Marine Studies of San Pedro Bay, California

PART III

THERMAL TOLERANCE AND SEDIMENT TOXICITY STUDIES

edited by
Dorothy F. Soule
and
Mikihiko Oguri

Published by
The Allan Hancock Foundation
Harbors Environmental Projects
and
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University of Southern California
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Volume III

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The USC Marine Facility, Berth 186, Los Angeles Harbor, home of the Espoir, Golden West, and Velero IV, photograph by John D. Soule

MARINE STUDIES OF SAN PEDRO BAY, CALIFORNIA. PART III.

THERMAL EFFECTS AND SAN PEDRO BAY

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ABSTRACT. A summary of thermal effects research is presented in relationship to local considerations of a proposed cold water effluent from a liquid natural gas conversion plant. Criteria for evaluating effects are discussed. Local, regional, and Pacific west coast current patterns and thermal regimes are compared, and the potential for recolonization by cool-temperate biota is examined. The proposed cold water effluent might mimic areas of up-welling along the coast of southern and Baja California, where such biota have been recorded.

ACKNOWLEDGMENTS. Support for field and laboratory investigations has been supplied to Harbors Environmental Projects of the Allan Hancock Foundation by the Office of Sea Grant Programs, and by contracts with Pacific Lighting Service Company, the Los Angeles Harbor Department, the Tuna Research Foundation, and the U.S. Army Corps of Engineers. The many individuals involved in the multidisciplinary effort of Harbors Projects have made it productive in serving the community needs for scientific information. Their enthusiastic participation is greatly appreciated.

THERMAL EFFECTS AND SAN PEDRO BAY

INTRODUCTION. The tolerances of plants and animals to temperature extremes have been documented by various investigators in the past, but the great predominance of the reports in the literature deal with high temperature extremes rather than low extremes. Altman and Dittmer (1966) summarized literature dealing with aquatic invertebrates, in 256 entries of published thermal limits data taken from 53 citations dating from 1887. Of these, only 20 of the 256 entries dealt with low temperature extremes. There were 434 entries regarding fishes, of which 131 were involved with low temperature extremes. Coutant and Goodyear (1972) reviewed current literature on thermal tolerance, covering 92 citations in the 1969-1971 period; of these only 14 dealt with cold tolerance. However, their references on thermal effects of all sorts numbered 394, indicating the increased and widespread interest in various phases of the topic. Kinne's volume on marine ecology (1971) is extensively devoted to thermal effects.

In 1973, Coutant and Pfuderer reviewed a total of 428 recent references on thermal effects. They reported that there was a growing trend in the approach by various investigators attempting to determine the temperature criteria of ecologically important species in a given area (Jensen and Brady, 1971). Such information could be applied to the management of thermal wastes, while protecting significant species in the local ecosystem.

It was a similar approach that led Allan Hancock Foundation investigators to initiate, in 1972, studies on the possible effects which might be induced by lowered temperatures on important local harbor species in the event that a proposed liquid natural gas conversion plant is built. These are reported by Brewer, Norse, Oshida and Reish, and Hadley and Straughan in the present volume of Marine Studies of San Pedro Bay, California, Part III (1974). Although only a few of the harbor organisms could be studied with the resources available, the information obtained should be helpful in determining temperature criteria for the area of influence of the proposed low temperature plume.

Consideration should be given to the annual reproductive cycles of significant organisms and also to their food supply, although much of this information is still poorly known. Attention must be given to the relatively more severe effects of thermal shock, caused by intermittent or sudden thermal alterations of the environment on unacclimated populations, as opposed to long-term changes to which organisms might become acclimatized by gradual change.

THERMAL CRITERIA. There are three general criteria that may be considered in evaluating the state of the biota. The first is survival, without growth or reproduction, which usually includes the widest range of temperature which the organism can tolerate. The second is survival, with growth (activity), but without reproduction; this range of thermal accomodation is somewhat narrower. The third is survival, along with growth (activity) and with reproduction. This is the most important criterion of environmental quality and is the most limiting in range of temperature. Brewer (1974) discussed the rate of anchovy jaw development, which must be related to the amount of yolk stored, or juveniles will starve before they are able to feed. Both Norse (1974) and Hadley and Straughan (1974) have shown the importance of activity, which was equated with growth as a single criterion, for if the species investigated were not able to maintain their normal activities, the result was death due to ecological dislocation. Possibly growth and activity should be considered as separate criteria in view of the new importance thus attached to activity.

Although the foregoing types of thermal requirements are clearly defined, the temperature ranges at which they occur are not absolute within a single species. Various investigators have shown that different populations of the same nominal species are in fact genetically different in their ability to adjust to specific temperature ranges (Reish, 1964; Sastry, 1970; Kinney, 1969; Loukashkin, 1970; Loosanoff, 1969; Pearce, 1969). Furthermore, individuals in a single population can adapt to differing thermal regimes if they are subjected to differing acclimation temperatures, as shown in the present volume, Vernberg and Vernberg (1969), Caldwell and Vernberg (1970), and others. Animals may also differ in their responses at different stages in their life cycles, according to the Vernbergs and other authors (Moore and Reish, 1969; Bayne, 1973; Messersmith *et al*, 1969; Wood and Strachen, 1970). It is also evident that there is a distinct difference between the results of sudden thermal changes in the environment (thermal shock) and the results of gradual changes in temperature. Generally, it has been shown that a gradual change widens the range of thermal tolerance considerably.

Prosser was among the first to associate temperature with enzyme function (see Prosser, 1967). Coutant and Goodyear (1972) reviewed a number of papers that bear this out and indicate other factors involved. For example, in fishes, the effects of cold shock caused disruption of the ability to osmoregulate. The primary locus for acclimatory response was reported in another citation to be at the synapse in the nervous system. Coutant and Pfuderer (1973) added other citations related to further research on biochemical effects of temperature. Physical-chemical factors controlling the solubility of calcium carbonate correlate with the increased calcium carbonate utilization by gastropods in shell deposition in colder waters. In corals, however, warmer water favored carbonate deposition. Ion changes correlated with temperature involved sodium, chloride, magnesium, potassium, as well as trace and heavy metals. A number of investigations derived optimimum temperatures and ranges for various enzymes and noted changes in fatty acid and amino acid composition. Alteration of enzyme configurations may account for temperature acclimation.

Since invertebrates and many vertebrates are not able to regulate body temperature or have limited ranges of regulation, activity is closely related to temperature. In some cases, the relationship seems to lie with the direction of change, rather than the actual temperature. Periodicities such as light and tide also combine in some cases with temperature in affecting behavior, especially reproductive or migratory patterns. Torpid animals cannot, of course, avoid introduced environmental hazards. There are synergistic effects from temperature stress when combined with other stresses, especially for organisms occurring in harbors and estuaries, where high levels of pollutants, low oxygen, or fluctuations in salinity may occur. A growing body of data indicates that the ability of an organism to tolerate a given stress is decreased if it is already subjected to another stress (Kinne, 1963; Anderson and Reish, 1967; Brenko and Calabrese, 1969; Calabrese and Davis, 1970; Manzi, 1970; Hazel *et al*, 1971). The same may be said for organisms that are already existing at the extremes of their geographic (and hence thermal) range.

<u>CURRENT PATTERNS, TEMPERATURE, AND COLONIZATION</u>. Southern California waters vary from year to year, due in part to variation in the duration and extent of the warm water intrusion by the northward flowing Davidson undercurrent. Annual variations in wind, in rainfall, and in air temperature can

also affect the localized thermal patterns of the water. Since southern California is naturally arid, annual rainfall is limited and generally occurs in a few heavy rains during a short winter season.

The surface currents off the California coast south of Point Conception consist primarily of the southeastward flowing California Current and one or more slowly turning, counterclockwise gyres formed along its northeastern margin (Emery, 1960; Jones, 1971).

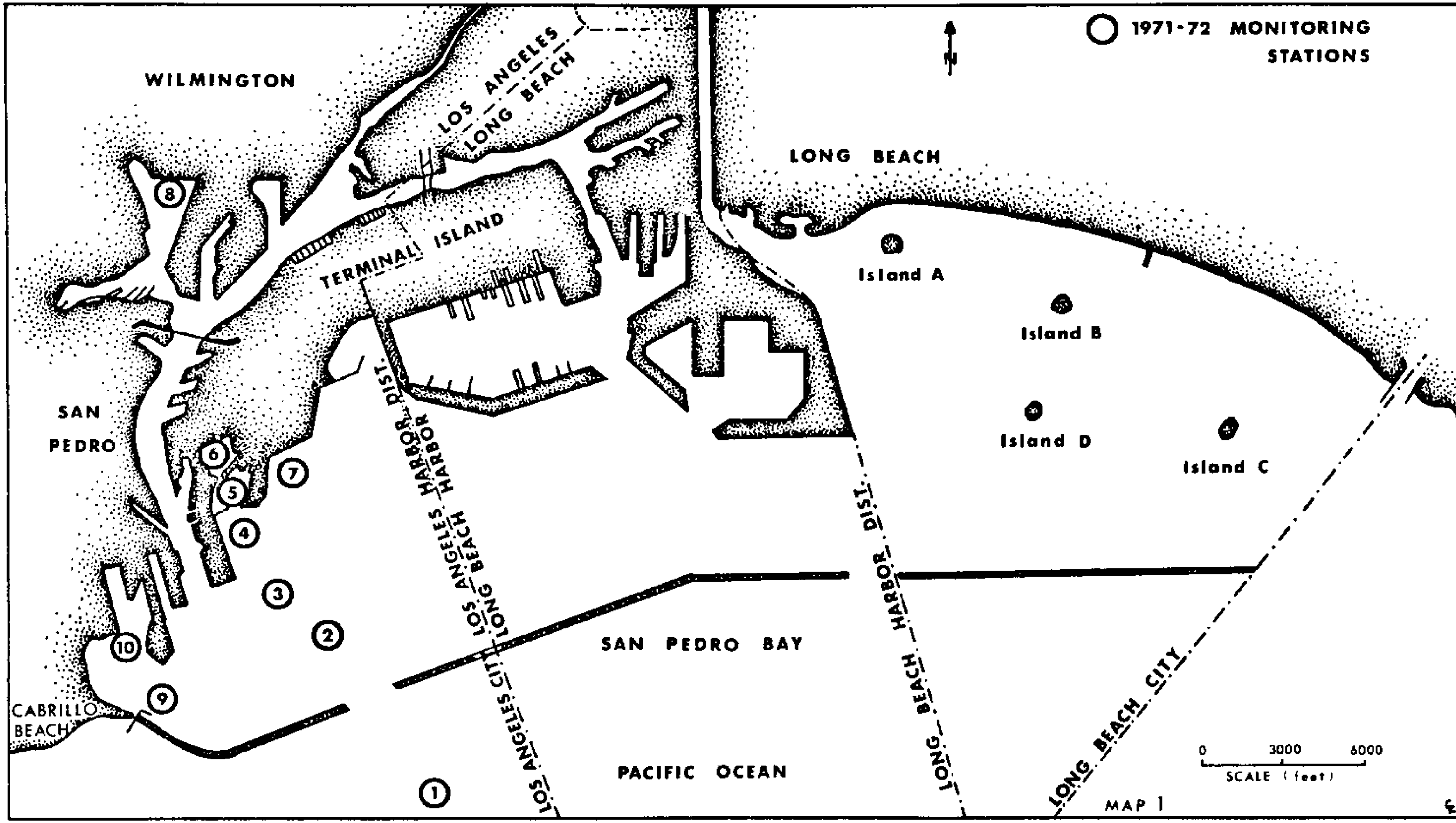
The northern gyre, located in the western end of the Santa Barbara Channel, is the most permanent of the three possible gyres. Water from this gyre can be found frequently along the northern coasts of the Channel Islands, which form the southeastern border of the channel. The eastern portion of the channel is dominated by water flowing into the channel past Hueneme in a northwestward direction and eddying into the northeastern section of the basin. These eddies often form a counter current flowing southeast along the coast.

To the south of the Channel Islands one and sometimes two counterclockwise gyres are apparent much of the time. The northernmost one of the two is centered roughly west of Catalina Island. This forms a large gyre which takes 10 to 20 days to make a half revolution. This results in a counter current flowing in a northwest direction and often extending into the coast. Other components of this gyre continue to revolve slowly or turn southeast down the coast.

The southernmost gyre, centered west of San Diego, may, in fact, be an extension of the one described above and appears more irregularly than either of the other two. In action it is similar to the others.

