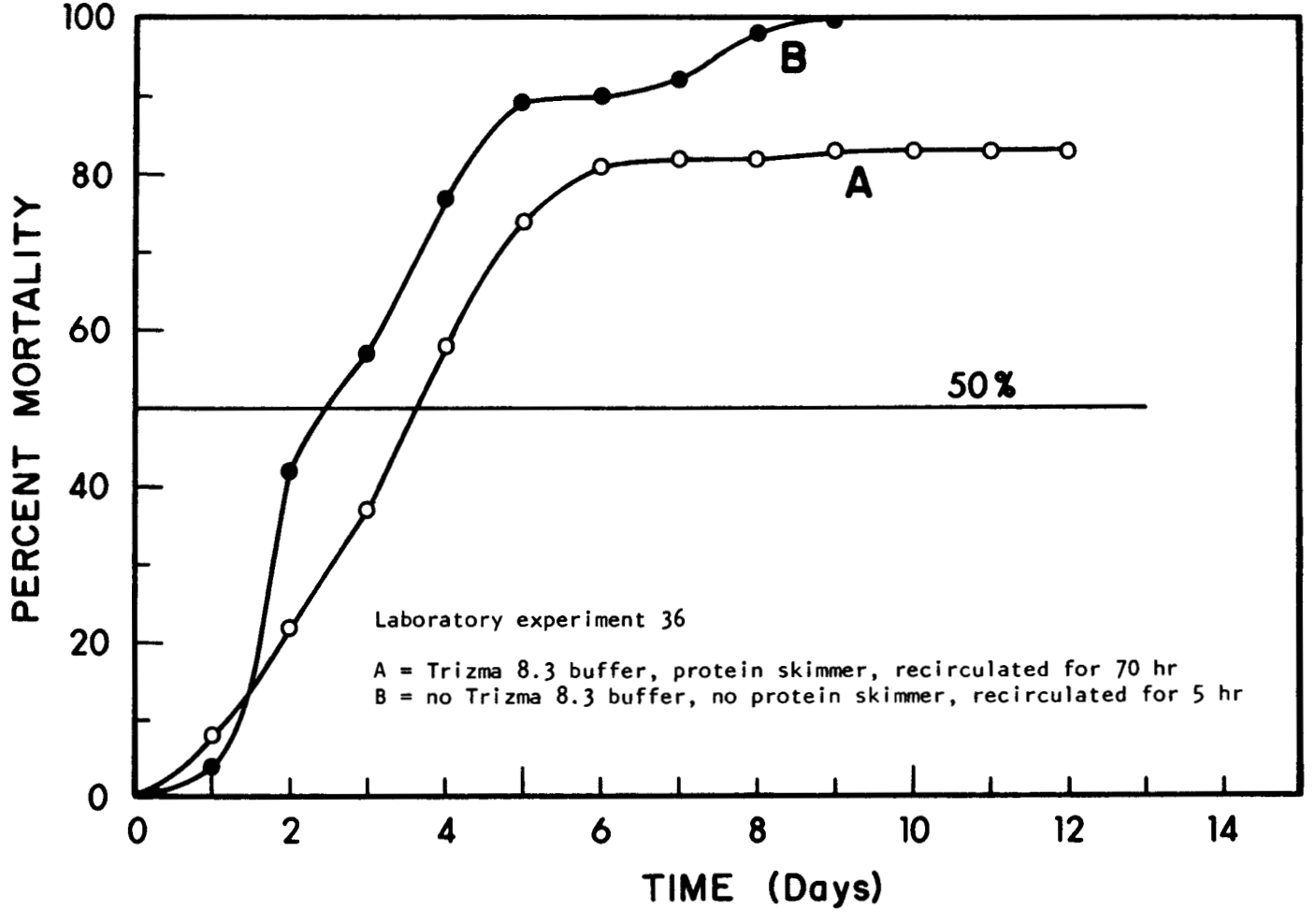


Figure 27. Effect of Trizma 8.3 buffer, protein skimmer, and reduced salinity on mortality of *S. purpureus* in closed, recirculated system.

in a closed system when 50 percent seawater is recirculated too long. Water in the tank with buffer and skimmer was recirculated for 70 hours; the pH only decreased to 7.90 (Figure 27, curve A). The tank with no buffer and skimmer was recirculated for 5 hours; the pH decreased to 7.45 (Figure 27, curve B). Fish treated with buffer and skimmer exhibited slightly higher survival, but fish in neither tank benefited greatly from recirculated 50 percent seawater. High mortality in the tank with buffer and skimmer was probably due to accumulation of toxic metabolites such as ammonia. In the tank with no additional treatment, pH was allowed to decrease below 7.50.

In conclusion, it seems best to recirculate water for no more than 3 to 4 hours (at appropriate density; Figure 23), regardless of addition of buffer or protein skimmer. Further, Trizma 8.3 buffer may maintain too high a pH, thereby increasing the accumulation rate of ammonia. A better treatment may be to use a buffer of lower pH. Further experimentation is needed to clarify this point.

9. Metabolites. Metabolites possibly affecting stress and mortality of nehu in the experiments are carbon dioxide and ammonia. In open systems with optimal environmental conditions (i.e., low density of nehu, oxygen saturation, and maximum turnover rate), it is unlikely that either metabolite will reach stress or toxic levels. Table 14 shows that in laboratory experiment 37, dissolved carbon dioxide concentration in nehu tanks with circulation, current, and supplemental oxygen or air did not significantly exceed that of water inflow control. The maximum carbon dioxide concentration measured over the 7-day experimental period was in tanks with circulation and air only (4.6×10^{-2} millimoles per liter; 2.12 ppm; partial pressure = 1.16 mm Hg). From these data, it would appear that

current may decrease carbon dioxide concentration. All concentrations of carbon dioxide were probably below a toxic level, but over a longer period of exposure this concentration might induce stress by affecting respiration. Unfortunately, it was not feasible to measure carbon dioxide concentrations daily. In some experiments with suboptimal environmental conditions, carbon dioxide may reach stress levels even in open systems. This is indicated by a few such experiments with low pH values (7.5 to 7.9). Further, in closed-system recirculated brackish water, carbon dioxide did accumulate, inducing stress and mortality.

Attempts to measure ammonia, which affects both stress and mortality in closed systems, were unsuccessful. Further experimentation and measurement are required to clarify the effect of carbon dioxide and ammonia on nehu in closed systems.

10. Density. Each experiment must be evaluated individually to determine suitable density. The optimum varies with (1) volume of water, (2) size of fish, (3) turnover rate, and (4) environmental factors such as oxygen, current, and visual environment.

Figure 28 summarizes data from three comparable experiments which vary the density of fish. Tanks were provided with supplemental air only, the current flow rate was 0.08 liter per second, and salinity was 34.0 to 36.0 ppt. Air was insufficient to maintain oxygen saturation and on Day 1, oxygen concentration fell to a stress level. Initial mortality was independent of density: most fish died from handling injuries and not from environmental stress effects. Further, time to delayed mortality and the 50 percent mortality level did not vary significantly between tanks of different concentrations, indicating that fish undergo stress mortality at the same time regardless of initial density. By Day 14, however, a greater percentage of

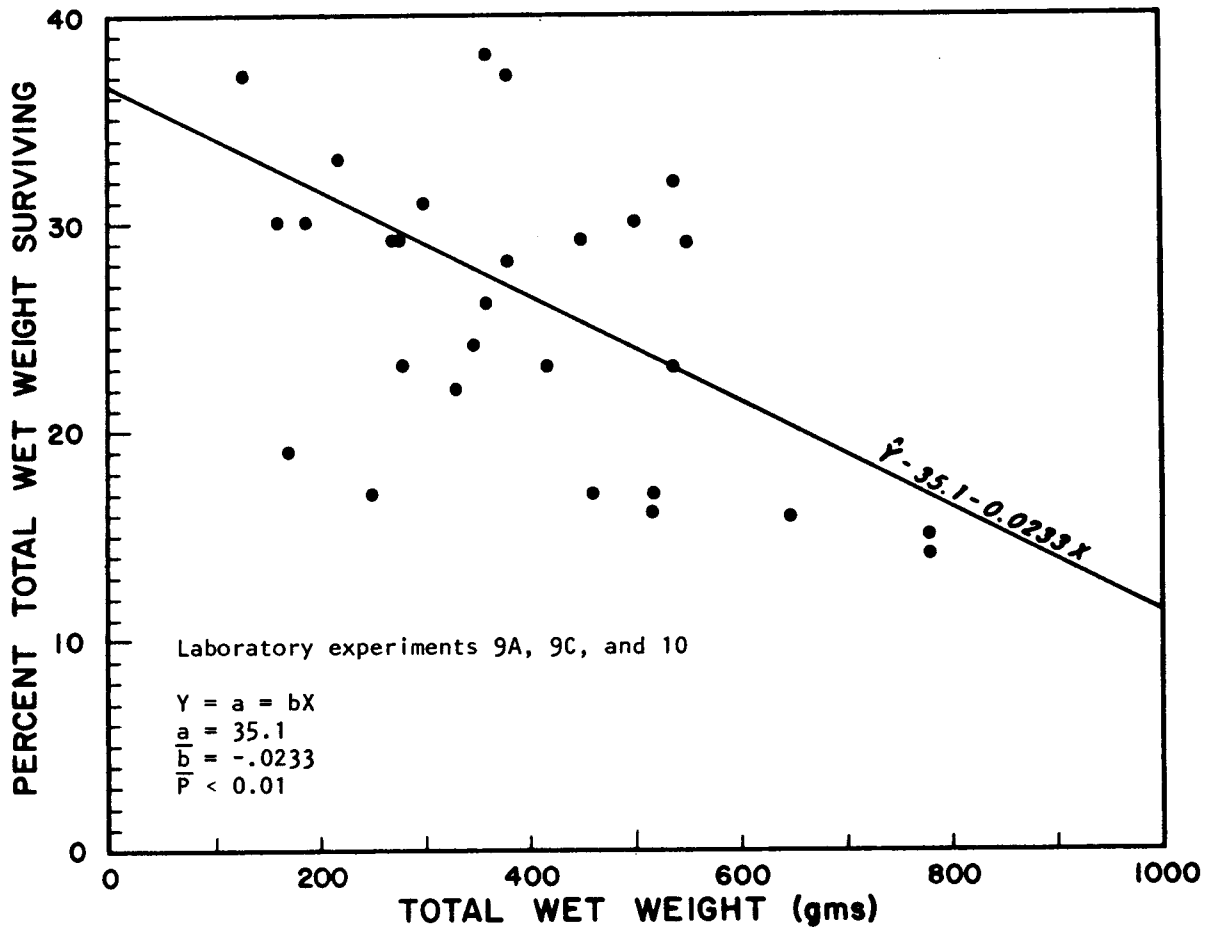


Figure 28. Effect of initial density on mortality of *S. purpureus*.

fish survived in tanks with lower initial density. Figure 28 shows that the relationship between percentage of survival to Day 14 and initial density in gram wet weight is approximately linear ($\underline{b} = 2.3343 \times 10^{-2}$; $P < 0.01$).

In Table 15, three capture environments are described and the approximate density of nehu appropriate for each is estimated. These estimates are based on groups of fish of mean wet weight from 0.500 to 0.700 gm. In bait wells with poor conditions (Table 15, item A) and a minimum turnover rate of about 25 percent per hour, density of fish is usually oxygen-limited. In this case, density should be approximately 80 to 130 gm per 100 liters. If bait well conditions are improved (Table 15, item B), density can be increased to about 130 to 260 gm per 100 liters. If 50 percent seawater is recirculated for 3 hours to give best conditions (Table 15, item C), density must be regulated to 200 to 210 gm per 100 liters or pH decreases to a lethal level too rapidly and other metabolites may reach stress or lethal concentrations. Density of fish in 50 percent seawater in an open system can be increased above that of a closed system. Densities in Table 15 are based on a minimum turnover rate (25 percent per hour). If flow rate is increased in items A and B (Table 15), density can be proportionately increased. If fish are injured from handling or if other environmental stress is great, densities should be decreased.

Results of all experiments (with supplemental oxygen available) indicate that, to a maximum density of approximately 260 gm per 100 liters (1,000 gm per 100 gal), density does not significantly affect the shape of the mortality curve if oxygen is at saturation in an open system. Greater densities proportionately increase mortality. Crowding fish at high densities would therefore simply result in additional work in removing dead fish.

11. Food. Juvenile and adult nehu usually feed on the dominant crustacean zooplankton. In Kaneohe Bay these are primarily copepod adults, nauplii, and eggs (Hiatt, 1951). On most fishing vessels where nehu are used as baitfish within a few days, feeding is unnecessary and probably undesirable. Food is costly and pollutes well water and nehu reduce the oxygen level more rapidly when feeding. Lack of food does not increase mortality until about 10 to 14 days after capture.

Because some experimental objectives require keeping nehu for longer periods, effects of various food treatments on survival and weight maintenance were contrasted. Experiments indicate that, of the various dried commercial fish meals, ground liver, ground skipjack tuna, Oregon moist pellet (OMP), and frozen brine shrimp (FBS), frozen brine shrimp elicit optimal feeding behavior and best weight maintenance.

Nehu prefer brine shrimp, feeding on them earlier than any other food tested. They also feed on Oregon moist pellet if sufficient time is allowed for training. Most difficulty with foods other than brine shrimp is behavioral. Nehu are slow to feed on dried or ground food, accepting it only after several days of training. Also, much dried or ground food is wasted: when missed by the fish, it falls to the bottom of the well and becomes a contamination problem.

How soon nehu begin feeding in captivity depends not only on food type, but also on the condition of the fish. Just after capture, when fish are stressed or injured, few will feed. If environmental conditions are good, a small percentage of nehu begin feeding on Day 2. The first significant feeding response usually begins on Day 3. About 25 percent of the fish feed at this time; the percentage gradually increases until most are feeding by Days 5 to 6. When delayed stress mortality begins,

feeding activity again decreases, although most fish continue feeding unless stress is excessive. After the asymptotic level of mortality is reached, fish feed readily for the remainder of the experimental period. In most experiments feeding began on Day 3 or Day 4 and the amount of food was decreased when fish were stressed or died.

Ideally, nehu should be fed several times daily, but experimental conditions limited the feeding schedule to 3 times daily. If fed more frequently, fish would learn to accept food earlier and less would be wasted. As noted, the amount of food varied daily, but the wet weight of food averaged 36 percent of the wet weight of nehu; dry weight of food averaged 3 to 5 percent of the wet weight (Table 16). The percentages of dry weight of brine shrimp and moist pellet are in Table 17.

In laboratory experiment 1 (Table 18; Figure 29), five food treatments were contrasted using acclimated fish (22 days). There was no significant difference in mortality between food treatments until approximately Day 10, when the effects of starvation increased mortality slightly in fish given no food (Figure 29, curve B) or only plankton (Figure 29, curve A). No significant increase in mortality was observed in fish fed trout food only (Figure 29, curve D), trout food plus frozen brine shrimp, or frozen brine shrimp only (Figure 29, curve C). Figure 30 shows the calculated regression curves (dry weight of a given standard length) at the end of 14 days for nehu given different food treatments. When treatment regressions were compared with the regression curve of the initial sample (Figure 30, curve E), the results showed that fish given trout food plus frozen brine shrimp or frozen brine shrimp only (Figure 30, curves B and C) increased in dry weight. Those fed trout food only gained somewhat less weight (Figure 30, curve D); those fed plankton or no food (Figure 30,

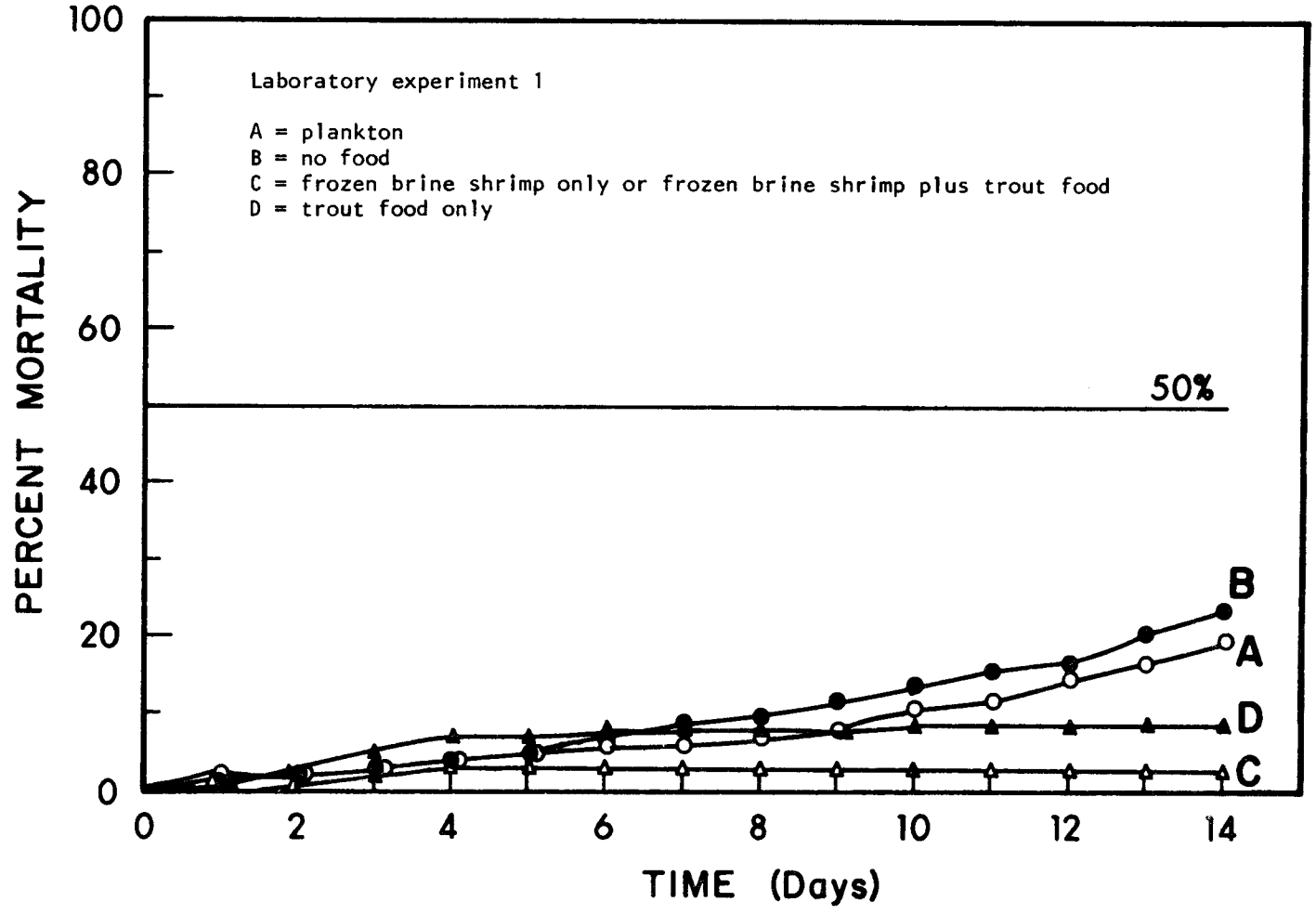


Figure 29. Effect of food type on mortality of acclimated *S. purpureus*.

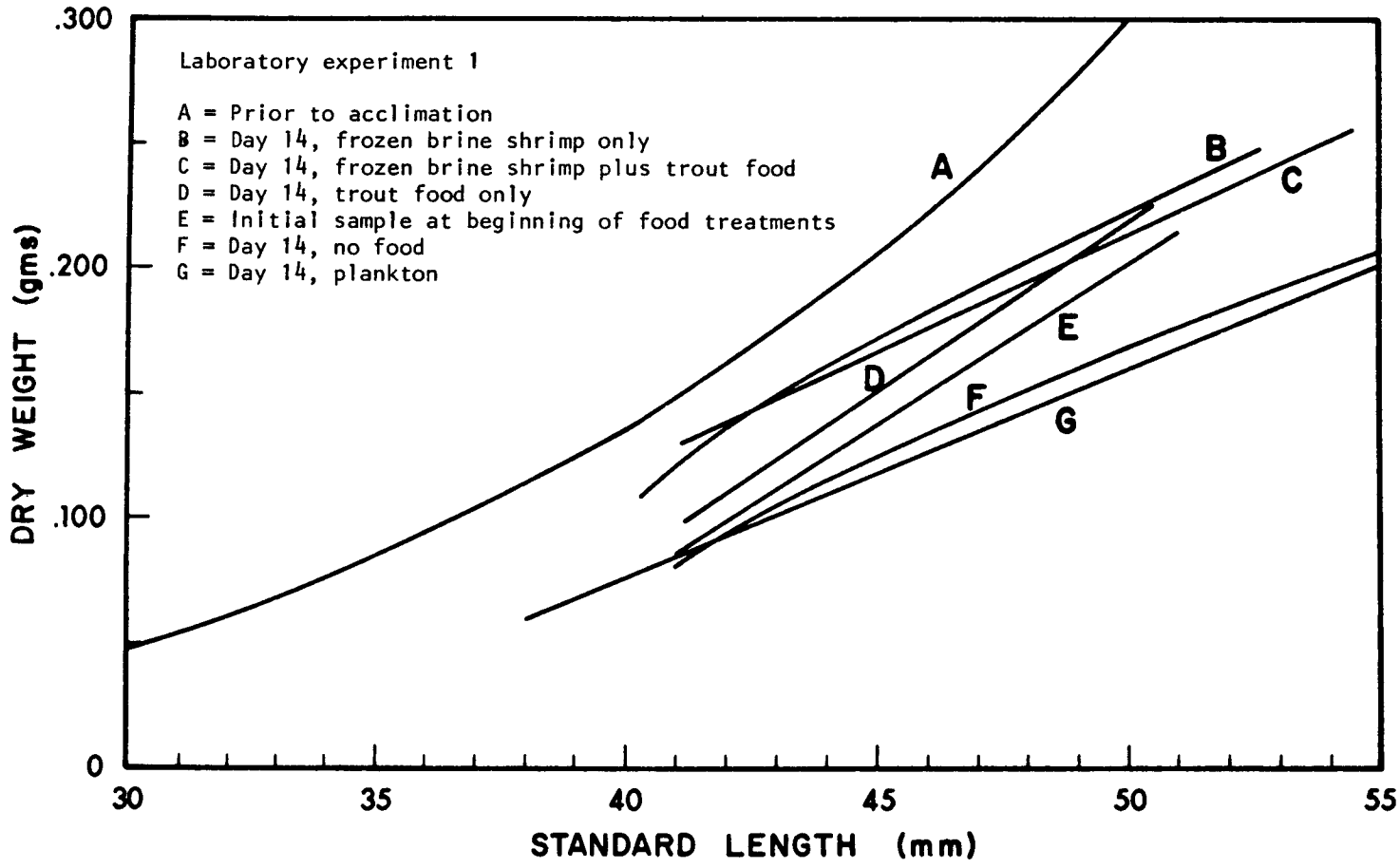


Figure 30. Calculated regression curves of dry weight on standard length of *S. purpureus* before and after different food treatments.

curves F and G) lost weight. Calculated dry weights ($\hat{Y}$) for a mean standard length of 45.0 mm (X) are in Table 18. Fish used in this experiment were given insufficient food during the acclimation period. The experimental initial length-weight regression curve (Figure 30, curve E) does not represent a true estimate for the natural population (Figure 30, curve A). Fish given food treatments resulting in weight gain actually regained weight lost during the acclimation period.