Water at intermediate depths is distinctly different from surface waters and has a southerly origin. At depths of 200 to 500 meters, the northern and southern waters are normally mixed, although failure of mixing has been noted. This is expressed by temperature inversions, which have been reported in the San Pedro and Santa Monica Basins. Subsurface currents in the near coastal region can often show vastly different directions and velocities compared to the currents at the surface.

Because of the variations in physical environmental parameters, the species composition of the harbor and shore communities may also differ from year to year. The predominant local species are probably more tolerant to a wider range of temperature than those species in communities located either


1971-72 MONITORING STATIONS
N
WILMINGTON
LOS ANGELES
LONG BEACH
LONG BEACH
TERMINAL ISLAND
SAN PEDRO
Island A
Island B
Island D
Island C
LOS ANGELES HARBOR DIST.
LONG BEACH HARBOR
LOS ANGELES CITY
LONG BEACH
LONG BEACH HARBOR DIST.
LONG BEACH CITY
SAN PEDRO BAY
PACIFIC OCEAN
CABRILLO BEACH
1
2
3
4
5
6
7
8
9
10
0
3000
6000
SCALE (feet)

MAP 1

in the cool-temperate northern California waters or the tropical and subtropical Mexican waters. Some species that flourish locally in warm years may be absent in cooler years and vice versa (Berner and Reid, 1961).

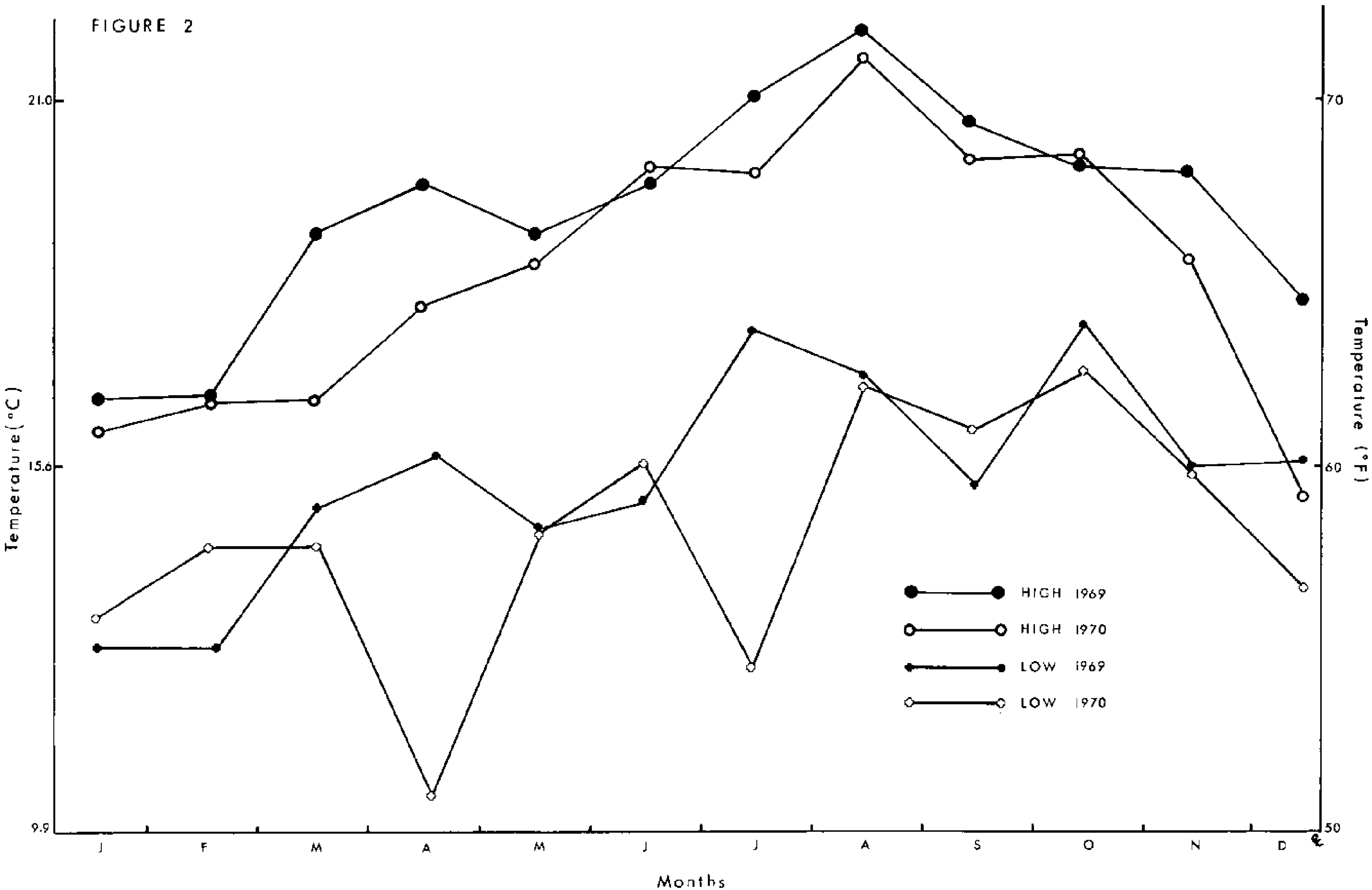
To determine the characteristics of local waters more precisely, seven stations in the outer Los Angeles Harbor (Map, Figure 1) have been monitored on a monthly basis by the Allan Hancock Foundation since June, 1971. The data and station locations are presented in a Data Report, Volume V of Marine Studies of San Pedro Bay. Temperature (in degrees Celsius and Farenheit), salinity, oxygen, and pH readings were taken at one meter increments to provide profiles with depth. Graphs of the high and low monthly temperatures at each of the stations show that there has been considerable variation in the seasonal cycles. At Station 5, for instance, the August 1972 temperature was almost 7° (F) lower than in August of 1971 and 4-6° (F) lower than in August 1973. For comparisons, temperatures in the inner harbor near the power plant outfall in West Basin are also presented.

Temperature readings taken by the Port of Los Angeles and the Department of Water and Power point up differences in annual seasonal temperatures. For example, the December high in 1970 was lower than the December low in 1969 (Figures 2, 3). Lows in April and August of 1970 were nearly 10° F below the lows for those months in the previous year.

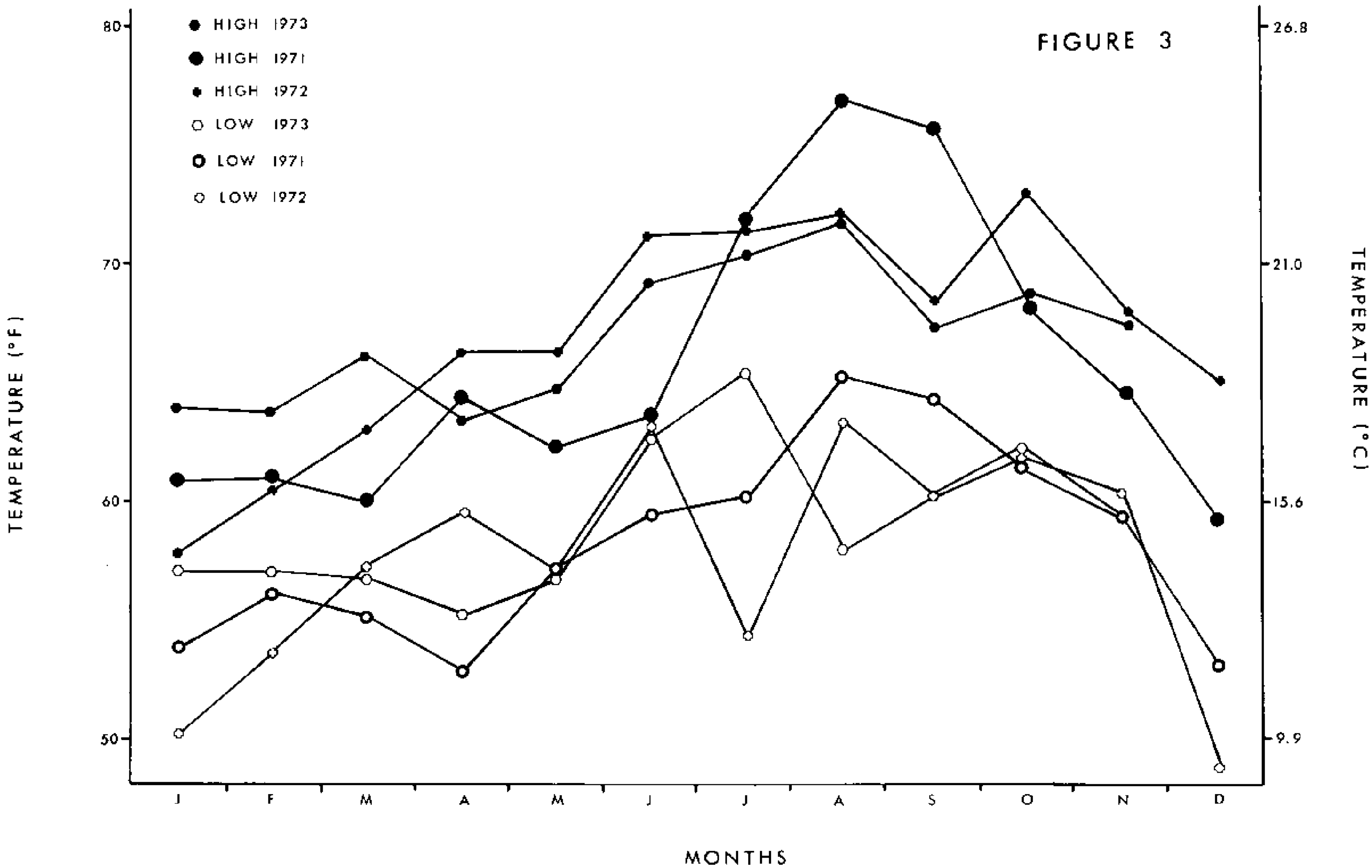
Thermal inversions have sometimes occurred in spring and fall due to rapid changes in surface water temperature, possibly initiating or at least enhancing, the phytoplankton blooms which commonly occur at those times. In the warmer years, patches of Red Tide or other phytoplankton populations may be found in the harbor area year around, especially in the vicinity of the thermally elevated power plant effluent plumes. Any stirring action, such as high winds, which disturbs bottom sediments can also cause a rise in biochemical oxygen demand when the organic sediments beneath the surface are exposed. The slower anaerobic bacterial action is replaced by aerobic bacterial action, speeding breakdown of the organic material. A phytoplankton bloom often seems to follow shortly.

Although the outer Los Angeles Harbor has a relatively low circulation (Soule and Oguri, 1972) there are differences in the thermal profiles at the various stations reported herein.

The lowest temperatures recorded in the June 1971 - November 1973 period reported were an 8.1° C (46.6° F) in December 1971 and an 8.2° C (46.8° F) in April of 1973, taken at the Sea Buoy, outside the entrance to Angel's Gate, Los Angeles Harbor. In December 1971, the low at Station 2 was



TEMPERATURE EXTREMES IN THE HARBOR AT ANY DEPTH OR LOCATION SAMPLED, 1969-1970 COMPARED



TEMPERATURE EXTREMES IN THE HARBOR AT ANY DEPTH OR LOCATION SAMPLED
1971 - 1973 COMPARED

8.3° C, Station 3 was 9.1° C, Station 4 was 10.3° C; Outer Fish Harbor Station 5 was 10.1° C, and Inner Fish Harbor was 10.9° C. At that time, the temperature was 11.5° C in West Basin. In April 1973, however, the lows in the harbor were higher; for example, 9.5° C at Station 2, 11.0° C at Station 3.

The possibility of recolonization of stressed areas or of colonization of newly exposed surfaces by differing biota is good in the harbor area because of the normal variation present in the local current structure and in annual water temperature cycles. With thermal effluents probably being added along the coast in the next few years, it may be that localized populations of tropical and subtropical species will eventually be found in warm water plumes, while populations of cool-temperate fauna will occur in the vicinity of cool water plumes. Similar conditions occur naturally in southern and Baja California, where up-welling of cold water occurs and a northern biota develops (Dawson, 1952; Emerson, 1956).

Eastern Pacific coastal waters are divided into faunal (biological) provinces based largely on temperature. Arctic-subarctic waters lie above the Puget Sound-San Juan Straits areas and tend to gyre in the area of the Gulf of Alaska.

From the San Juan Straits south, cool-temperate waters extend to the area between Monterey and Point Conception, carried by the current system. The plants and animals in this region are mostly different species from those found south of Point Conception. The greater proportion of these species are not able to survive in warmer water, although some species are able to acclimate to the wider range of temperatures.

The waters from Point Conception south are warm-temperate, extending down Baja California to the zone of transition into subtropical-tropical waters. Different species are found in each province, but a very few are common to all.

Within each province, different species occur in different areas, depending on the local temperatures, salinities, the kinds of substrates available, the strength of water flow, and suspended sediment. In the inshore areas, great differences in salinities and temperatures in harbors and estuaries limit the numbers of species present. There are relatively small numbers of species adapted to the very particular conditions in each small area; great numbers of individuals of a single or a few species may be found.

If the temperature of the proposed LNG plant effluent permanently reduces the local temperature so that the normal warm-temperate fauna will not survive, it will be necessary to depend on reseeding from northern waters with cool-temperate fauna.

On the basis of literature, it has been estimated that 70 to 80 per cent of the pelagic larvae (larvae carried floating in the currents) will settle out of the currents and either develop into adults or die in 6 to 7 weeks. Only about 5 per cent of the total larvae are so-called long-distance larvae, surviving from 13 to 26 weeks (Thorson, 1961). Based only on estimates of the speed of the southerly flow, it is possible for about 5 per cent of the most northern, Alaskan cold-water larvae to travel as far as southern California.

Gradual warming of a current causes more larvae to drop out of the current than gradual cooling. Some plankton are known to migrate vertically within a water mass so as to remain at a relatively uniform temperature.

It is of some importance to note that seasonal water temperature lows of about 45° to 46° F at the surface in Oregon are about equal to lows recorded in Monterey Bay at 33 feet in May and June (Sverdrup *et al*, 1942). Areas of cold water up-welling along the southern coast are also known where the fauna is more typical of northern areas than southern ones (Allan Hancock Foundation, 1965). This offers the possibility of cold water larvae entering the current system much closer to the southern California area than Washington and Oregon.

The only local southern area with lows similar to those in northern waters is from Las Pitas Point to Point Hueneme, where the temperature approaches 45° F at 200 feet.

Animals living in a range of 2-4° C (36-39° F) lows to 15° C (59° F) highs found off Washington State may not be able to survive transport in warmer waters or the higher summer temperatures in southern waters.

Minimum temperatures generally rise gradually from the mouth of the Columbia River south, although this varies from year to year. According to Scripps Institute of Oceanography reports in 1969, the Seaside, Oregon minimum was 5.4° C above Neah Bay, Washington, at the northern tip of the Olympic Peninsula. From Seaside to Crescent City, California, the minima were fairly uniform at about 7° C (45° F). Point Lobos, south of Monterey Bay, may be affected by up-welling (Sverdrup, 1942), since the minimum and maximum temperatures there are slightly below those of Pacific Grove to the north and Avila (Morro Bay) to the south. South of Morro Bay, the minima generally remain above 10° C (50° F), although lower temperatures of surface waters have been reported in other locations than those monitored by Scripps Institute of Oceanography.

In 1970 (SIO, 1971), the maximum temperatures were generally about 1° C lower, except at Seaside, Oregon, Avila, and Santa Monica. In 1971 (SIO, 1972), maxima were similar to 1969 or higher by 1-2° C.

However, in 1972, the minima were the same as or warmer than 1969 in Washington and Newport, Oregon, but colder at the Columbia River and all of the stations recorded south of Bodega Bay. Mean temperatures in 1970 and 1971 were generally 1-2° C lower than in 1969 (Figures 4, 5, and 6).

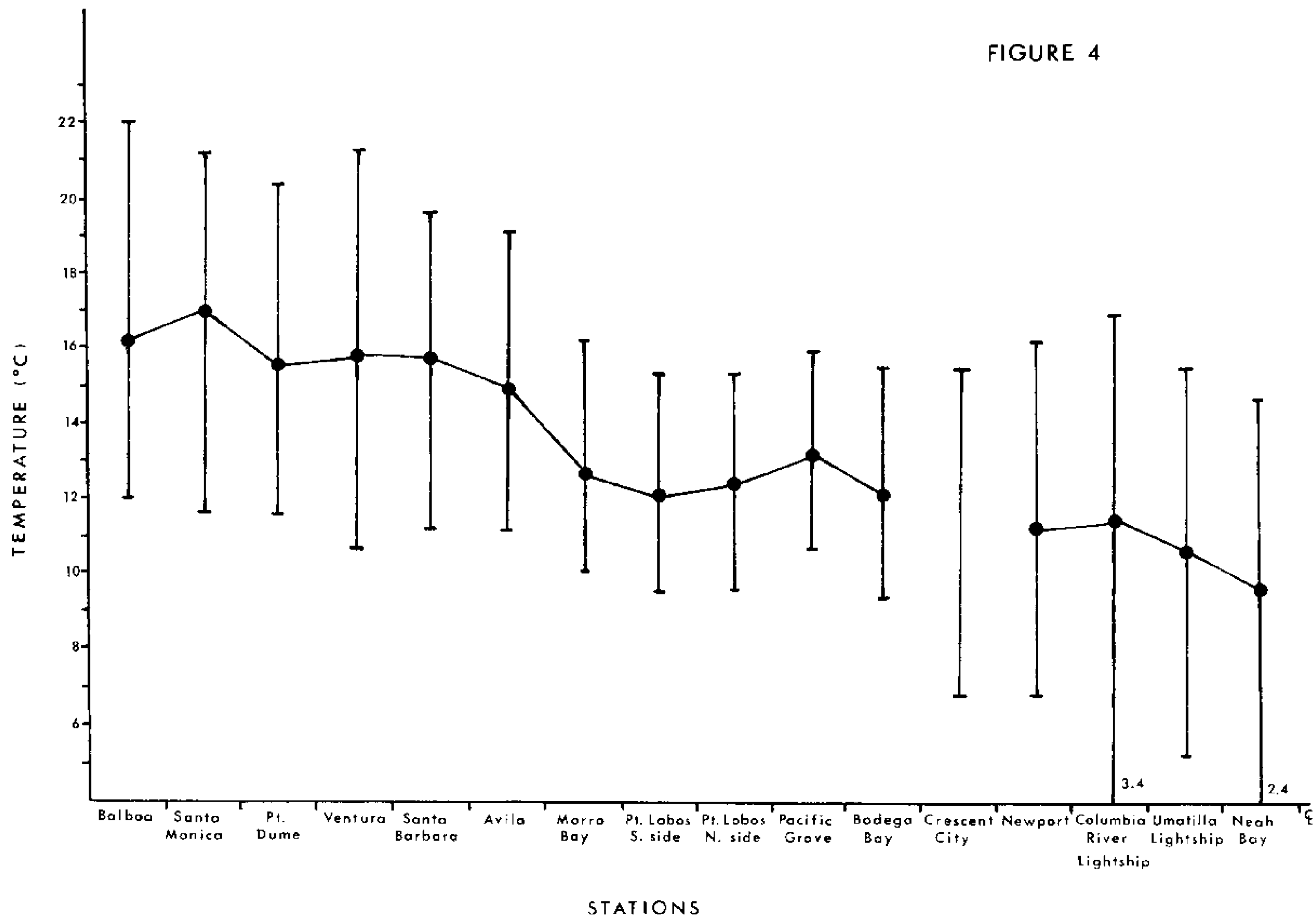
Although no extreme shifts were seen in the three years, it is clear that considerable variation does occur annually, and these variations may well fall within the 2.8° C (5° F) usually applied as permissible change due to thermal effluent by State regulatory agencies. Lower temperatures such as are seen at Point Lobos and Point Dume suggest that a cool-temperate biota could well develop in a limited area of the harbor, should the LNG plant effluent be established there.

That new communities would be established can hardly be doubted (Reish, 1964). The clear danger, however, is that valuable or essential species, from the commercial or ecological viewpoint, may be eliminated if great care is not exercised in developing realistic thermal guidelines for effluents. Guidelines must be based on obtaining as much knowledge of the thermal tolerances of species as rapidly as possible.

Also of importance is more knowledge of the food chains or food webs of harbor organisms. If the normal seasonal reproduction period of a given species is advanced or retarded by temperature effects, the food that larvae, juveniles, or newly developed adults depend on may not be present in adequate amounts at the new period. Similarly, eggs, larvae, and juveniles may be fed upon by a new predator who was not normally present in great numbers at the old reproductive period.

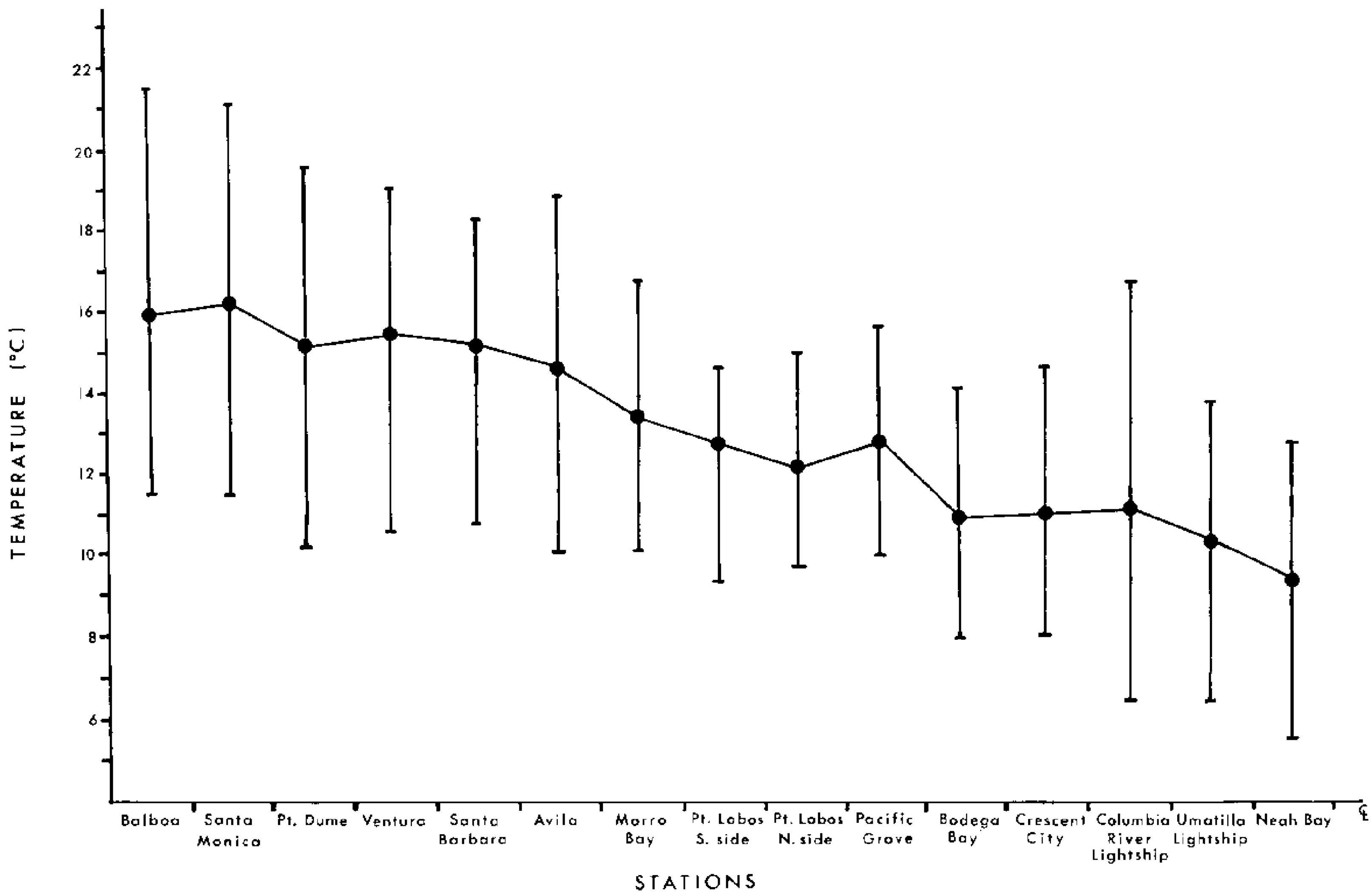
The harbor community does not seem to be a stable or climax community at present. Since the cessation of dumping of refinery wastes in 1968-69 in Dominguez Channel, the biota has increased dramatically in both numbers of individuals and numbers of species. The growth peak probably has not yet been reached, since removal of some of the most polluted sediments in the shallower waters and the possible shut down of additional toxic industrial waste discharges may further enhance the community. The proposed liquid natural gas (LNG) conversion plant presently plans an effluent of approximately 100-150 million gallons per day. The State Water Resources Board has not yet set standards for cooler than ambient