In laboratory experiment 2 (Table 18) the same food treatments were tested on unacclimated fish. These nehu were particularly well-handled night bait which were transferred by swimming into barge wells. Delayed mortality did not begin until about Days 10 and 11 (Figure 31). An interesting result is that those given food (Figure 31, curves A, B, and D) underwent delayed mortality earlier than those given no food (Figure 31, curve C). This would not have occurred if oxygen concentration did not fall below the critical level. In this experiment, however, oxygen stress occurred on Day 8, with oxygen concentration falling below 5.0 ppm. It appears that if low oxygen stresses fish, feeding them results in further stress and ealier delayed mortality. If oxygen is at saturation, no difference in mortality occurs between those fed and those not fed. Starvation mortality in nehu given no food (Figure 31, curve C) began about Days 16 to 18; all fish were dead by Day 20. Length-weight regressions of fish sampled on Day 16, when compared with the regression curve of the initial sample (Figure 32, curve A), showed that fish fed frozen brine shrimp maintained weight (Figure 32, curve B), those fed trout food or trout food plus frozen brine shrimp (Figure 32, curves C and D) lost some weight, and those given no food (Figure 32, curve E) lost most weight. Percentage

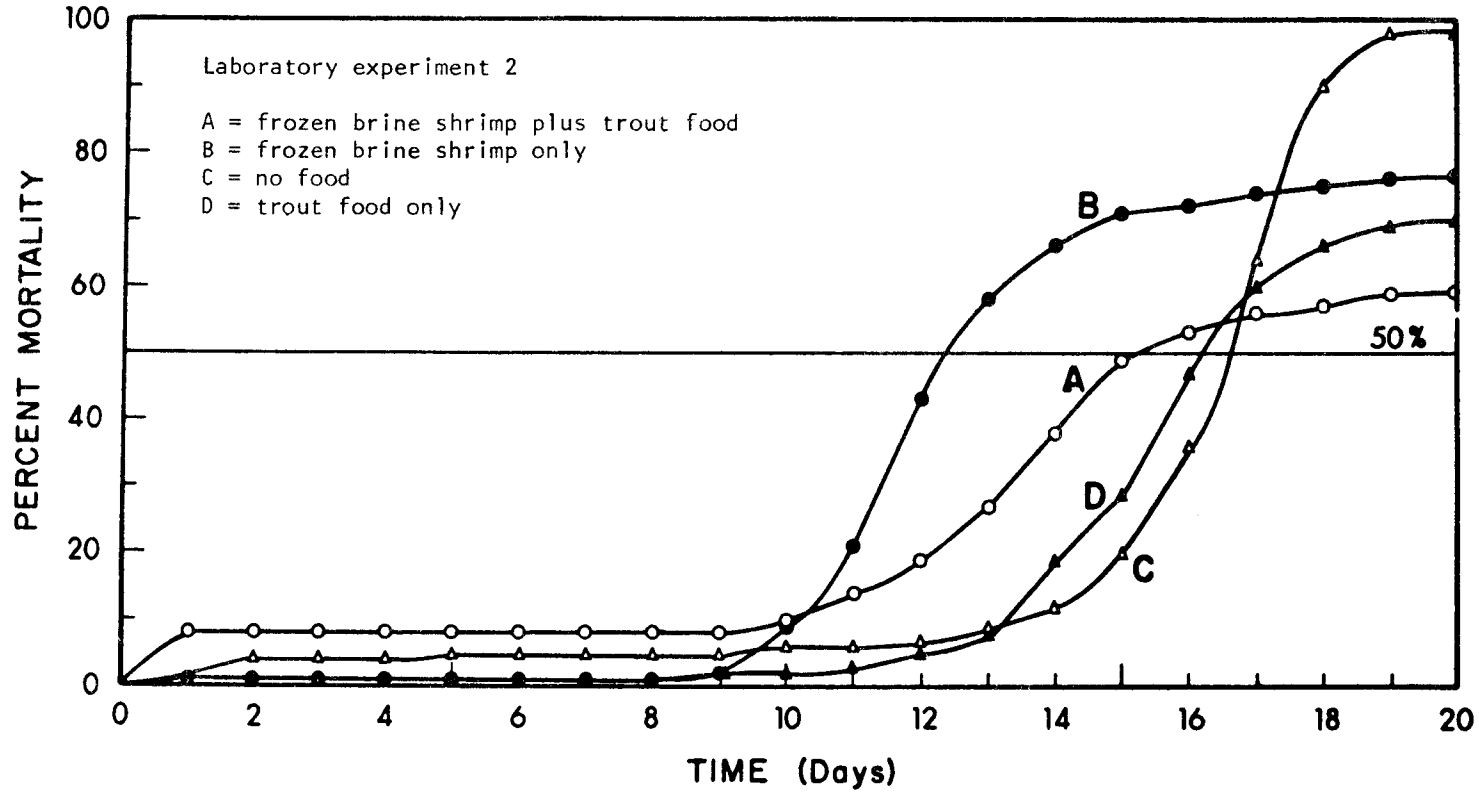


Figure 31. Effect of food type on mortality of unacclimated *S. purpureus*.

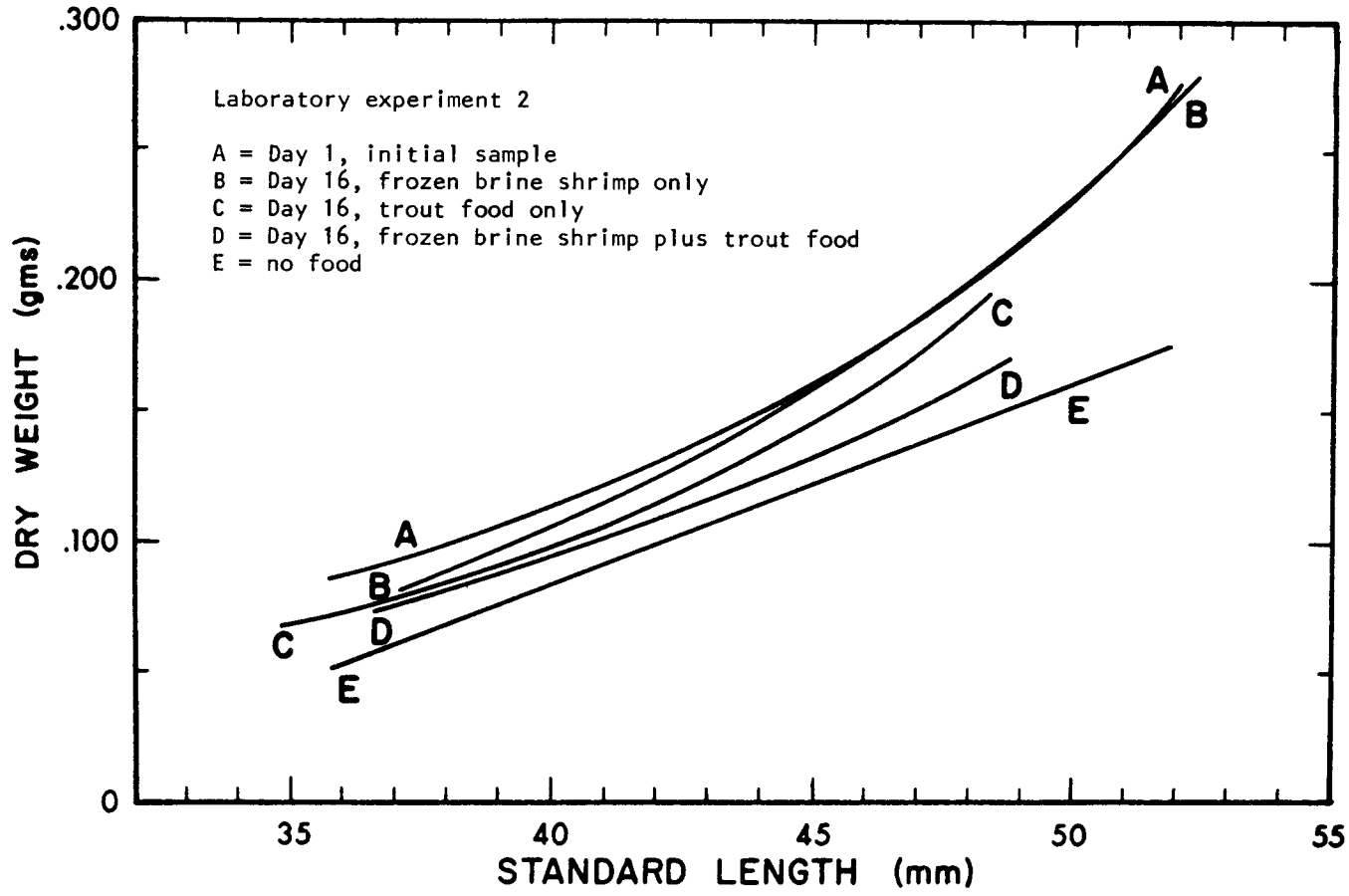


Figure 32. Calculated regression curves of dry weight on standard length of *S. purpureus* before and after different food treatments.

of weight loss or weight gain for different food treatments is compared in Table 18. The only food resulting in no weight loss was frozen brine shrimp.

In barge experiments 7 and 8, all fish were given frozen brine shrimp (wet weight of food approximately 36 percent per gram wet weight of fish; dry weight of food approximately 5 percent per gram wet weight of fish). Figures 33 and 34 and Table 18 show that most fish maintained weight with this food treatment.

Frozen brine shrimp should be washed in fresh water instead of seawater to thaw. This results in brine shrimp sinking more slowly so that fewer are missed by the fish. Excess brine shrimp should be removed from the bottom of the tank after feeding.

If fish are to be held for 10 days or less and used for bait purposes such as on fishing vessels, they should not be fed.

12. Other fishes. Other species of fish are commonly caught with nehu by both day and night capture methods (Table 34). Some are schooling fishes which are not predatory on nehu, such as iao (*Pranesus insularum*) and makiawa (*Etrumeus teres*). Others prey on nehu, such as the carangids (*Caranx mate*, *C. melampygus*, *Gnathanodon speciosus*, and *Scomberoides lysan*). It is best to remove other fish from the bait well, except those which school and are difficult to separate from the nehu. Most large predators should be removed when bait is placed in the tank. Predators stress nehu by attacking them and disrupting normal milling. The final factor analysis shows that the presence of other unpredatory schooling fish may significantly enhance survival by possibly promoting earlier milling.