FIGURE 4



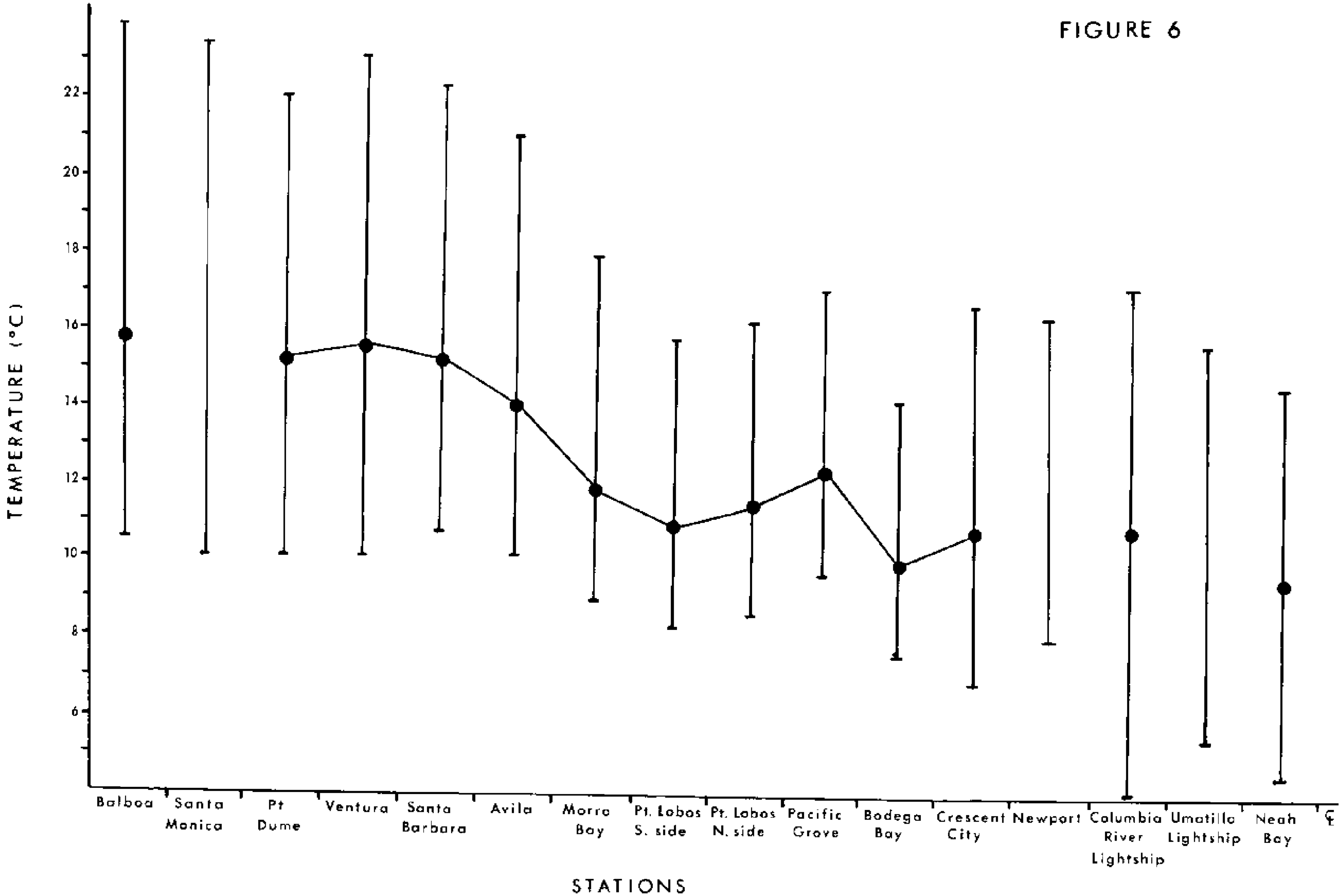
SURFACE WATER TEMPERATURES FOR THE WESTERN UNITED STATES - 1969

FIGURE 5



SURFACE WATER TEMPERATURES FOR THE WESTERN UNITED STATES - 1970

FIGURE 6



SURFACE WATER TEMPERATURES FOR THE WESTERN UNITED STATES - 1971

effluents. Present design criteria are based on assuming a 5° F (2.8° C) differential, in keeping with current standards of 5° F above ambient for warmer-water effluents such as those at nuclear and fossil fuel plants. Regulations similar to those presently in effect on the distance from the point of effluent for reaching compliance temperatures would probably also be set. Concerted cooperative research efforts are required if dislocation and destruction of valuable species are to be avoided during this period of changing technology and development in the harbor.

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MARINE STUDIES OF SAN PEDRO BAY, CALIFORNIA. PART III.

PRELIMINARY OBSERVATIONS ON THE LOWER MINIMUM TEMPERATURE REQUIREMENTS OF THE NORTHERN ANCHOVY

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ABSTRACT. The proposed installation of a liquified natural gas facility within the Los Angeles-Long Beach Harbor and the subsequent discharge of chilled sea water prompted this study of the lower minimum temperature requirements of the northern anchovy, *Engraulis mordax*, an abundant and commercially valuable species, particularly in the harbor area. Juvenile anchovies averaging 70 mm standard length, held for a minimum of two weeks at acclimation temperatures of 19.5°, 16.0°, and 12.0°. were subjected to various degrees of thermal stress from 17.0° to 7.0°C. A lower lethal temperature of approximately 7.0°C was estimated from two experimental procedures.

Anchovy eggs, collected by plankton net, were held at incubation temperatures between 9.0° and 15.0°C. At incubation temperatures below 10.0°C, complete yolk absorption and starvation occurred in larvae before development of pigmented eyes and a functional jaw was completed.

Behavior of juvenile anchovies in a 2.3 m horizontal thermal gradient with a temperature differential of 5.0°C indicated significant selection of the warmest water available to the fish at experimental temperatures between 13.0° and 25.0°C. Anchovies actively avoided temperatures above 25.0°C.

Experiments designed to determine the effects of subnormal temperatures on reproductive potential had to be postponed due to malfunctioning water refrigeration equipment. However, field studies indicate threshold spawning temperatures between 11° and 12°C.

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PRELIMINARY OBSERVATIONS ON THE LOWER MINIMUM TERMPERATURE REQUIREMENTS OF THE NORTHERN ANCHOVY

INTRODUCTION. The potential threat of thermally altering existing lakes, streams, bays, and estuaries, as a resultant by-product of our increasing demand for energy production, has prompted a number of studies to determine the thermal requirements of important aquatic species. For the most part, these studies have concerned fresh-water organisms and the majority of such studies have focused on the effects of high temperatures, exclusively.

The possible installation of a liquified natural gas (LNG) facility within the Los-Angeles-Long Beach Harbor, and the subsequent discharge of substantial volumes of cooled water has motivated the present study of the lower minimum temperature requirements of the northern anchovy (*Engraulis mordax*). Although over 100 species of fish inhabit the Los Angeles-Long Beach Harbor (Chamberlain, 1973), the anchovy has been selected for study because of its abundance and commercial importance.

For reasons that are not yet clear, the Los Angeles-Long Beach Harbor provides an inshore habitat that is especially attractive to anchovies throughout the year. According to a recent California Department of Fish and Game publication (Anonymous, 1971),...."it is not unusual to find every live-bait dealer from Point Conception to the Mexican border fishing in one spot (in the Los Angeles-Long Beach Harbor) on summer nights."

Approximately 80% of the live-bait (98% of which is *E. mordax*) captured in southern California in recent years has been taken within the Los Angeles-Long Beach Harbor. This commercial fishery supplies bait to thousands of southern California fishermen on private and sportfishing boats. Sales of the live-bait have been estimated at $750,000 per year (Baxter, 1967).

The general biology of the northern anchovy was reviewed by Baxter (1967), and an annotated bibliography was prepared by Brewer (1973). Oliphant (1973) has recently compiled catch statistics for anchovies landed in California.

The northern anchovy is a pelagic schooling species found in coastal waters from British Columbia to Cabo San Lucas, Baja California. A species that is distributed over such a broad area must, by necessity, withstand a wide range of temperatures. Baxter (1967) indicates water temperatures ranged from 8.5° to 25.0° C, as recorded from locations where over 600 samples of anchovies were captured;

however, 75.0% of the catches occurred in water temperatures between 14.5° and 20.0° C. Anchovy larvae were shown by Ahlstrom (1959) to be more restricted in their distribution. Larvae were taken in water temperatures between 10.0° and 19.7° C, with 95% taken between 14.0° and 17.4° C.

Field observations are one source of data that may provide important insights and comparisons for laboratory studies, but it is unsound to draw conclusions from field data alone, as the following discussion will illustrate.

A study of meristic characters (i.e., number of vertebrae, fin-rays, gill rakers, etc.) by McHugh (1951) and later a genetic analysis using serum transferrins by Vrooman and Smith (1971) indicated the presence of three distinct populations of *E. mordax*, spatially separated along the coast. The central population, which is the concern of the present study, apparently extends from San Francisco Bay to Ensenada, Baja California. Hence, the temperature data given above which encompasses all three populations may not adequately describe the requirements of each separate population. It is conceivable that each population has genetically determined temperature optima for reproduction and different upper and lower lethal temperature limits. Such "genetic adaptation" for different populations of a single species has been described previously (Kinne, 1963).

Two procedures have generally been used to determine lethal temperatures in fishes. One method has been to heat or cool slowly a vessel of water containing the test animal until the animal dies. The second method requires that test animals be taken from holding tanks and placed directly into a series of vessels previously equilibrated to specific test temperatures (Gift and Westman, 1971). The lethal temperature has generally been considered to be that temperature level at which 50% of the test animals succumbed in a certain period of time, usually 12, 24, or 48 hours.

Critics have attacked both of the above procedures. Fry (1947) suggests that the first method is useful only for broad preliminary comparisons, since two variables, time and temperature, make interpretation of results difficult. The second method, which has been used extensively by Fry and his co-workers (see Fry, 1971 for a review), has been criticized by Tyler (1966) because sudden temperature changes may result in shock effects. Preliminary tests in the present study have used both of the above techniques.

Determinations of lethality are only one aspect of the thermal requirements of fishes. Upper and lower lethal temperatures can be considered only as end-points of a temperature spectrum within which subtle biochemical changes occur that may profoundly alter the animal's normal physiology. Therefore, it is important to consider other aspects of the animal's ecology in relation to temperature.

An extensive literature exists on the relationship of temperature (often interacting with photoperiod) to reproductive cycles in fishes. Virtually all such studies have shown that reproduction is inhibited outside a relatively narrow temperature range, and well within the upper and lower lethal temperatures (Fry, 1971). Data collected by Brewer (unpublished) have shown large numbers of eggs present throughout the Los Angeles-Long Beach Harbor in late winter, early spring, and fall, indicating active spawning within the confines of the breakwater. Studies on the effects of temperature on fish reproduction require long term experiments that encompass seasonal reproductive cycles (Atz, 1957). A comprehensive study of this nature is impossible at this time.

The thermal requirements of anchovy eggs and larvae for embryonic development may be quite different than those temperatures tolerated by adults. Some observations on the development of anchovy eggs and larvae in cold water are described in the present report.

Fishes are active, sensitive organisms which are capable of discriminating and avoiding unfavorable environments. Fish may respond to temperature as a directive factor when temperature-gradients exist (Bull, 1928; Doudoroff, 1938; Alabaster and Robertson, 1961; Coutant, 1969). Therefore, it is important to determine at what temperature the anchovy will actively avoid a cold water stress and move into a more favorable or "preferred" temperature region. Initial experiments concerning anchovy avoidance reactions to cold water in thermal gradients are also discussed herein.

It must be emphasized that the present study can only be considered preliminary because of the scope of this complex problem on thermal requirements. The study was hampered in its early stages by mechanical problems which forced termination of a number of experiments. Furthermore, experiments were subject to the availability of adult anchovies and freshly spawned eggs.

MATERIALS AND METHODS. All tests were conducted in a small experimental sea water system described by Brewer (manuscript submitted for publication). The system delivers filtered, ultraviolet sterilized sea water from the Los Angeles-Long Beach Harbor, to five round, fiberglas aquaria, each with a capacity of 950 liters (250 gallons) and a single rectangular, fiberglas water table with a capacity of 380 liters (100 gallons). Three separate sea water lines run to each aquarium carrying heated, ambient, and chilled sea water respectively. Desired temperatures were maintained by adjusting the flow rates of the three sea water lines. An exchange rate of 2-4 liters per minute was maintained in each aquarium. Overflow drainage, into standpipes, kept water levels constant. Waste water was not recirculated.