Adult *Scomberoides lysan* or lae are mid-water or surface carnivores found along coastal areas (Gosline and Brock, 1960). Juveniles approximately 10 to 80 mm in length are found in shallow waters associated with

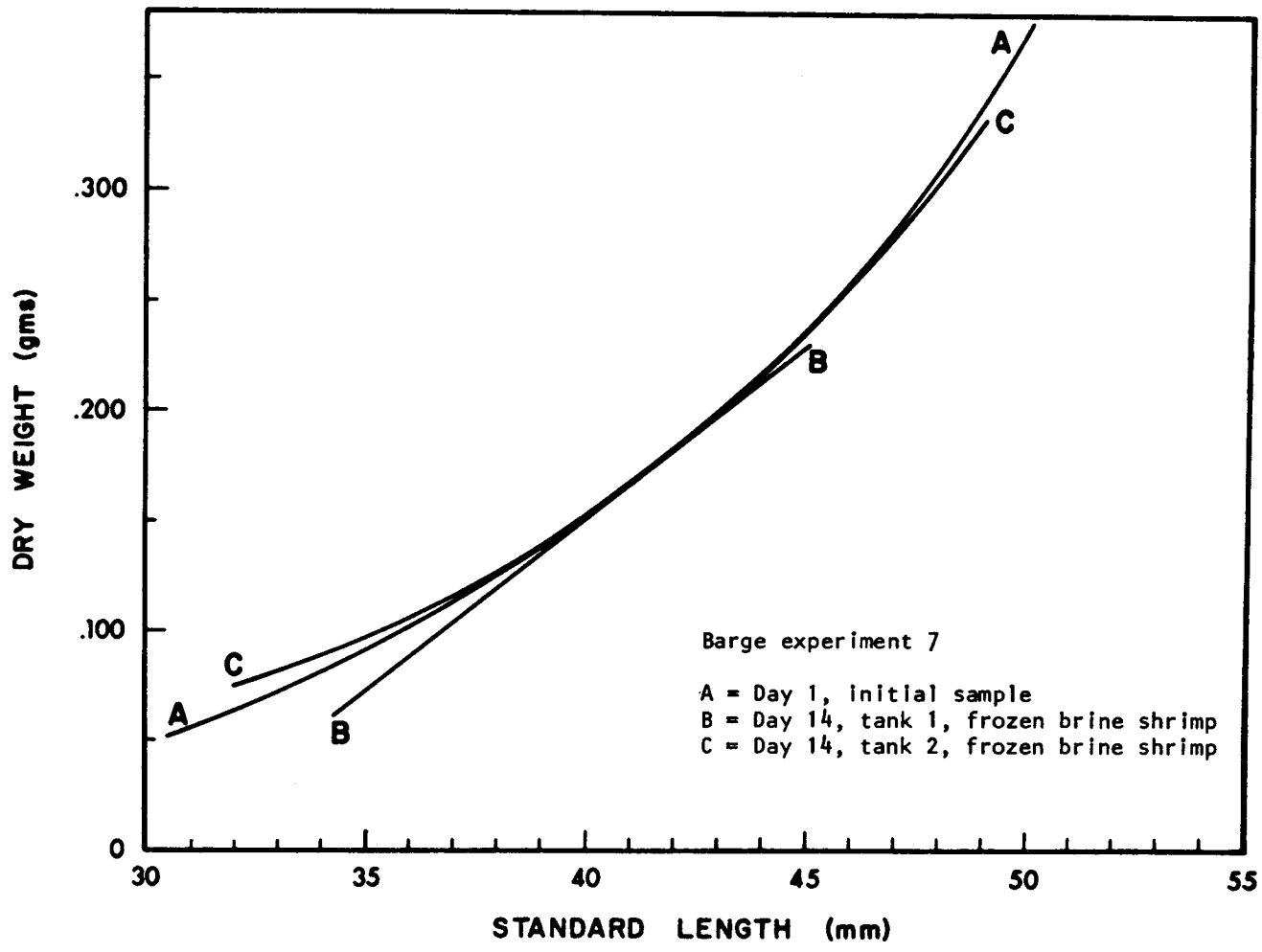


Figure 33. Calculated regression curves of dry weight on standard length of *S. purpureus* before and after fed frozen brine shrimp in both tanks.

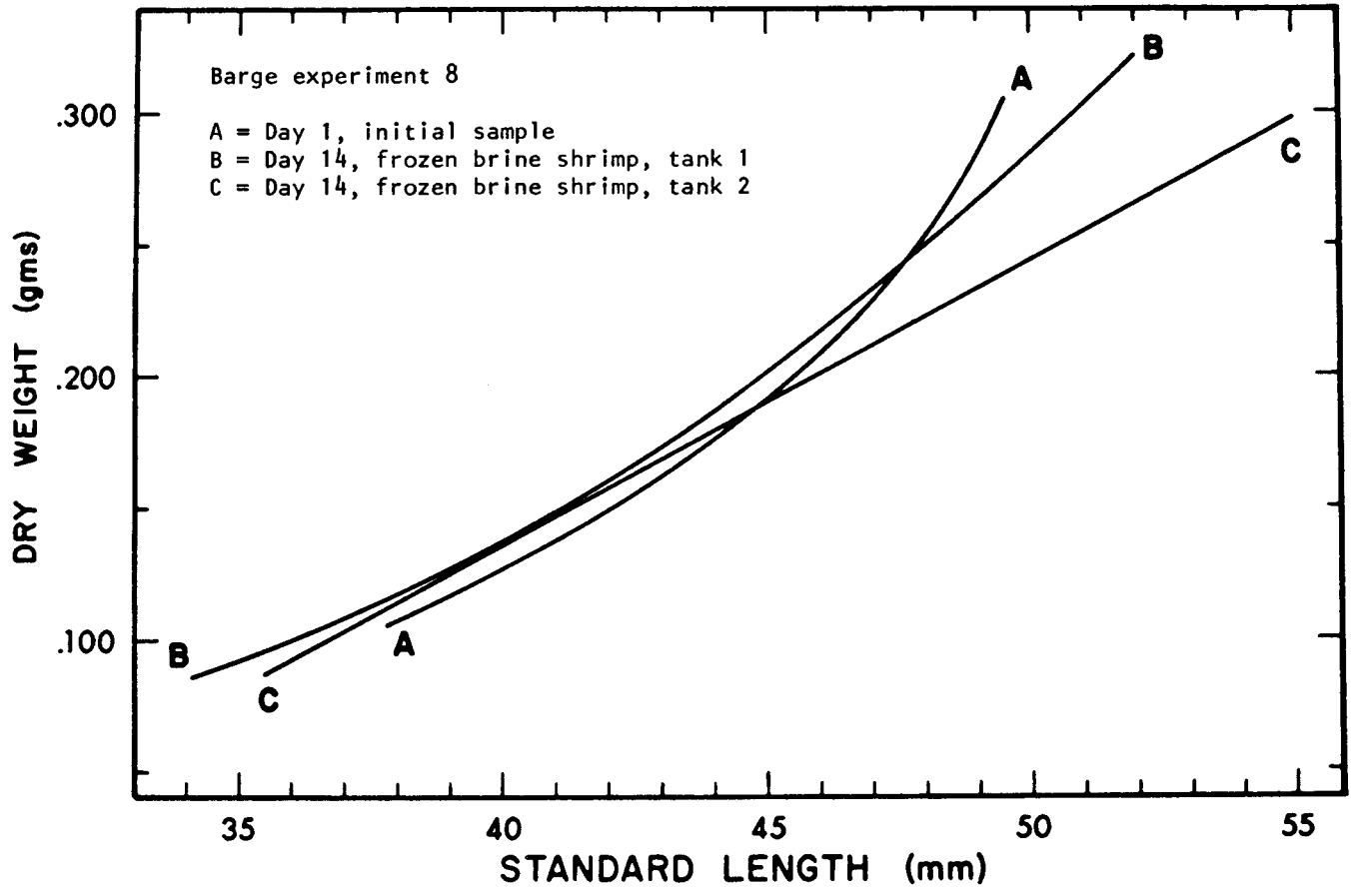


Figure 34. Calculated regression curves of dry weight on standard length of *S. purpureus* before and after fed frozen brine shrimp in both tanks.

schooling fish including nehu. Juvenile lae are frequently captured with nehu from about March to December, but are caught in greatest numbers during July, August, and September, when they become a major stress and mortality factor of nehu in bait wells. During this period, the predominant size is from about 25 to 40 mm. Figure 35 shows the length frequency distribution of a sample of 91 juvenile lae captured with nehu in September. The length of the lae ranged from 23.0 to 63.0 mm; the mean length was 35.2 mm. Lae from about 20 to 50 mm in length feed predominantly on nehu scales. Some scale feeding continues until they reach 140 mm in length (Major, 1973). As they increase in size through the fall and winter months, they begin feeding on juvenile and finally adult nehu. On the other hand, the number of lae captured with nehu rapidly decreases from October through March and bait well mortality caused by lae becomes less.

In a typical attack sequence, lae swim parallel to or above the nehu for about 20 seconds, then dart toward the dorsal area behind the head making contact with the mouth. Simultaneously, the venomous anal spines (Halstead et al., 1972) are lowered although movies made of the attack sequence indicate that lae do not hit or "sting" nehu with the spine, but strike only with the mouth. During the attack, many nehu scales are dislodged and lae eat the free-floating scales. In confinement, lae continually attack and stress nehu until they die. Examinations of dead nehu in tanks with lae reveal that numerous scales in the dorsal area are removed.

Table 19 summarizes laboratory experiments in which lae were present in at least some tanks (180-gal size). Results show that the presence of lae significantly increased mortality of nehu by the end of 13 to 16 days ($P < 0.01$; A vs B and C). Although one to five lae significantly increased mortality ($P < 0.05$), mortality was still less than when six or

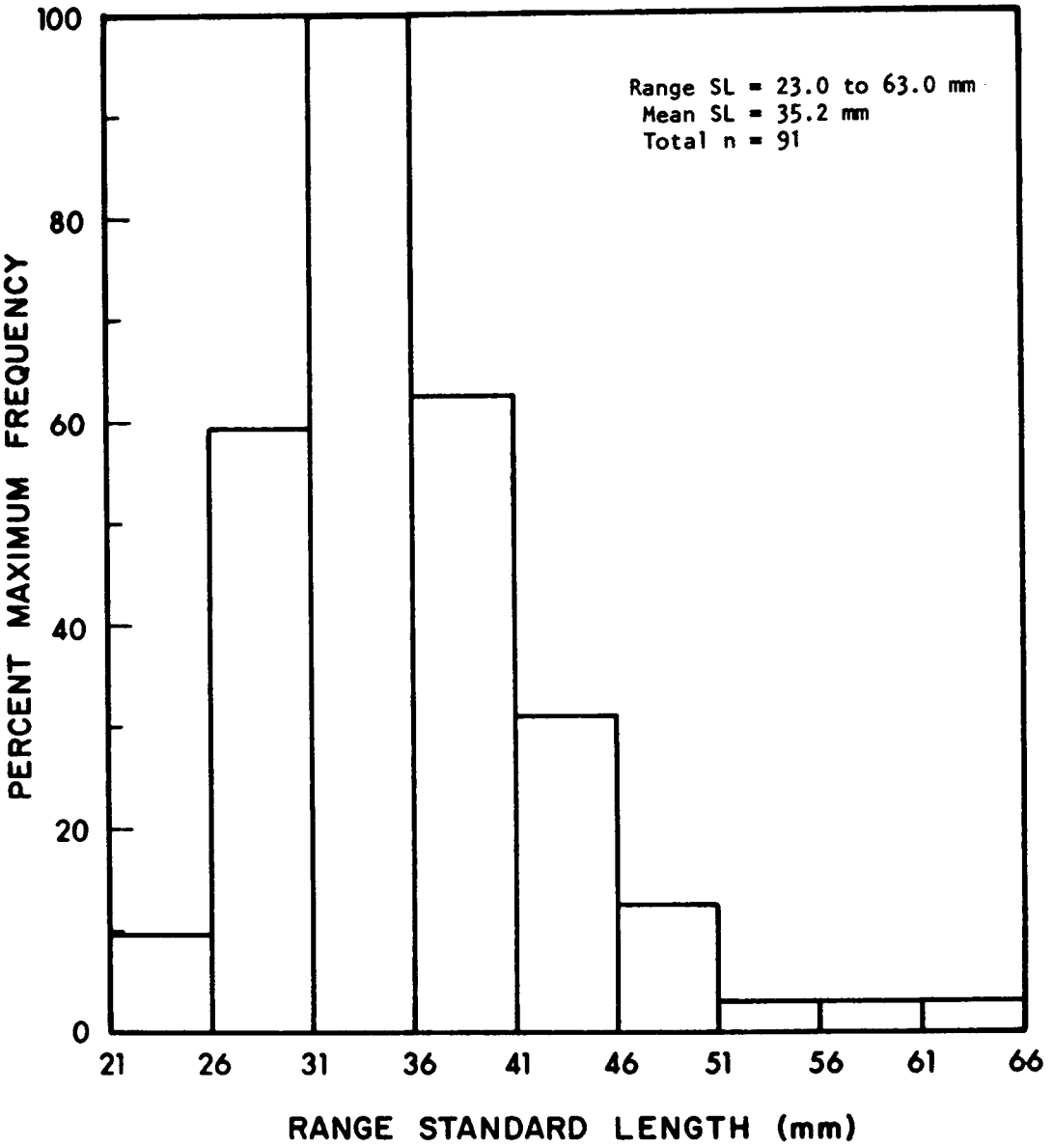


Figure 35. Frequency distribution of standard length of *Scomberoides lysan* captured with *S. purpureus* in September 1970.

more lae were present. More than ten lae in a tank usually effected total mortality of nehu by 13 to 16 days.

The gut contents of lae which are approximately 10 to 50 mm in length were composed mostly of nehu scales and traces of epidermal tissue (C. Moldenhauer, 1967: personal communication). When scales were removed from the nehu and ashed, they contained about 42 to 48 percent organic material. Extracts of homogenized lae intestine were added to samples of nehu scales. The intestinal brie contained enzymes which break down the scales. Observations made so far in addition to other work by Major (1973) on structural changes in teeth with increasing body size strongly support the contention that juvenile *Scomberoides lysan* are predominantly scale-feeders.

Several attempts to eliminate lae from bait wells containing nehu were generally unsuccessful or inefficient. Lowering salinity to a level tolerated by nehu did not kill lae and small traps baited with nehu did not capture them. Fishermen often leave larger fish in bait wells because lae aggregate around them and can then be easily removed with a net. In the laboratory, lae were fed brine shrimp to concentrate them at one end of the tank. When they aggregated where the shrimp were released, they were easily removed with a hand net. Further research on lae would be of interest, not only because of their importance in bait fish mortality, but also because of their unusual scale-feeding behavior during the juvenile stage.

13. Disease and parasites. Although pathogenic bacteria were undoubtedly present in the experiments (Struhsaker et al., 1973), it is unlikely that bacterial disease is a major mortality factor of nehu relative to previously discussed environmental factors. If nehu are kept in

captivity for periods longer than 14 days, however, stress and mortality from bacterial disease may occur, especially in injured fish. Obvious symptoms of common bacterial disease of fish in captivity were not observed in the experiments, but it is possible that if appropriate studies had been made, such diseases may have been revealed.

During warmer months--approximately March through November--nehu are frequently infected by a protozoan, probably a species of *Cryptocaryon* (Nigrelli and Ruggieri, 1966; Wilkie and Gordin, 1969). The infection resembles the common "white spot disease" or "ich" of freshwater fish. Opaque white cysts of diameters varying to a maximum of about 1 mm occur under the gill epithelium. Infections of gills immediately after capture were usually light, 1 to 5 cysts (observed without magnification) in each gill chamber. During the 2 weeks of captivity, the infection increased until a large number of cysts was found in the gills and eventually on the fins and body. The cysts probably seriously impede respiration, causing stress and mortality. Areas infected by protozoan cysts are also possible foci for bacterial infection.

In laboratory experiment 19, it was discovered that reduced salinity (50 percent seawater) effectively eliminated cysts; most after 5 days, all after 10 to 11 days. Figure 36 compares mortality of nehu treated with 100 percent and 50 percent seawater and also shows, for daily samples of 10 fish, percentage of nehu infected by cysts over the experimental period. In the initial sample, all fish were lightly infected with a few cysts. The percentage of nehu infected by at least a few cysts decreased rapidly in 50 percent seawater until most were uninfected by Day 5 and none were infected by Day 11 and throughout the remainder of the experiment (Figure 36, curve A^1). It is probable that cysts develop and fall

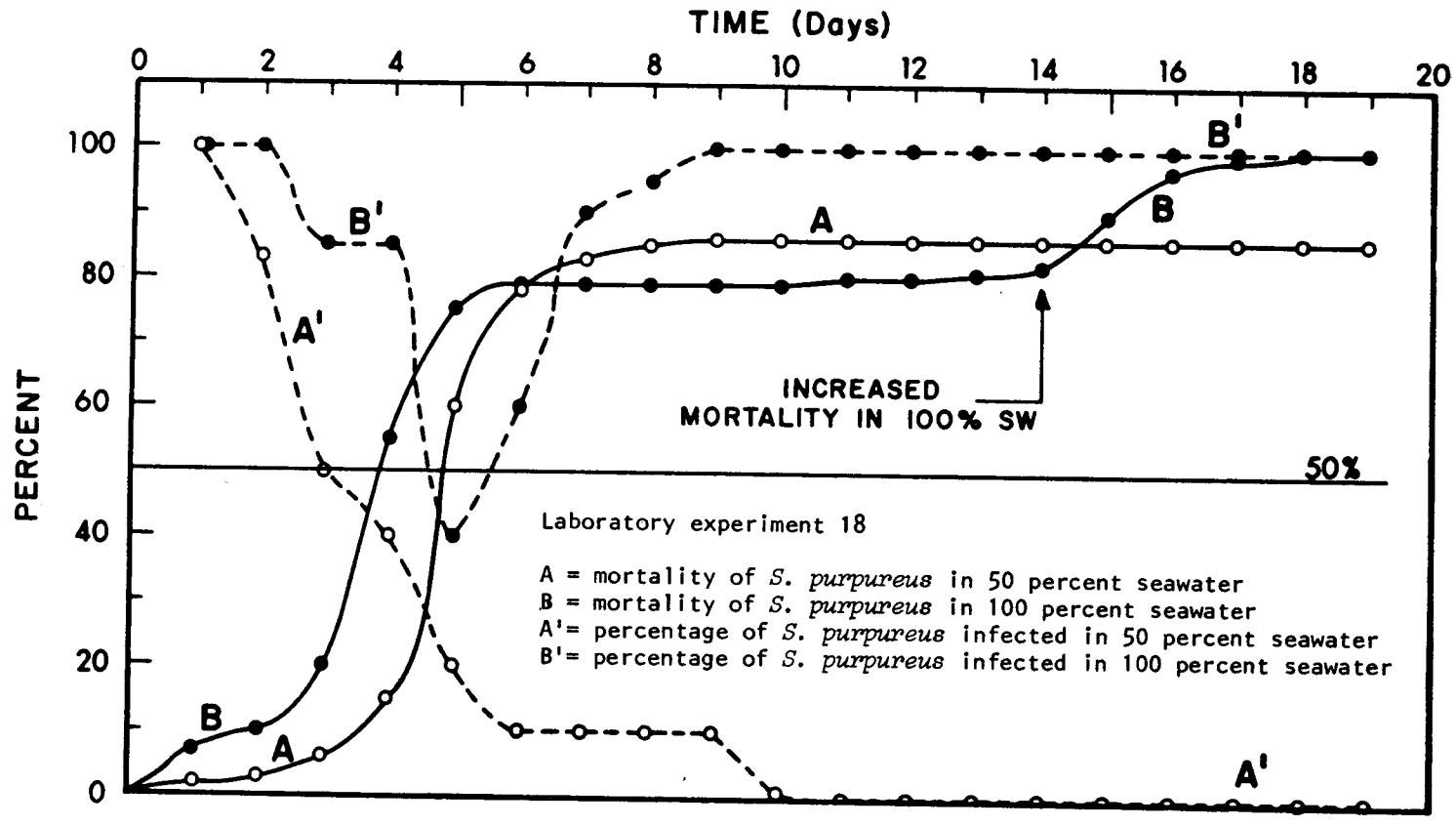


Figure 36. Effect of *Cryptocaryon* sp. protozoan cysts on mortality of *S. purpureus* and the effect of reduced salinity on infection.

off and the emerging free-swimming stages die without reinfecting fish in reduced salinity. No increase in mortality occurred after Day 14 (Figure 36, curve A). In nehu maintained in 100 percent seawater, the infection at first decreased as cysts developed and dropped off. The free-swimming stages survived, however, and began reinfecting fish so that new cysts appeared and increased in number until all fish were heavily infected by Day 9 (Figure 36, curve B^1). An increase in mortality began on about Days 14 to 15, until all fish died by Day 19 (Figure 36, curve B). Infection by this protozoan may contribute to the "weak bait" phenomenon in which nehu die more rapidly during summer months. Continuous exposure to 50 percent seawater in an open system completely eliminates this protozoan disease and may also reduce bacterial infection.

Ectoparasitic isopods of family Cymothoidae are commonly associated with nehu. Although they were observed to injure nehu by biting them, relative mortality from these ectoparasites is probably negligible.

If bait-well conditions are optimal (i.e., if 50 percent seawater is provided, oxygen is at saturation, and density is appropriate), stress and mortality from disease and parasites are probably minimized.

Final Data Analysis of Laboratory and Barge Experiments

A final analysis was performed to determine interrelationships or patterns of environmental variables and their relative effect in producing stress and mortality of *S. purpureus* in captivity. Component factor analysis--analyzing all data variance--was the analytical technique used. Resultant factors are basic dimensions of the vector space of the data. The analysis was accomplished with a 360 IBM Computer using a California Biomedical Program (BMD 03M) for component factor analysis (Dixon, 1970).

A complete treatment of this technique is given by Rummel (1970). Only information pertinent to interpretation of results is given here.