Photoperiod was maintained throughout the study at 12 hours light and 12 hours dark unless otherwise noted. Two incandescent light bulbs above each aquarium were controlled by separate dimmer controls, and were turned on and off by an automatic timer. One bulb served as a "daylight bulb" and supplied the equivalent of 65 foot candles of light to the center of each tank; the "night bulb" supplied the equivalent of 1.5 foot candles. Some illumination during the night cycle was required to enable fish to discriminate the sides of the aquaria and thus avoid contact and possible injury.

The sea water system has proved successful in holding stocks of northern anchovies for periods of several months.

Thermal Lethality Experiments on Juveniles. A Long Beach live-bait dealer provided anchovies for the experiments. Anchovies were netted from holding tanks in the bait boat and placed into a single 950 liter aquarium which was maintained as a stock tank. A random sample of fish was removed from the stock tank each time new animals were received, and several measurements were recorded. Sacrificed fish were measured to the nearest millimeter of standard length and weighed to the nearest 0.01 g. Finally, the gonads were preserved in Bouin's solution for later histological examination.

Mechanical injury caused some initial mortality, but within a few days mortality became insignificant. The anchovies were offered a revolving diet of chopped anchovies, chopped squid, and frozen brine shrimp equal to 4% of their wet weight per day. In addition, the fish were fed a dry commercial preparation called Trout Chow, size no. 2, equal to 1% of their wet weight per day.

All fish were held for a minimum of two weeks in the stock acclimation tank at constant temperature (plus or minus 0.5° C) before being subjected to any thermal tests. For each thermal test, two samples of anchovies were removed from the stock aquarium and placed in separate aquaria. The water temperature in one of the tanks was regulated according to the experimental plan described below, while the other tank served as a control. The water temperature in the control tank was maintained equal to the acclimation tank temperature.

The first experiment was designed to obtain a relative measure of the lower thermal tolerance limit of the juvenile northern anchovy. In this test, anchovies were removed from the acclimation tank (19.5° C) and placed into a test and a control tank. Both tanks were held for 96 hours at 19.5° C in order to remove those fish obviously injured during transfer from the acclimation tank. The temperature of the test tank was then lowered 1.0° C per day. Mortalities were removed from the tanks as they occurred each day, and the death was noted on data sheets. Fish were considered dead when direct mechanical stimulation resulted in no response of the animal.

The second experiment on juvenile anchovies was actually a series of tests to determine the lower lethal temperature of *E. mordax*. The techniques used for this determination were similar to those developed by Fry, Brett, and Clawson (1942) for the determination of upper lethal temperatures. The procedure called for animals to be removed from the acclimation tank and placed directly into test tanks previously cooled to certain test temperatures. Mortality was noted at intervals of 24, 48, 72, and 96 hours and dead fish were removed at these times. Three different acclimation temperatures (12.0°, 16.0°, and 19.5° C), and several test temperatures (between 7.0° and 17.0° C) were utilized. Control tanks were maintained for each thermal test.

After considering the results of the first two experiements a third experiment was initiated. A sample of anchovies was removed from the acclimation tank (19.5° C) and placed in another tank at acclimation temperature. The temperature in the test tank was then lowered steadily during a 24 hour period until a temperature of 11.0° C was reached. The tank was then maintained at 11° C (plus or minus 0.5° C) for 96 hours. Mortality was noted at 24, 48, 72, and 96 hour intervals.

Thermal Lethality Experiments on Eggs and Larvae. Anchovy eggs were collected by a standard 0.5 meter conical plankton net (0.333 mm mesh) within or just outside the Los Angeles-Long Beach Harbor. All collections were made between 8 AM and 11 AM. Plankton collections were quickly transferred to large, wide mouth glass jars and transported in styrofoam insulated containers to the sea water system. Anchovy eggs at the same stage of development were sorted with the aid of a dissecting microscope, and individual eggs were placed in 25 ml vials containing sea water at ambient temperature. Vials containing single eggs were randomly placed in water baths at test temperatures between 9° and 15° C and allowed to equilibrate to the bath temperature over a period of 60 minutes.

Lasker (1964) used similar techniques for studying sardine (*Sardinops caerulea*) eggs and larvae. He found that at low temperatures sardine larvae were extremely susceptible to mechanical injury from handling during daily measurement. In the present study, to avoid possible injury to the fragile larvae, the animals were not removed from the vials until their development was complete through yolk-sac absorption and starvation had occurred. At that time, the animals were observed under magnification and their state of development was recorded.

Low Temperature Avoidance Experiments on Juveniles. The fiberglas rectangular water table, 2.3 meters long, 0.7 meters wide, and 0.3 meters deep, was used for experiments on temperature avoidance by juvenile anchovies. The water table was divided into four equal compartments by placing three perforated wooden partitions, 0.5 meters long, across the width of the table. Uniform lighting was provided by suspending two 40 watt florescent bulbs, 0.8 m above the water's surface, longitudinally over the center of the water table.

Unless special precautions are taken in the design of horizontal temperature gradients, vertical temperature stratification occurs and test animals are subjected to conflicting thermal gradients (Doudoroff, 1938). In an attempt to overcome this problem, incoming water from the warm, cold, and intermediate temperature sea water lines flowed into sections of polyvinyl chloride (PVC) pipes lying across the bottom of each compartment. The warm and cold water lines were directed into compartments at opposite ends of the table, and water of intermediate temperature flowed into the two central compartments. The water, flowing through holes in the plastic pipe in each compartment, created gentle streams directed upward and caused a gentle overturn of the bottom water. As a further measure to prevent density stratification, a single airstone was placed on the bottom center of each compartment and equal volumes of air forced through each. With a horizontal temperature gradient established, the maximum vertical gradient was approximately 0.3° C. Overflow drainage of the water table was provided by a central standpipe.

Fish were introduced into the water table after the water temperature was adjusted to equal the temperature of the acclimation tank (16.0° C). After a period of 60 minutes, water of acclimation temperature was replaced by warm and cold water in the lines at opposite ends of the water table and the thermal gradient was allowed to establish. Approximately 30 minutes were required for the gradient to become stabilized, and observations were begun 60 minutes after temperature stabilization. At precisely 5 minute intervals the location of the test fish in the four compartments was recorded and the temperature in each compartment noted. Between 6 and 8 fish were used for each observation period, and each fish was considered a single observation. Observation periods continued for approximately 30 minutes.

The design limitations of the water table precluded the establishment of a gradient greater than 5.0° C. Therefore, three different test series were run with gradients between 13° and 18° C, 16° and 21° C, and 22° and 27° C, respectively.

To be certain the fish were responding to the temperature directive and to no other extraneous factor, a series of control observations were made without the thermal gradient present. Each of the four sea water lines carried water at acclimation temperature in these control experiments. As a further control measure, one half of all observations for each of the three 5.0° C gradient test series were run with the warm and cold water polarity reversed; the warm end of the water table became the cold end, and the cold end became the warm end.

<u>Thermal Effects on the Reproductive Potential of Adults.</u> This experiment was an attempt to determine what effects continuous exposure to sub-normal water temperatures might have on the reproductive cycle of the anchovy. In March 1973, aquariums were stocked with adult anchovies and a random sample was removed and processed as described previously. Gross examination of the fish's gonads showed all the sample fish to be in ripe condition. The tanks

were then held at ambient temperatures for one week.

After the acclimation period, the water temperature in one tank was lowered 1.0°C per day until 11.0°C was reached. Water temperatures in the second tank were maintained at ambient temperatures (average 15° C) and recorded daily.

Photoperiod (i.e. day length) has been shown to be a major factor in seasonal maturation of gonads in some fishes (see Schwassman, 1971, for a review). Since the effects of photoperiod have not been studied for *E. mordax*, samples of fish were subjected to two different photoperiods for these experiments. In the first test, control animals and test animals were held under 12 hours of light and 12 hours of dark (as described previously). In the second test, control animals and test animals were held under a short day cycle with 4 hours of light and 20 hours of dark. The short day cycle was selected because a study by the National Marine Fisheries Service (Reuben Lasker, personnal communication) has shown that anchovies held at 17.0°C and under short day cycle were maintained in full spawning condition throughout the year.

The adult anchovies were held under the conditions described and fed as discussed previously, until mechanical difficulties forced the termination of the experiment.

RESULTS.

Thermal Lethality Experiments on Juveniles. Results obtained from the first experiment on low temperature lethality, in which the aquarium water temperature was lowered 1°C per day, are given in Table 1. All experiments on lethality utilized juvenile fish obtained during the summer and early fall, which averaged 70 mm standard length (range 60-86 mm) and weighed an average 3.39 g (range 2.07-5.94 g). All temperatures are within plus or minus 0.5° C. Survival percentages of test animals were adjusted for mortality in the control aquarium. Survival was zero percent on day 14 at temperatures between 7° and 8°C.

Table 2 lists the results obtained when juvenile fish were transferred from acclimation tanks held at 19.5°, 16.0°, and 12.0 °C and placed directly into test tanks at various test temperatures from 19.5° to 7.0° C. Again, percent survival has been adjusted for control mortality. Data on

Day	Test Temp. Degrees °C	Accumulated Mortality	Percent Survival	% Survival Adjusted for Deaths in Controls
1	19.5	0	100	105.8
2	19.0	0	100	105.8
3	18.0	0	100	105.8
4	17.0	0	100	105.8
5	16.0	0	100	105.8
6	15.0	0	100	105.8
7	14.0	0	100	105.8
8	13.0	0	100	105.8
9	12.0	0	100	105.8
10	11.0	0	100	105.8
11	10.0	1	93.0	98.7
12	9.0	1	93.0	98.7
13	8.0	3	80.0	84.6
14	7.0	10	0.0	0.0

Table 1. Results of Experiment 1 on lethality in which the water temperature was lowered 1.0° C per day. Temperatures are within plus or minus 0.5° C. Survival and Adjusted Survival values are based on 15 test fish and 17 control fish.

Acclimation Temperature Degrees °C	Test Temperature Degrees °C	Cumulative Mortality (hours) 24	48	72	96	Percent Survival	Percent Survival Adjusted for Deaths in Controls
19.5	19.5 control	4	5	9	15	81.7	100.0
	17.0	0	1	1	1	81.7	100.0
	15.0	0	1	3	3	85.7	104.8
	14.0	0	0	0	0	100.0	122.4
	13.0	3	7	8	9	43.8	53.6
	12.0	0	0	3	8	46.7	57.2
	11.0	4	10	18	21	12.5	15.3
	9.0	0	9	11	12	0.0	0.0
16.0	16.0 control	0	1	1	1	97.2	100.0
	12.0	0	0	1	1	95.2	97.9
	10.0	0	1	2	6	66.7	68.6
	9.0	0	1	4	10	41.2	42.4
	8.0	9	13	13	16	0.0	0.0
12.0	12.0 control	6	10	13	14	81.1	100.0
	10.0	3	5	5	5	66.7	82.2
	9.0	3	7	8	8	60.0	73.9
	8.0	11	16	21	21	0.0	0.0
	7.0	10	16	18	18	0.0	0.0

Table 2. Results of Experiment 2 on lethality in which anchovies were removed from acclimation tanks and placed directly into tanks at various test temperatures. Temperatures are within plus or minus 0.5° C.

control mortality from acclimation temperature test series have been combined under the heading Cumulative Mortality. Improved techniques were developed for transferring fish from one aquarium to another after relatively high mortalities were noted in control tanks during the 19.5° C test series. Hence, control moratality for the 16.0° C test series was significantly lower than control mortality for the 19.5° C series. Relatively high control mortality in the 12.0° C test series may result from increased susceptability of fish held at low temperatures.

Ninety-six hour LD_{50} temperatures (when adjusted for control mortality) were 11.0°, 9.0°, and 8.0°C, with acclimation temperatures of 19.5°, 16.0°, and 12.0° C, respectively. These LD_{50} values are probably biased downward since intermediate temperatures of 11.5°, 9.5°, and 8.5° were not utilized. Extrapolation of data would indicate these slightly higher values.

The lower lethal temperature of *E. mordax* juveniles was estimated to be approximately 7.0° C (Figure 1). The lower lethal temperature is considered to be that point on the line where the acclimation temperature equals the lethal temperature (Fry, Brett, and Clawson, 1942; Fry, 1971; Gift and Westman, 1971).