A preliminary analysis of combined data from laboratory and barge experiments disclosed that influence of environmental variables differed between the two locations. Separate analyses were then performed on laboratory and barge data; results are presented separately below. Variables comprehended in analysis of laboratory and barge data are listed in Tables 20 and 21. For laboratory experiments, 34 variables--27 independent and 7 dependent (Table 20)--are included with 184 cases (tanks). For barge experiments, 31 variables--24 independent and 7 dependent (Table 21)--are included with 28 cases (tanks). Some independent variables measured in the laboratory were not measured on the barge. Measurement techniques for independent environmental variables are delineated in the "methods" section. Derivation of dependent variables measuring stress and mortality is described below.

Figure 2 (curves A and D) showed typical mortality curves of *S. purpureus* in captivity. Mortality (Y) is a function of time in days (X) and the relationship is described by the logistic function $\hat{Y}(X) = \frac{Y^{\infty}}{1 + e^{a-rX}}$. Dependent variables used in the factor analysis are parameters of the logistic curve. The day that 50 percent mortality is reached (Y = 50% or Day 50%), the time that delayed or stress mortality begins (X = m or X1-DM), the time that delayed or stress mortality ends (X = n or X2-DM), the total duration of delayed mortality (X_n - X_m or Tot X-DM), the total delayed mortality (Y_n - Y_m or Tot Y-DM), and the time that midpoint of delayed mortality is reached (a/r or A/R) are derived from the logistic function. Initial mortality (Y at X = 1 or %IM) and number surviving (Y at end of experiment or % Survival) are

obtained directly from the mortality data.

Stress parameters were derived by putting the logistic functions in the equivalent form of hyperbolic tangent functions (Preisendorfer, 1970; J. Caperon, 1970: personal communication), and then taking the first, second, and third derivatives of these functions. The derivatives are summarized in Table 22. The constants a (Y-intercept) and r (instantaneous rate of increase) were calculated from data using the logarithmic form of the logistic function: $\ln \frac{Y^{\infty} - Y(X)}{Y(X)} = a - rX$. The point at which the second derivative equals zero ($\frac{d^2Y(X)}{dX^2} = 0$) corresponds to the midpoint of delayed mortality ($A/R = a/r$) in the logistic function; in the hyperbolic tangent function it corresponds to the origin ($A = 0$). The points where the third derivative equals zero ($\frac{d^3Y(X)}{dX^3} = 0$) correspond to the points of flexure in the logistic curve, or to the beginning and end of delayed mortality, X_n and X_m. X_n and X_m were calculated from the equation for the third derivative using a Basic Program (ZEROES: Hewlett-Packard Program Library) which determines values of X for $F(X) = 0$, maxima and minima of a defined function over a given X-interval. The value of X at Y = 50%, Total $X(X_n - X_m)$, Y_n, Y_m, and Total $Y(Y_n - Y_m)$ were then calculated for the values of X_n and X_m.

Tables 23 to 27 contain results of component factor analysis of laboratory data. Table 23 includes means, standard deviations, coefficients of variation, and communalities for 34 variables. Coefficients of variation were determined by dividing standard deviation by the mean (Sokal and Rohlf, 1969) and provide a basis for comparing variation within variables of different means. Coefficients of variation for dependent variables (Table 23) indicate less variation in total days of delayed mortality (Tot X-DM) and total delayed mortality (Tot Y-DM) than in level of initial mortality (%IM), time delayed mortality begins (XI-DM),

day 50 percent mortality is reached (Day 50%), and number surviving (% Survival). This accords with the observation that, in most experimental environments, duration and extent of delayed stress mortality are relatively invariable; most variation occurs with the onset of delayed mortality and number of fish already dead when delayed mortality begins.

Communalities (Rummel, 1970) represent the proportion of a variable's total variance accounted for by factors and is the sum of squared factor loadings for a variable (Table 25). The sum of communalities divided by the number of variables multiplied by 100 equals the percentage of total variation in all data that is patterned, or the total variance accounted for by factors. It measures order, uniformity, or regularity of data. For the 34 laboratory variables, 82 percent of the total variance is patterned (Table 23).

The correlation matrix for 34 laboratory variables is presented in Table 24. Many significant correlations are not biologically meaningful; some result from experimental and statistical design. Of the biologically interpretable correlations, most variables interrelate in patterns, emerging as significant factors in the rotated factor matrix (Table 25). Others, although not involved in major factors, may significantly correlate with dependent variables. Significant correlations of biological interest which are not included in the discussion of factors are summarized as follows (from Table 24):

1. With current, 50 percent mortality is reached later.
2. With current, delayed mortality is less.
3. With current, number surviving is greater.
4. With increase in hours of recirculated 50 percent seawater, 50 percent mortality occurs later.
5. With addition of protein skimmer, delayed mortality begins later.

6. When fish are fed more, delayed mortality is less and number surviving is greater.

7. With increased exposure to 50 percent seawater, initial mortality is less and delayed mortality begins later.

8. With greater light intensity, 50 percent mortality is reached later, delayed mortality begins later, and a greater percentage survives.

9. With less variation in light, a greater percentage survives.

Other correlations are discussed below in the context of factors.

Orthogonal matrix rotation was employed in this analysis (Rummel, 1970). Factors with eigenvalues equal to or greater than 1.000 were rotated. For laboratory variables, 12 factors were rotated, accounting for 82 percent of the total variance. The orthogonally rotated factor matrix is in Table 25. Some of the 12 factors are not biologically interpretable, relating to variation between cases, i.e., to variation in experimental design from beginning to end of the experiments. Factor 1, for example, relating current, light, total wet weight of fish, and total days in experiment (factor loadings greater than 0.400), is a consequence of modification throughout experiments. In initial experiments, there was no current, less light, fewer fish, and more days. Other factors resulting from variation in experimental or statistical design are Factors 5, 6, 10, 11, and 12.

A summary of biologically significant factors (from Table 25) is given in Table 26. Only variables with factor loadings greater than 0.400 are included, arranged in order from high to low factor loadings. For each factor, the independent and dependent variables are separated. The sign of factor loading gives the direction of the relationship (positive or negative).

In Table 27 the dependent variables are expressed as functions of factors (related independent variables) using the component factor analysis model. Factor 2, the Seasonal Factor, relates handling, baiting, oxygen, month, and mean wet weight. Factor 2 may be interpreted as follows: baiting is a function of month--night baiting is less successful during spring months; bait handling is a function of time of bait capture--night bait is handled less than day bait; mean wet weight of nehu is also a function of time of bait capture--night bait is larger; and finally, a high concentration of oxygen is a function of handling--increased handling requires administering more oxygen. Factor 3, the Temperature Factor, shows temperature as a function of month. Factor 4 is the Other Fish Factor; Factor 7, the Oxygen Factor; Factor 8, the Salinity Factor; and Factor 9, the Predation Factor. Factor 12 is omitted from the following interpretation because it appears to have no biological meaning.

Initial mortality (%IM) shows significant loadings with Factors 2 and 9, the Seasonal and Predation Factors (Table 27). With increased handling, day bait, decreased mean wet weight, high oxygen, and more lae (*Scomberoides lysan*), initial mortality is greater.

The day 50 percent mortality (Day 50%) is reached relates to the Other Fish Factor 4 (Table 27). Other fish in this case are schooling fish, primarily *Pranesus insularum*. Their presence appears to influence survival of *S. purpureus* with 50 percent mortality occurring later and with greater survival. A possible interpretation is that *P. insularum* are often calmer in captivity, mill earlier, and may behaviorally influence nehu survival by promoting earlier milling, thus reducing stress.

The time that delayed mortality begins (XI-DM) relates to Temperature Factor 3 and Predation Factor 9 (Table 27). During summer months when the

temperature increases and there is an increase in the number of lae, delayed mortality begins earlier.

The total number of days over which delayed mortality occurs (Tot X-DM) relates to Salinity Factor 8 (Table 27); the higher the salinity, the greater the duration of delayed mortality.

The midpoint of delayed mortality (A/R) relates to Oxygen Factor 7; with critical to lethal levels of oxygen, the midpoint occurs at a higher mortality.

As mentioned above, total mortality (Tot Y-DM) and percentage of fish surviving (% Survival) relate to the Other Fish Factor.

Tables 28 to 32 contain results of component factor analysis of barge data. Tables are comparable with those for laboratory data discussed above. Table 28 includes means, standard deviations, coefficients of variation, and communalities for 31 barge variables. As for laboratory data, coefficients of variation indicate that duration and extent of delayed mortality were relatively invariable among different barge experiments. The total data variance accounted for by the factors (communality) is 92 percent.

The correlation matrix for 31 barge variables is presented in Table 29. Correlations of biological significance not included in the discussions of factors are as follows:

1. In later months--summer and fall--more lae occur.
2. With day bait, a greater duration of delayed mortality occurs.
3. With increase in hours of recirculated 50 percent seawater, a greater percentage of fish survive.
4. With increased exposure to 50 percent seawater, a greater percentage of fish survive.

For barge variables, 9 factors were rotated, accounting for 92 percent of the total variance. The orthogonally rotated factor matrix is in Table 30.

As in the laboratory analysis, some factors are not biologically interpretable, resulting from modification of experimental design. These are Factors 2, 4, 6, 8, and 9.

A summary of biologically significant factors from barge data (Table 30) is in Table 31, arranged in order from high to low factor loadings and with independent and dependent variables separated.

In Table 32, the dependent variables are expressed as functions of factors (related independent environmental variables). Factor 2, the Mixed Factor, associates maximum temperature, mean wet weight, lae, and mean salinity during the experimental period. Mean wet weight is a function of maximum temperature and mean salinity; during summer months, mean wet weight is less and in experiments with reduced salinity, fish do not die as rapidly, resulting in lower mean wet weight. Lae are a function of maximum temperature; during the higher temperatures of summer months more lae occur.