The third experiment on lethality considered the survival of a sample of anchovies when the water temperature was lowered from 19.5° to 11.0° C over a 24 hour period. Results are given in Table 3. Survival was 95% after a 96 hour test period. These results are in contrast to the results of the first experiment in which 11.0° C resulted in only 12.5% survival after 96 hours exposure of fish acclimated to 19.5°C. The differences undoubtedly result from the rapidity with which the test fish were subjected to the temperature change. This problem is discussed later.

Thermal Lethality Experiments on Eggs and Larvae. Approximately 6 to 12 days are required for anchovy eggs to hatch and larvae to develop pigmented eyes and a functional jaw at temperatures between 10.0° and 15.0° C. Incubation time decreases and developmental rate increases with increasing temperature.

The formation of pigmented eyes and a functional jaw are required in order for the larva to begin capturing prey before its yolk supply is completely absorbed. Lasker (1964) showed that starvation occurs in sardine larvae at temperatures below 13.0° C because the yolk supply was completely utilized before development of these important

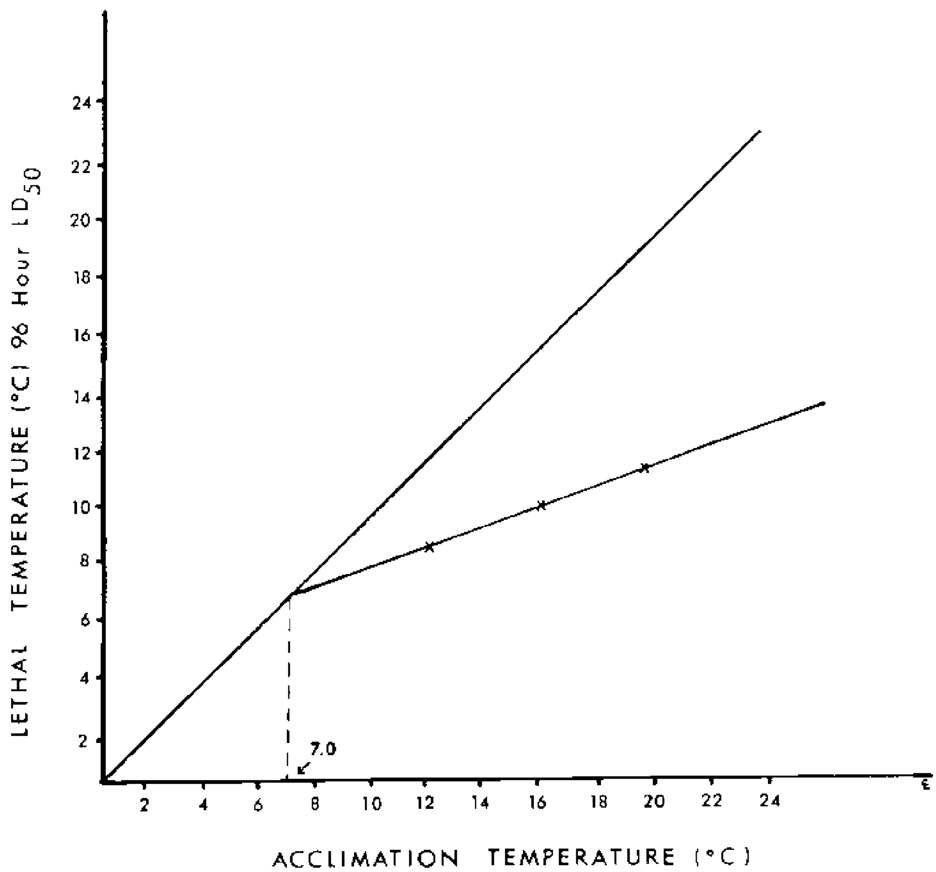

Figure 1. Estimation of lower lethal temperature of *Engraulis mordax* juveniles. The lower lethal temperature is the point on the line where the acclimation temperature equals the lethal temperature.

Acclimation Temperature Degrees °C	Test Temperature Degrees °C	Cumulative Mortality (hours) 24	48	72	96	Percent Survival	Percent Survival Adjusted for Death in Control
19.5	19.5 control	0	0	0	0	100.0	100.0
19.5	11.0	0	0	1	1	95.0	95.0

Table 3. Results of Experiment 3 on lethality in which the water temperature was lowered from 19.5° C to 11.0° C during a 24 hour period. Temperatures are within plus or minus 0.5° C.

organs was complete. In the same paper (Lasker, 1964), anchovy development at low temperatures was compared with sardine development. Lasker found that development through jaw formation and pigmented eyes was complete in the anchovy at temperatures as low as 11.0° C; temperatures below this were not utilized. In the present study, development was studied at temperatures of 9.0° , 9.5° , 10.0° , 10.5° , 11.0° , 11.5° , 12.0° , and 15.0° C. Mortality (death before complete yolk-sac absorption) was approximately 30% at all test temperatures. Only those larvae developing through complete yolk utilization were considered in these experiments. Results indicated that temperatures below 9.5° C (plus or minus 0.5° C) were lethal to anchovy larvae, as evidenced by starvation of animals before eye pigmentation and jaw formation occurred when held at such temperatures.

<u>Low Temperature Avoidance Experiments on Juveniles</u>. Results of experiments on temperature avoidance are presented graphically in Figures 2 and 3. Figure 2 presents the results of control tests in which no thermal gradient existed. The percent time that samples of fish occurred in each of the four compartments are not significantly different (Chi^2 value = 1.72; Chi$^2_{.05}$ = 7.82 with 3 d.f.). Figure 3 presents the responses of the fish when confronted with gradients between 13° and 18° C, 16° and 21° C, and 22° and 27° C.

Anchovies responded to the thermal gradients significantly by selecting the highest temperature made available to them except in that case where the highest temperature gradient was used. When gradient temperatures were between 22° and 27° C, the anchovies avoided the 27° C compartment and selected instead a temperature range between approximately 24.5° and 26.0° C.

<u>Thermal Effect on the Reproductive Potential of Adults</u>. Unfortunately, tests used to determine the effects of cold water on reproductive potential had to be suspended because of malfunctioning water refrigeration equipment. The test animals were subjected to fluctuating water temperatures and many fish were ultimately killed by oil contamination from the faulty refrigeration equipment. Adult, sexually mature anchovies were not available from the live-bait catch during the summer months, and therefore, these experiments could not be resumed, even after installation of new refrigeration equipment. Results of such experiments will have to await further studies.

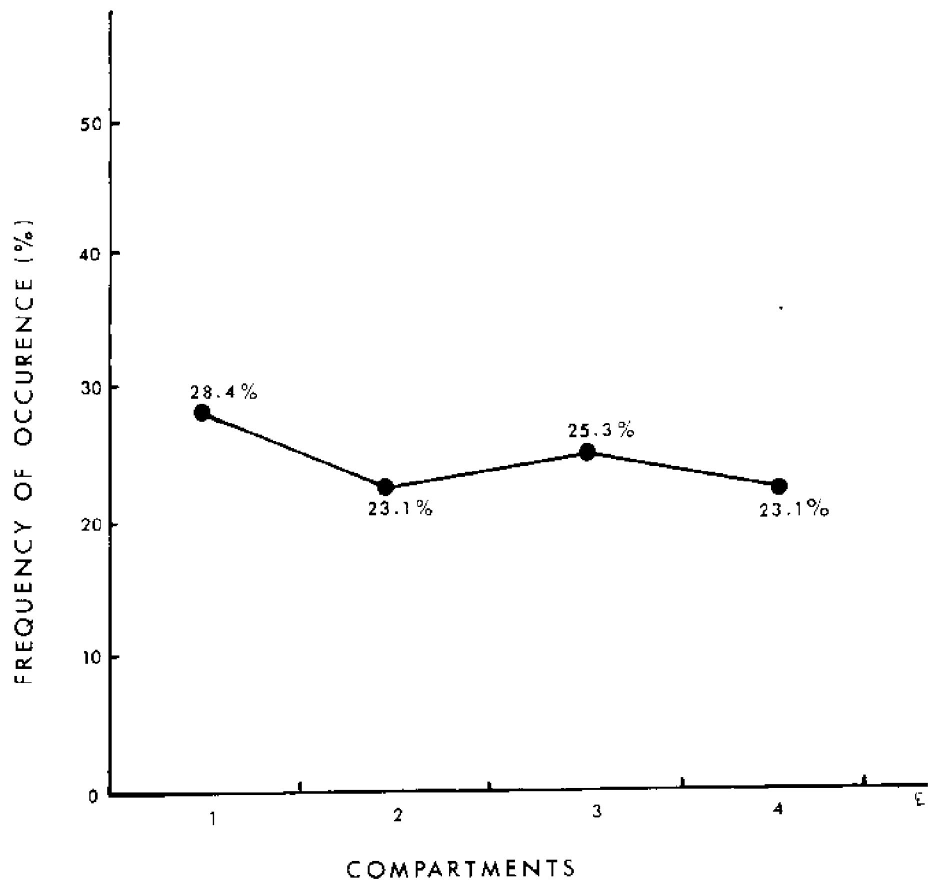

Figure 2. Frequency of anchovy occurrences in different compartments of the water table with no thermal gradient present. Temperature of the tank was equal to the acclimation temperature (16.0° C). The graph is based on 225 observations.

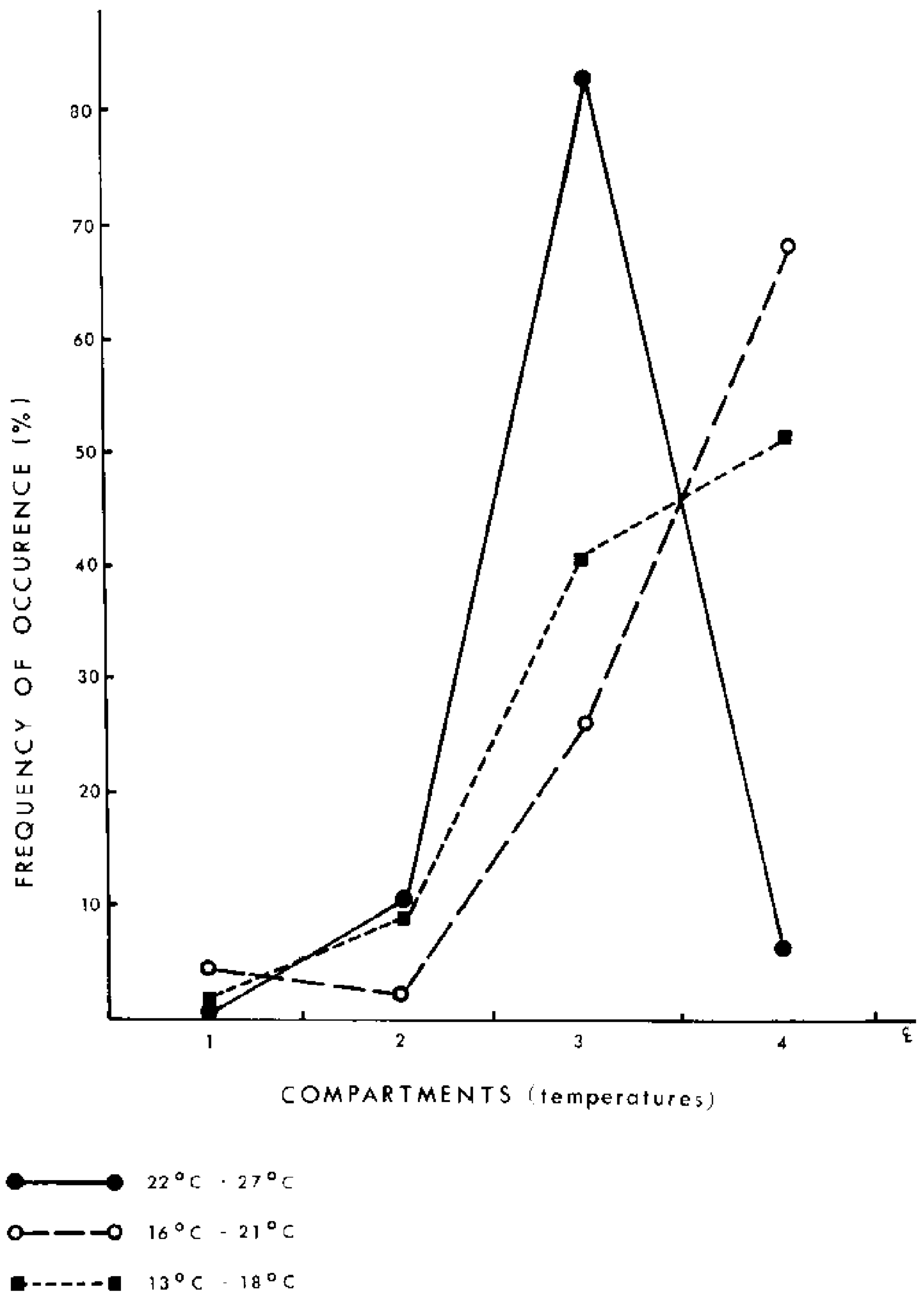

Figure 3. Frequency of anchovy occurrences in different water table compartments, with a thermal gradient of 5° C present. Over 100 observations were made for each thermal gradient test. All fish had been acclimated to 16.0° C.

SUMMARY AND DISCUSSION. A number of factors concerning the lower lethal limits of the anchovy remain unresolved. Hoar and Robertson (1959), Tyler (1966), and Lewis and Hettler (1968) have shown that season of the year or photoperiod affects the ability of some fishes to survive thermal stress. Other studies suggest that the thermal tolerance limits of some species varies with the size of the fish (Albaster, 1967; Spass, 1960). Other, more subtle factors may also influence a fish's ability to withstand thermal stress, such as oxygen concentration, noise stresses, diet, and disease. One or more of these parameters may have affected the results of this study.

Further work is needed before any conclusive statements can be made regarding "safe" low temperature criteria for the northern anchovy *Engraulis mordax*. Tests conducted to date do not adequately answer the question of how time interacts with temperature stress to determine lethality. It was noted that 11.0° C proved lethal to a majority of the test fish when animals acclimated to 19.5° C were placed directly in the cold water, while 11.0° C was not lethal for fish acclimated to 19.5° C, when the water temperature was dropped over a 24 hour period. *E. mordax* is able to adjust to some cold water stress, but experiments on rate-of-gain of cold tolerance, such as those conducted by Brett (1946) on goldfish (*Carassius auratus*), should be carried out.

No data are yet available regarding the effects of acute (but not continuous) exposure to cold temperatures on anchovies. For example, can anchovies survive an exposure to temperatures of 5.0° C, for 2 minutes when acclimated to 19.5° C? Answers to questions such as these will give us a better understanding of the thermal requirements of the anchovy.

Two procedures were used in the present study to indicate that the lower temperature limit for the anchovy is 7.0° C (plus or minus 0.5° C). This lower temperature tolerance limit is analogous to what Fry (1947) called the lower incipient lethal level and is defined as that temperature level beyond which the anchovy can no longer live for an indefinite period of time.

Using procedures that have been documented by previous workers, this study has shown the inability of juvenile anchovies to tolerate temperatures below 11.5°, 9.5°, and 8.5° C when acclimated for a minimum period of two weeks to temperatures of 19.5°, 16.0°, and 12.0° C, respectively.

Anchovy eggs and larvae failed to develop pigmented eyes of a functional jaw when held at temperatures below 9.5° C. With yolk reserves gone, anchovy fry would not have the capacity to capture food and would starve at these low temperatures.

It is impossible to maintain fishes or any other organism under laboratory conditions which duplicate the animal's natural habitat. For this reason, critics have attacked laboratory behavior studies because artificial stimuli, necessarily introduced in order to observe test animals, may elicit unnatural responses in the animals. Similar criticisms have been addressed to experiments on fishes placed in artificial temperature gradients. The most common criticism has been over the use of small tanks with sharp temperature gradients. Such gradients are almost never found in natural waters (Gift and Westman, 1971).

Juvenile anchovies placed in a 5° C temperature gradient, 2.3 meters long, responded by selecting the warmest end of the gradient up to temperatures of approximately 25° C. These results do not imply that anchovies under natural conditions will preferentially select such warm water and avoid cold water. Under natural conditions fish respond to a host of directive factors other than temperature, including food, oxygen concentration, current, and light intensities. Indeed, ocean water temperatures along the coast of California and Baja California seldom reach such extremes, except at the southernmost extent of the anchovies' range. "Preferred temperature" selection is a behavioral response to a thermal gradient, when no other behavioral activity overrides the selection of some particular temperature. Fry (1964) and Ferguson (1958) also present data showing fish do not always select their "preferred temperature" in the field.

Unfortunately, experiments designed to analyze the effect of cold water on the reproductive potential of sexually mature anchovies had to be postponed due to mechanical failures. It would be unwise to speculate on the exact role of temperature in regulating reproduction without a thorough study encompassing the entire reproductive cycle of the anchovy with both temperature and photoperiod under precise regulation. For the present, we must rely on field data by Ahlstrom (1956), which indicates threshold spawning temperatures between 11° and 12° C.

In conclusion, with average annual surface water temperatures in the Los Angeles-Long Beach Harbor ranging from about 12° to 20° C, preliminary observations on the effects of cold water on the anchovy indicate a relatively narrow temperature-buffer zone for anchovy development, survival, and spawning within the harbor. Reduction of normal wintertime lows by addition of cold temperature effluent could endanger anchovy survival if an appreciable area of the harbor presented lethal limit temperatures of 7.0° C or below. Lethality due to starvation before adequate development can occur and is more restrictive at about 9.5° C.

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MARINE STUDIES OF SAN PEDRO BAY, CALIFORNIA. PART III.

EFFECTS OF SUBNORMAL TEMPERATURES ON SOME COMMON LOS ANGELES HARBOR ANIMALS

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ABSTRACT. Four species of animals belonging to three phyla (Coelenterata, Mollusca and Arthropoda) were tested to demonstrate effects of lowered temperatures. Adult *Mytilus edulis* and zoeal larvae of *Pachygrapsus crassipes* were acclimated at 21°C; medusae of *Cladonema sp.* were acclimated at 17°C, and adult and late copepodite stages of *Tisbe sp.* were acclimated at 21, 17, 13, and 9°C. Animals were then transferred to 21, 17, 13, 9, or 5°C for 24 hours. Significant mortalities occurred in 19.2% of *Tisbe sp.* acclimated at 21°C, and 100.0% of *Cladonema sp.* acclimated at 17°C died when transferred to 5°C.

Sublethal effects during survival experiments were the following: *Tisbe sp.* from 17°C acclimation could not swim, clean their appendages, or feed at 5°C, and locomotion was lessened in *Tisbe sp.* transferred to temperatures 8°C lower than acclimation. *Cladonema sp.* had swimming ability decreased when the temperature was lowered 4°C and could not hold on to substrates normally or swim with an 8°C drop. *Pachygrapsus crassipes* zoeae lost mobility with a 4°C drop in temperature and could hardly swim with an 8°C drop. Byssus thread formation was impaired in *Mytilus edulis* with an 8°C drop, and no byssus were formed in the first 24 hours after transfers to 12 or 16°C lower than acclimation. Mussels with a drop of 12 or 16°C could not move by byssus attachment and reattachment. *Tisbe sp.* had diminished reproductive rates when transferred from 17°C to lower temperatures and fed. Transfers to 21°C had an increase in reproductive rate to a minimum generation time of 5 days; control rates were 9 days, while transfers to 13 and 9°C had minimum generation times of 18 to 30 days respectively. No reproduction occurred at 5°C.

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EFFECTS OF SUBNORMAL TEMPERATURES ON SOME COMMON LOS ANGELES HARBOR ANIMALS

INTRODUCTION. The effects of high temperatures on marine organisms have been the subject of numerous studies in the last few years, but studies on the effects of subnormal temperatures *per se* are few. One work (Mangum, 1969), concerned with physiological differences in congeners, demonstrated the effects of low temperatures on feeding in two polychaetes, but in general, information on effects of low temperatures have been incidental to studies on the biology of organisms over their ambient temperature ranges.

In this study, the effects of subnormal temperatures were investigated using four species living in the inner areas of Los Angeles Harbor. The principal organism used was *Tisbe sp.*, a benthic marine and estuarine harpacticoid copepod living primarily on hard substrates. Adults of *Tisbe sp.* can swim but spend little time in the water column. They are extremely abundant in samples of mussel clumps taken from inner harbor docks and are capable of feeding on wood (Barnard & Reish, 1960). The species is not positively identified because of the suspected large number of sympatric species in some temperate latitude localities; some of the species are virtually identical morphologically and can be separated only by means of cross-breeding experiments (Volkmann-Rocco, 1971). Examination of several characters in the females, (males are almost impossible to distinguish) did not justify assigning these to *Tisbe gracilis*, a species reported in Los Angeles Harbor by Barnard and Reish (1960). In addition to the existence of sibling species, some species are polymorphic (Battaglia, 1956), further complicating their taxonomy. In addition, polymorphs may differ in aspects of their physiology, such as osmoregulation (Battaglia & Bryan, 1964). Despite these difficulties, *Tisbe sp.* proves to be an excellent bioassay organism because of its rapid reproduction in the laboratory, lack of morphological variation with environmental conditions (Volkmann-Rocco, 1969), and simple nutritional needs. It can be cultured in vessels of 20 cc capacity and above, and can be fed the easily cultured green alga *Dunaliella* (Battaglia, 1970 and Betouhim-El and Kahan, 1972).

Cladonema sp. is an anthomedusan hydrozoan which is unusual among medusae in that it leads a semi-benthic existence. It can be found on mussel clumps as a medusa, and in aquaria is much more often observed sitting on the glass than swimming. As in the case of *Tisbe sp.*, the specific identification has not been made. This species of *Cladonema* more closely resembles *C. radiatum* from British waters (Russell, 1953) than *C. californica*, described by Hyman (1947) from Tomales Bay, California.

Pachygrapsus crassipes is the abundant, lined shore crab of California intertidal areas, ranging from Oregon to Baja California on this coast and also occurring in the Galapagos, Japan and Korea (Rathbun, 1918). Very little is known of the ecology of the zoeal and megalopa stages, which are planktonic. However, Villalobos (1971b) studied the influence of temperature on *P. crassipes* first zoeae. Because crab larvae are quite difficult to rear in the laboratory, freshly hatched larvae were used in this study and were not fed prior to the onset of the experiment.

Mytilus edulis, the bay mussel, appears to be cosmopolitan and penetrates into cold water in both the Atlantic and Pacific. It is extremely abundant on docks and pilings in the inner harbors. Recent studies show that *M. edulis* suffers changes in a number of biological functions when subjected to a rapid 5°C change in temperature but filtering rates, oxygen consumption, and other parameters reach normal values at the new temperatures after about 21 days (Widdows and Bain, 1971). These authors did not calculate survival, and it is probable that lowering temperatures from 15°C to 10°C, or from 10°C to 5°C, is not a sufficient shock to cause mortality in the British mussels studied. Reish and Ayres (1968) used another criterion, the degree of byssus thread formation, as an indicator of chlorinity and dissolved oxygen and found this to be more sensitive than survival criteria. For all four species in the present study, behavioral and/or physiological criteria are used in conjunction with survival and reproduction data because the former are earlier indicators of diminishing physiological condition than direct survival.

MATERIALS AND METHODS. The harpacticoid copepods, *Tisbe sp.*, were cultured through several generations in the laboratory at 17°C, in well-aerated 55 liter aquaria. The alga *Dunaliella tertiolecta* served as a food for the nauplius larvae, the intermediate copepodite stages, and adults. In all experiments with *Tisbe*, acclimation times of at least 21 days were used. The organisms were transferred into aerated jars with 750 ml of seawater. There were two types of experiments: those designed to show survival ability 24 hours after transfer from acclimation temperature to experimental temperature, and reproduction experiments to show the effects of chronically lowered temperatures on reproduction. Observations of activity were made during the course of the studies. Temperatures in all experiments were 21°C, 17°C, 13°C, 9°C, and 5°C. Organisms were not fed during survival experiments, but were fed in reproduction tests.

Tisbe sp. were counted at the end of the survival experiments by placing the glass jars on a black cloth and illuminating from the side. The copepods appear as tiny, bright, white spots. Actively swimming copepods were removed; still animals were placed in petri dishes and allowed to warm; examination was then made for moribund but living animals and for exuviae (shed exoskeletons). Exuviae were distinguished from dead animals by their clear appearance, while dead animals are translucent gray. Movement of limbs and heart were the criteria for survival.

In reproduction experiments, five egg-carrying females acclimated at 17°C were placed into each of two aerated jars with 750 ml of seawater at each of the following temperatures: 21°C, 17°C, 13°C, 9°C, and 5°C. Before transfer and at two day intervals thereafter, approximately equal amounts of the alga *Dunaliella* were placed in the jars. The "minimum generation time" is herein defined as the time in days necessary for the production of new egg-bearing females, and was determined by frequent visual examination.