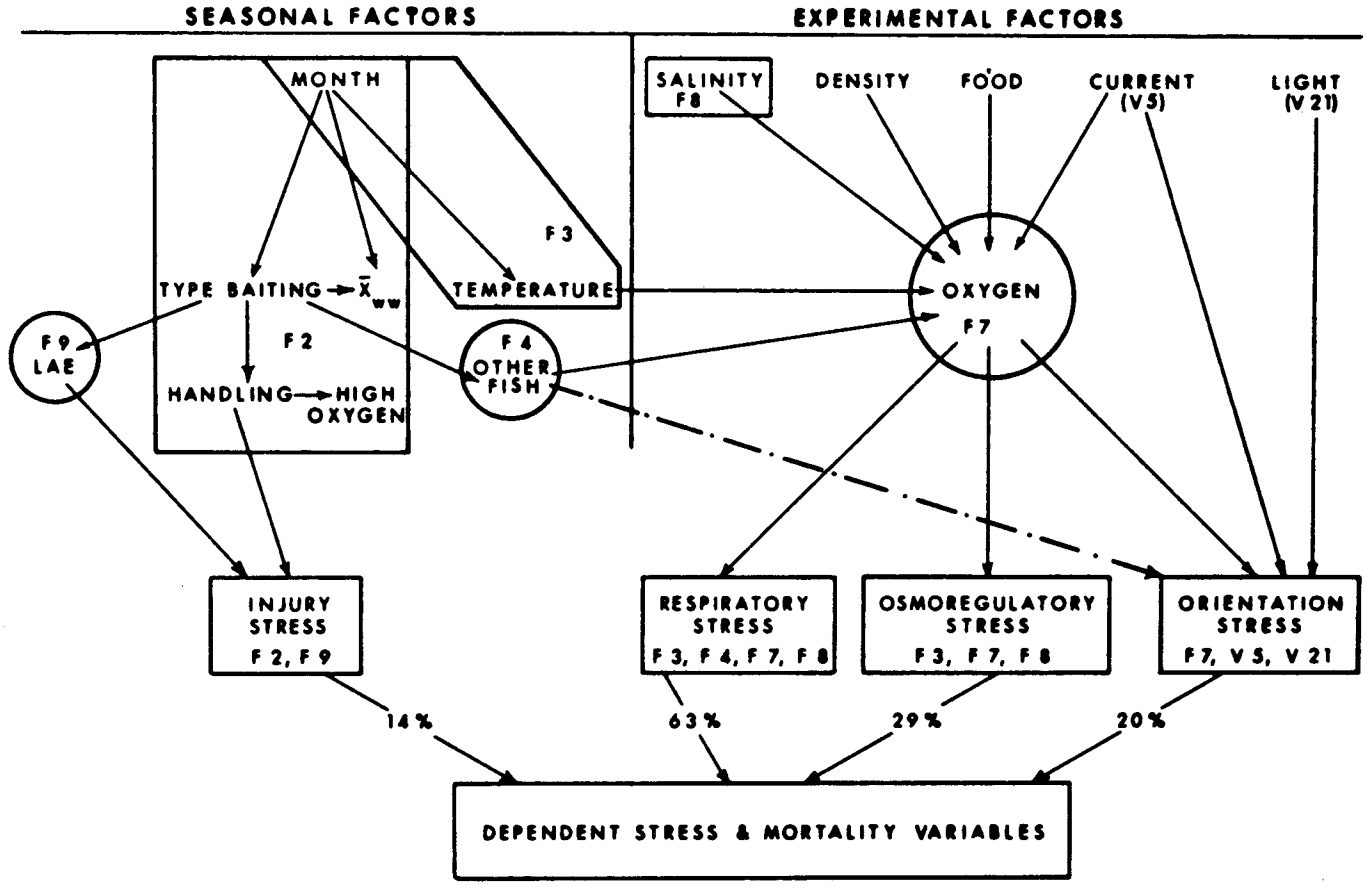
Factor 7 is the Current Factor. Initial mortality shows significant loading with the Current Factor; with current added, initial mortality is less. The remaining dependent variables show significant loadings with the Mixed Factor; the day that 50 percent mortality is reached is later, the time that delayed mortality begins (XI-DM) is later, the total duration of delayed mortality (Tot X-DM) is less, the total mortality (Tot Y-DM) is less, the midpoint of delayed mortality (A/R) is later, and survival is greater with lower maximum temperature, fewer lae, lower mean salinity during experimental period, and greater mean wet weight.

A comparison of factor results for laboratory and barge data is difficult since the number of cases varied greatly between the two locations (184 cases in the laboratory vs 28 cases on the barge). Temperature appears to increase stress and mortality significantly at both locations.

Other variables similarly affected dependent stress variables in the laboratory and on the barge (see correlation matrices, Tables 24 and 29). Differences in degree of stress and mortality between locations are largely a result of different experimental situations, particularly volume of water and circulation rate (both greater in barge wells). Generally, fish survived better in barge wells. The amount of variance accounted for by the rotated factors was greater for barge data (92 percent; Table 28) than laboratory data (82 percent; Table 23).

Further interpretation of factor results could take the form of step-like multiple regressions of significantly related variables within factors. However, since much of the data is non-parametric, further regression analysis appears unjustified.

Figure 37 exhibits a final interpretation of independent variables affecting stress and mortality in terms of causal relationships. Factors and variables are divided into two categories. Seasonal factors ensue from monthly variation in time of bait capture and temperature and experimental factors from experimental design; for example, whether or not current is added or salinity reduced. Factors and variables are then connected by arrows in a hypothetical causal nexus. The strength and direction of the relationships may be determined by reference to the matrix of correlation coefficients in Table 24. Factors and variables are classified into effects on four major types of stress: injury, respiratory, osmoregulatory, and orientation. The relative contribution of each type to total stress and mortality (measured by dependent variables) is shown. Percentage of total variance among dependent mortality variables accounted for by injury is approximately 14 percent; by respiratory stress, 63 percent; by osmoregulatory stress, 29 percent; and by



Factors (related variables) are enclosed.
Percentage of total variance among dependent variables accounted for by factors and variables in shown for four types of stress.
Dashed line indicates an unclear relationship.

Figure 37. Summary diagram showing hypothesized causal relationships between significant independent variables.

orientation stress, 20 percent. A comprehensive partitioning of variance due to factors in the four stress categories is shown in Table 33. A similar figure for barge experiments is omitted because the effect of environmental variables on *S. purpureus* in barge wells is approximately the same as in laboratory tanks.

DISCUSSION

For brevity, discussion of other work relating to individual variables was included under the appropriate variables in the "results and conclusions" section. Interaction of variables was discussed under the "final data analysis" results. Further discussion on the definition and significance of stress follows.

In this paper stress is considered in terms of the effect of environmental stress variables on mortality of *S. purpureus* in captivity. The effect of stress on morphological and functional changes of the endocrine system was not studied, although this would be of interest for future research. The General Adaptation Syndrome (GAS) as defined by Selye (1950) applies in a general way to results of this study in that mortality curves of *S. purpureus* show characteristics in common with the GAS curve. As described by Selye (1950) there is a period of shock with lowered resistance (corresponding to initial mortality in this study); a period of countershock entering into a stage of resistance (early period of no mortality); and finally a stage of exhaustion with decreased resistance (corresponding to the period of a delayed increase in mortality). According to Selye, this syndrome is accompanied in vertebrates by simultaneous changes in the endocrine system which is associated with adaptation to stress conditions. Essentially, the reaction pattern is a failure of part

or all of the captive population to adapt to stress. A pattern of negative feedback occurs in which fish initially respond to stress by adapting, but ultimately succumbing. The underlying physiological-hormonal mechanism of the syndrome is not clearly understood in fish.

A definition of stress applicable to this research is that of Brett (1958):

> "...stress is a state produced by an environmental or other factor which extends the adaptive responses of an animal beyond the normal range, or which disturbs the normal functioning to such an extent that, in either case, the chances of survival are significantly reduced. By this definition the magnitude of the stress then becomes possible of quantitative expression by an estimate of the chances of survival (where actual losses can be recorded) or by a measure of reduction in capacity of normal performance."

A systematization of factors into types of stress produced is also given by Brett (1958). To avoid confusion with this analysis in which variables are grouped into factors, it should be made clear that the environmental variables of this study correspond to Brett's factors. The major distinction made by Brett is between discriminate and indiscriminate stress. Discriminate stress is defined as "one which applies at any one time to individuals, singly within a population and not to a group or stock as a whole." Indiscriminate stress is defined as "one which applies to every member and is not discrete in its action." Further distinction is made in four other categories which are defined below with a listing of effective variables from the results given under each category: D = discriminate stress and I = indiscriminate stress.

1. Lethal stress: "The extreme effect of any stress is to destroy the organism."
   a. Handling injury--D
   b. Lethal oxygen level--I
   c. Lethal metabolite levels, i.e., ammonia, carbon dioxide (usually in closed systems)--I
   d. Predation by *Scomberoides lysan*--I

2. Limiting stress: "These are factors which affect the supply of essential metabolites or interfere with the chain of energy release."

   a. Oxygen below saturation, but not at critical level--I

   b. High density (related to oxygen availability)--I

   c. Insufficient food--I

3. Inhibiting stress: "Inhibiting factors reduce the ability of the organism to carry out normal functions and, insofar as this introduces significantly lowered chances of survival, a stress is imposed."

   a. Poor orientation and visual environment (low light intensity, wrong tank color, no current)--I

   b. Parasitism of gills by *Cryptocaryon* sp.--I

   c. Organic particles adhering to gills, e.g., mucus, scales, etc.--I

4. Loading stress: "Any environmental factor which places an undue burden on an organism necessitating the rapid or steady release of energy invokes a stress."

   a. High temperature--I

   b. High salinity, injured integument, shock--I

Under extreme environmental conditions, classification of these variables may change. For example, under poor capture conditions variables listed under limiting, inhibiting, or loading stress may produce lethal stress. The above classification pertains to the capture environment most commonly encountered on fishing vessels.

In most experiments (with the exception of those with optimal environment), approximately 75 percent of the nehu die and 25 percent survive. This survival may correspond to the percentage of older fish in the captive population (mean wet weight approximately 0.7 gm or more), as older fish are less susceptible to stress. Genetic variation is another probable factor of susceptibility to stress.

SUMMARY

With a maximum reduction in handling and the addition of improvements to bait wells, mortality of nehu was reduced from approximately 75 percent to only 10 percent over a period of 14 days. The environmental factors and facility modifications that will give increased survival are summarized below.

1. Time of bait capture. Night bait should be used when possible; fish are usually larger and survive longer. Night capture is most successful in summer and fall, during first quarter-moon phase, and in good weather.

2. Handling. Transfer bait by swimming whenever possible. Avoid bucketing and do not overcrowd buckets.

3. Salinity. Maintain 50 percent seawater (approximately 16.0 to 18.0 ppt) for as long as possible, especially during the first 3 hours after capture. Open system circulation is preferable. In closed systems, recirculate brackish water for no more than 3 hours at a density not to exceed approximately 200 gm per 100 liters (1.6 to 1.8 lb per 100 gal; 8/10 to 9/10 buckets per 100 gal). The pH should not decrease below 7.5.

4. Oxygen. Use supplementary oxygen; concentration should not exceed 10.0 ppm or fall below 5.0 ppm. Ideally, oxygen concentration should be kept at saturation level.

5. Temperature. A range of from 21° to 24°C appears optimal. Higher temperatures should be avoided if possible.

6. Current. Provide horizontal current at approximately 0.10 m/sec (15 to 20 ft/min); not to exceed 0.13 m/sec (25 ft/min). Decrease current speed for smaller or injured fish to minimum required for orientation

on Day 1. Maintain current at least until period of delayed mortality is over.

7. Light. Provide white or green light of at least 1,080 lumens/m (100 footcandles at surface) and of uniform intensity over surface. Higher light intensities may give even better results. Light should also be provided at night.

8. Tank color. Green tanks appear best. Green should approximate Turquoise Green (#25, #75 in *Webster's New International Dictionary of the English Language*, 2nd ed., 1961). Contrasting visual cues on sides of tank may prove helpful. Avoid dark colors, especially black.

9. Tank shape. Circular or rectangular with rounded corners will promote a more uniform, less turbulent current. A central bottom drain for removal of dead should be added.

10. Circulation, flow rate in open system. The maximum flow rate possible without exceeding optimal current speed should be maintained. A turnover rate of at least 50 to 60 percent per hour is optimal; turnover rate should be not less than 40 percent per hour.

11. Recirculation in closed system. Fifty percent seawater should be recirculated for no more than 3 hours at a density of not more than 200 gm per 100 liters (1.6 to 1.8 lb per 100 gal; 8/10 to 9/10 buckets per 100 gal) unless a filtration system is added to remove ammonia and a Trizma buffer is used to sustain pH at about 8.0. Add protein skimmer to remove organic particles, mucus, and scales.

12. pH. Maintain pH at about 8.0 to 8.1. Do not allow pH to fall below 7.6 since a pH of 7.5 is the approximate lethal level. The addition of current may decrease dissolved carbon dioxide and increase pH. Trizma buffer may be used to sustain pH.

13. Density. In an open system, density should not exceed approximately 260 gm per 100 liters (2.2 lb per 100 gal; 1/2 to 1 bucket per 100 gal) unless the flow rate is substantially increased over a minimum turnover rate of 25 percent per hour. In a closed system density should not exceed 200 gm per 100 liters (1.6 to 1.8 lb per 100 gal; 8/10 to 9/10 buckets per 100 gal).

14. Food. Food should not be provided if nehu are to be maintained for short periods. Starvation mortality begins about 10 days after capture. For longer periods in captivity, feed wet weight of brine shrimp (San Francisco frozen brine shrimp) amounting to approximately 36 percent per gram wet weight of nehu, or dry weight of brine shrimp amounting to approximately 3 to 5 percent per gram wet weight of nehu. Begin feeding on Day 3 and feed 3 times daily or more.

15. Other fishes. Remove large predators captured with nehu. Remove lae as described in the "results" section. Leave other schooling fish in the bait wells, as they appear to increase the survival of nehu.

16. Disease. Fifty percent seawater (16.0 to 18.0 ppt) effectively eliminates *Cryptocaryon* sp. cysts from gills of nehu in about one week. To reduce possible bacterial stress, all dead nehu, debris, and excess food should be removed twice a day.

17. Bait well design. The above improvements have been incorporated into a new bait well design (Baldwin, 1973). A brochure for fishermen outlining procedures and improvements was prepared by Baldwin et al. (1972).

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REFERENCES CITED

Alderdice, D.F., J.R. Brett, and D.B. Sutherland. 1966. Design of a small holding tank for fish. *J. Fish. Res. Bd. Can.* 23(9):1447-1450.

Au, D.W. 1965. Survey of the distribution of the eggs and larvae of the nehu *(Stolephorus purpureus)* in Pearl Harbor, Hawaii. M.S. Thesis, University of Hawaii, Honolulu.

Bachman, R. 1963. Fluctuations and trends in the abundance of nehu *(Stolephorus purpureus* Fowler*)* as determined from catch statistics. M.S. Thesis, University of Hawaii, Honolulu.

Baldwin, W.J. 1970. Oxygenating device for live-bait wells. *J. Fish. Res. Bd. Can.* 27:1172-1174.

Baldwin, W.J. 1973. Baitfish can be kept alive longer on Hawaiian skipjack tuna boats. *National Fisherman* 54(6):6-7,9, Section C.

Baldwin, W.J., J.W. Struhsaker, and G. Akiyama. 1972. *Longer life for nehu.* Translated into Japanese by Seiyei Wakukawa. UNIHI-SEAGRANT-MS-71-01A. Sea Grant Program, University of Hawaii, Honolulu.

Brett, J.R. 1958. Implications and assessments of environmental stress. In: *The investigation of fish-power problems.* H.R. MacMillan Lectures in Fisheries, ed. P. Larkin. Vancouver: University of British Columbia. pp. 69-83.

Brock, V.E., and M. Takata. 1955. *Contribution to the problems of baitfish capture and mortality, together with experiments in the use of Tilapia as live bait.* Grant No. 49. Indust. Res. Advis. Coun., Hawaii.

Brock, V.E., and R.N. Uchida. 1968. *Some operational aspects of the Hawaiian live-bait fishery for skipjack tuna (Katsuwonus pelamis).* Spec. Sci. Rep. Fish. 574. U.S. Fish and Wildlife Service.

Burdick, J.E. 1969. The feeding habits of nehu (Hawaiian anchovy) larvae. M.S. Thesis, University of Hawaii, Honolulu.

Dixon, W.J., ed. 1970. *BMD Biomedical Computer Programs.* University of California Publ. in Automatic Computation No. 2. Berkeley: University of California Press.

Gosline, W.A., and V.E. Brock. 1960. *Handbook of Hawaiian Fishes.* Honolulu: University of Hawaii Press.

Halstead, B.W., D.D. Danielson, W.J. Baldwin, and P.C. Engen. 1972. Morphology of the venom apparatus of the leatherback fish *Scomberoides sancti petri* (Cuvier). *Toxicon* 10:249-258.

Hiatt, R.W. 1951. Food and feeding habits of the nehu *Stolephorus purpureus* Fowler. *Pac. Sci.* 5(4):347-457.

June, F.C. 1951. Preliminary fisheries survey of the Hawaiian-Line Islands area. Part III--the live-bait skipjack fishery of the Hawaiian Islands. *Commer. Fish. Rev.* 13:1-18.

Loukashkin, A.S., and N. Grant. 1965. Behavior and natural reactions of the northern anchovy, *Engraulis mordax* Girard, under the influence of light of different wave lengths and intensities and total darkness. *Proc. Calif. Acad. Sci.* 31(24):631-692.

Major, P.F. 1973. Scale feeding behavior of the leatherjacket, *Scomberoides lysan* and two species of the genus Oligoplites (pisces: Carangidae). *Copeia* 1:151-154.

Maginniss, L.A. 1970. Osmotic and ionic regulation of the Hawaiian anchovy, *Stolephorus purpureus*. M.S. Thesis, University of Hawaii, Honolulu.

McFarland, W.N., and K.S. Norris. 1961. The use of amine buffers in the transportation of fishes. *Ann. N.Y. Acad. Sci.* 92:446-456.

Nakamura, E.L. 1965. *Synopsis of biological data on Hawaiian species of Stolephorus*. U.S. Bur. Comm. Fish., Biol. Lab., Honolulu.

Nigrelli, R.F., and G.D. Ruggieri. 1966. Enzootics in the New York Aquarium caused by *Cryptocaryon irritans* Brown 1951 *(Icthyopthirius marinus* Sakima, 1961), a histophagous ciliate in the skin, eyes and gills of marine fishes. *Zoologica* 51(9):97-102.

Preisendorfer, R.W. 1970. Calculus Lectures. Lecture 22: A Model of Biological Population Growth. Naval Postgraduate School, Monterey, California. pp. 212-219.

Pritchard, A. 1955. *Oxygen requirements of some Hawaiian tuna baitfish.* Spec. Sci. Rep. No. 146. U.S. Fish and Wildlife Service. pp. 1-30.

Prosser, C.L., and F.A. Brown, Jr. 1961. *Comparative Animal Physiology.* 2nd ed. Philadelphia: W.B. Saunders Co.

Rummel, R.J. 1970. *Applied Factor Analysis*. Evanston: Northwestern University Press.

Selye, H. 1950. Stress and the general adaptation syndrome. *Brit. Med. J.* 1:1383-1392.

Sokal, R.R., and F.J. Rohlf. 1969. *Biometry: the principles and practice of statistics in biological research.* San Francisco: W.H. Freeman and Co.

Strickland, J.D.H., and T.R. Parsons. 1968. *A Practical Handbook of Seawater Analysis*. Fish. Res. Bd. Can. Bull. 167.

Struhsaker, J.W., D.Y. Hashimoto, S.M. Girard, F.T. Prior, and T.D. Cooney. 1973. Effect of antibiotics on survival of carangid fish larvae *(Caranx mate)* reared in the laboratory. *Aquaculture* 2(1):53-88.

Tester, A.L. 1951. The distribution of eggs and larvae of the anchovy, *Stolephorus purpureus* Fowler, in Kaneohe Bay, Oahu, with a consideration of the sampling problem. *Pac. Sci.* 5(4):321-346.

Tester, A.L. 1955. Variation in egg and larva production of the anchovy, *Stolephorus purpureus* Fowler, in Kaneohe Bay, Oahu, during 1950-1952. *Pac. Sci.* 9(1):31-41.

Tyler, J.E. 1971. Book Review of: Kinne, O., ed. 1970, *Marine Ecology*, v. 1; Environmental factors, part 1. *Limnol. & Oceangr.* 16(5):841-842.

[U.S.] Bureau of Commercial Fisheries. 1969. *A separate baitfish facility in Kaneohe Bay for the Hawaiian skipjack tuna (aku) fishery.* Final Report. U.S. Bureau of Commercial Fisheries, Biological Laboratory, Honolulu.

Wilkie, D.W., and H. Gordin. 1969. Outbreak of cryptocaryoniasis in marine aquaria at Scripps Institution of Oceanography. *Calif. Fish & Game* 55:227-236.

Yamashita, D.T. 1951. The embryological and larval development of the nehu, an engraulid baitfish of the Hawaiian Islands. M.S. Thesis, University of Hawaii, Honolulu.

APPENDIX

CAPTIONS FOR TEXT TABLES

Table

1 Night baiting, factor analysis; correlation matrix

2 Night baiting, factor analysis; factor matrix

3 Analysis of variance between buckets of nehu captured at different seasons

4 Analysis of variance between buckets of nehu captured at different moon phases

5 Summary of standard length, wet weight, dry weight, and regressions of wet and dry weight on standard length

6 Differential mortality of size

7 Summary of initial mortality in laboratory tanks with 50 percent as opposed to 100 percent seawater

8 Summary of selected experiments with reduced salinity; open system

9 Recovery of body water in nehu by Day 14; different oxygen levels

10 Approximate rates of oxygen consumption of *Stolephorus purpureus* under different experimental conditions

11 Estimated total oxygen consumed by an average bucket of nehu at different rates of oxygen consumption and mean wet weights of fish

12 Mean number of days to 50 percent mortality; maximum and minimum temperature ranges

13 Summary of laboratory experiments with 50 percent recirculated seawater; closed system

14 Maximum concentrations of dissolved carbon dioxide present under different experimental conditions

15 Minimum densities of nehu estimated appropriate for varying environmental conditions in captivity

16 Summary of amount of food given daily

17 Grams dry weight per gram wet weight of food

18 Summary of food experiments

19 Effect of lae on mortality of nehu

Table

20 List of variables measured in laboratory experiments included in factor analysis

21 List of variables measured in barge experiments included in factor analysis

22 Derivation of first, second and third derivatives of logistic equation in form of hyperbolic tangent function

23 Factor analysis, laboratory experiments; means, standard deviations, coefficients of variation and communalities

24 Factor analysis, laboratory experiments; correlation matrix

25 Factor analysis, laboratory experiments; factor matrix

26 Factor analysis, laboratory experiments; summary of factors

27 Factor analysis, laboratory experiments; dependent variables as functions of factors

28 Factor analysis, barge experiments; means, standard deviations, coefficients of variation and communalities

29 Factor analysis, barge experiments; correlation matrix

30 Factor analysis, barge experiments; factor matrix

31 Factor analysis, barge experiments; summary of factors

32 Factor analysis, barge experiments; dependent variables as functions of factors

33 Percentage of variances of dependent variables accounted for by factors affecting four categories of stress

34 List of fish species captured during night and day baiting operations

35 Oxygen line pressure necessary to provide sufficient oxygen for nehu consuming from 100 - 170 cc oxygen/hr at different concentrations in buckets

36 Flow rate necessary to provide 1,270 cc oxygen/hr for one bucket of nehu, no supplemental oxygen