The hydrozoan (jellyfish) *Cladonema sp.* were acclimated for at least 21 days at 17°C. Individuals were kept in a well-aerated 55 liter aquarium and were fed *Tisbe sp. Cladonema* were transferred into aerated jars with 750 ml of seawater at 21°C, 17°C, 13°C, 9°C, or 5°C, and at the end of 24 hours they were examined for mortality. Individuals which were not actively swimming were placed in a petri dish and allowed to warm. Contractions of the "bell" were criteria for determining survival. Observations on the activity of the medusae were made at the start and finish of the experiment.

First zoeal larvae from the lined shore crab, *Pachygrapsus crassipes,* were obtained from larvae hatched in the aquarium of an ovigerous female caught in the harbor on the previous day. The acclimation temperature for the period before the female was caught is, therefore, unknown, and thus only a one day acclimation for the larvae at 21°C can be claimed. However, the control temperature of 21°C was probably close to the actual field acclimation value, since July temperatures in inner harbors approximate this value. Zoeae were transferred into aerated jars with 750 ml of seawater, and survival was checked at 24 hours. Motionless animals were removed to petri dishes and allowed to warm before final counts were made. Movement of limbs was the criterion of survival, and observations for this were made at intervals during the experiment.

Bay mussels, *Mytilus edulis,* from 10 mm to 20 mm in width were acclimated for at least 21 days at 21°C in a 55 liter aquarium during which time they were fed *Dunaliella tertiolecta*. The byssus threads were cut, the mussels were cleared of fouling organisms and transferred to aerated jars containing 2 liters of seawater at 21°C, 17°C, 13°C, 9°C, and 5°C. At the end of the 24 hours, all were opened and checked for ciliary movement with carmine particles. Ciliary movement and movements of the foot and mantle were criteria used for determining survival. One physiological criterion, the ability to form new byssus threads, was also examined.

RESULTS. Quantitative results from 24 hour survival experiments are given in Table 1; Chi^2 analyses of the significance of differences between control and experimental values are given in Table 2.

Examination of the data in Tables 1 and 2 shows no effect of lowered temperatures on survival for *Tisbe sp.* acclimated at 9°C, 13°C, or 17°C. Those acclimated to 21°C showed a highly significant mortality when transferred for one day to 5°C. These results may be misleading unless viewed in conjunction with observations of activity. *Tisbe sp.* transferred from 21°C to 17°C, 17°C to 13°C, 13°C to 9°C or from 9°C to 5°C suffered no losses of locomotion. However, when transferred from 21°C to 13°C, 17°C to 9°C, or from 13°C to 5°C, locomotion was slowed. Those transferred from 21°C to 9°C, or from 17°C to 5°C could move along the bottom only with great difficulty and could not swim at all. Part of the locomotory difficulty was due to their inability to remove strings of detritus which had been transferred along with the copepods from the acclimation tank, implying diminished success in cleaning behavior. Transfers from 21°C to 5°C showed no locomotion and the movements of the limbs and hearts of survivors were very slow and irregular.

Tisbe sp., adapted at 17°C and fed *Dunaliella,* reproduced most rapidly at 21°C, with the first ovigerous females occurring on the fifth day. At 17°C, the generation time was

9 days; at 13°C it was 18 days; and at 9°C, the first new ovigers were seen at 30 days (see Figure 1). No *Tisbe sp.* survived transfer to 5°C for more than five days, and therefore no reproduction took place. Fecal pellets, an indicator of feeding, were seen in all cultures except at 5°C, and it can be inferred that *Tisbe* did not feed at this temperature. Generation times for *Tisbe pori* from the Mediterranean (Betouhim-El and Kahan, 1972) were similar over a range from 17°C to 26°C to those found for local *Tisbe sp.* at a range from 14°C to 21°C. The local *Tisbe* were acclimated at 17°C, but the acclimation temperature of the Mediterranean organisms is not known, although the range suggests a higher acclimation temperature.

Tables 1 and 2 show no significant mortality for *Cladonema sp.* medusae acclimated at 17°C except when transferred to 5°C, where mortality was very highly significant. *Cladonema* appears to have a very sharply defined minimum survival temperature between 9°C and 5°C. However, this may be misleading because behavioral observations indicate swimming was impaired at 13°C. At 9°C, the medusae could not swim at all, and the great majority of individuals could not hold on to the substrate because most individual's tentacles were in a contracted state. At 5°C, the *Cladonema sp.* were thrown immediately into violent contraction, could neither hold on to the sides of the jars nor swim, and no pulsations of the bell could be seen. All died within 24 hours.

No significant differences were shown between transfers from acclimation temperature to experimental temperatures for the zoeae of the crab *Pachygrapsus crassipes* (Tables 1 and 2). However, these organisms exhibit behavior which suggests that survival data, taken alone, are misleading. Individuals transferred to 17°C showed diminished activity, as measured by the number of zoeae swimming above a line drawn 20 mm from the bottom of the jars. About 50% of the experimental zoeae were initially above the line, in contrast to 80% above the line in controls. At 13°C, the zoeae dropped quickly to the bottom or moved weakly above it; at this point, no larvae were above the 20 mm line, but by the end of 24 hours up to 5% were in the water column above the line. At 9°C, larvae dropped immediately to the bottom and all activity was halted except for sporadic twitching of appendages. At 5°C, there was no observable movement after larvae fell to the bottom.

No mortalities were recorded for *Mytilus edulis* in 24 hour survival experiments. However, bay mussels also show physiological characteristics which may indicate that survival

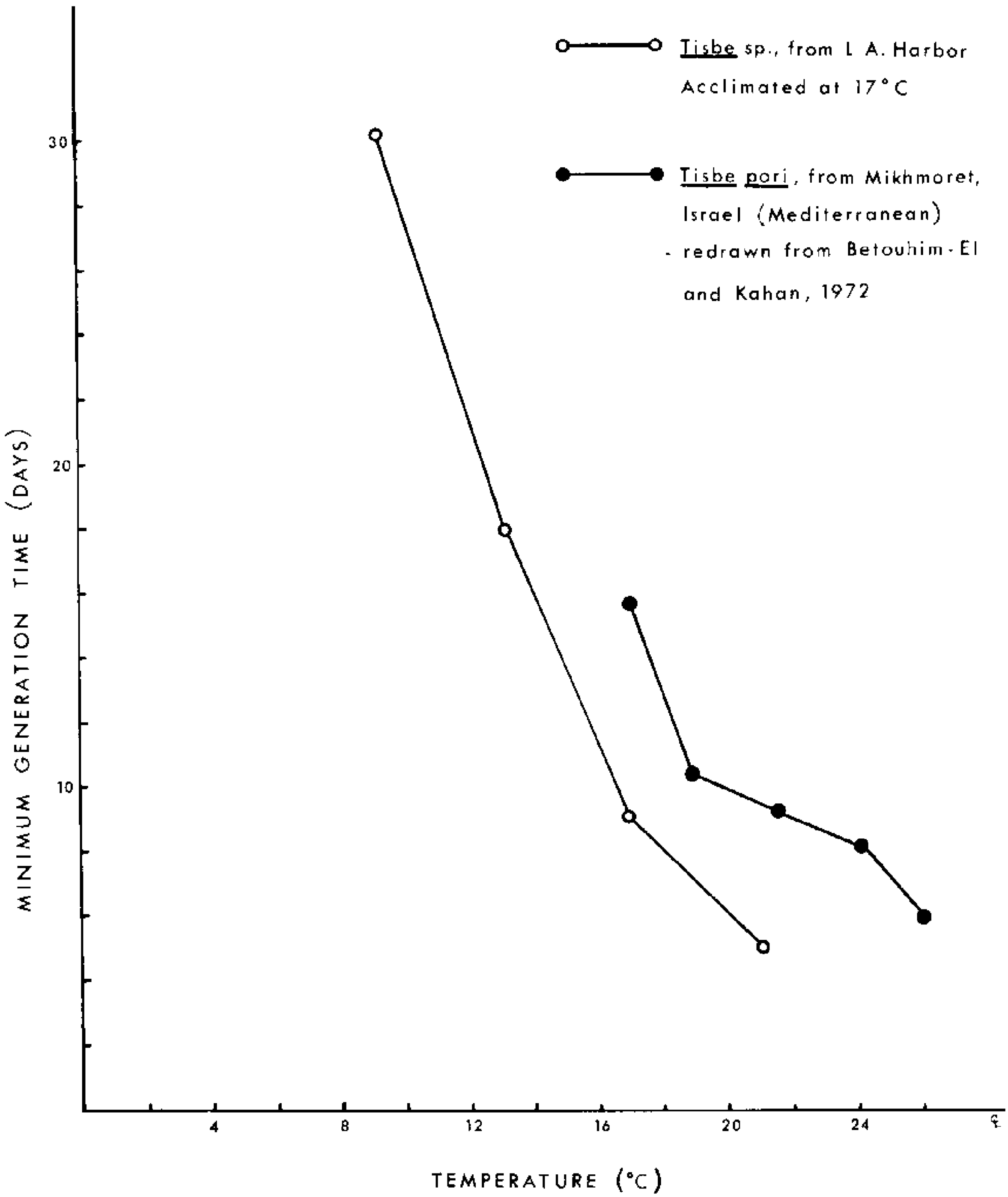

FIG. 1

MINIMUM GENERATION TIMES AT
DIFFERENT TEMPERATURES FOR TWO TISBE SPECIES

data taken alone may be misleading. Mussels transferred to control jars and to 17°C jars made numerous strong byssus threads. These appeared to be either fewer in number or weaker in 13°C animals, and no byssus were made by 9°C or 5°C animals. An inadvertent experiment occurred after the 24 hour test when the air supply for four of the jars failed, resulting in a situation of diminishing dissolved oxygen. In 21°C, 17°C and 13°C jars, mussels were able to move from the bottom to just under the water's surface. It appears that movement is effected by alternately forming a new byssus thread above the mussel and breaking one below until, in combination with contractions of the foot, the mussel is able to climb 10 cm or more. Mussels in the 9°C and 5°C tanks had, by this time, formed byssus (only two or three threads among the 22 mussels in the latter case), but were not able to climb to the surface to obtain more oxygen.

DISCUSSION. It would appear from the results of the survival experiments that abrupt changes in temperature from those which prevail in Los Angeles Harbor during the late Spring, Summer and early Autumn, to substantially lowered temperatures have limited effects on harbor organisms except in a few cases: The nearly 20% mortality for *Tisbe sp.* and 100% mortality for *Cladonema sp.* acclimated at 17°C when both species were transferred to 5°C were significant. However, observations on the behavior, physiology and reproduction of the study species are quite illuminating relative to what might occur if the temperature of the water were lowered a given amount during a certain season. These observations, along with results from the survival experiments and likely suppositions based on our knowledge of Los Angeles Harbor, were used to prepare Table 3, showing results which might be expected if such lowered temperatures actually occurred. Many other factors, such as predation on moribund animals, could result in considerably more serious occurrences (Thorson, 1950), but since these are not predictable even in the simplest and best known ecosystems, they are omitted. Conclusions thus presented must be considered to be very conservative.

Several assumptions have been made in preparing Table 3. First, it is assumed that organisms which become immobilized would settle out of the water column, landing on the soft substrates which cover the harbor bottom. Soft substrates are not optimally suitable for *Tisbe sp.* and *Cladonema sp.* and are completely unsuitable for *Pachygrapsus crassipes* zoeae and *Mytilus edulis* larvae.

Secondly, there is a high likelihood of burial and subsequent mortality, if small organisms normally on hard substrates or in the plankton drop to the soft bottom. In the present study, adult *Tisbe sp.*, medusae of *Cladonema sp.*, and zoeae of *P. Crassipes*, all of which spend some or all of their time in the water column, and the sessile adults of *M. edulis* were used. However, other stages of these organisms, such as the nauplius larvae of *Cladonema sp.* (these are not known from this genus but related anthomedusae have them), megalopae of *Pachycrapsus crassipes*, and veliger larvae of *Mytilus edulis* are all planktonic. Torpor would probably also drop these latter larval types from the water column, and into sediments unsuitable for settling, with subsequent burial and mortality. It is not known how quickly this would occur.

Third, because adult mussels were not able to form adequate numbers of byssus threads at 9°C and 5°C when acclimated at 21°C, it is assumed that the larvae would not be able to form initial attachment byssus threads, and thus fix themselves to hard substrates necessary for their survival. This assumption is probably justified, since most larval marine organisms have narrower tolerances than do adults of the same species.

The results of other studies tend to confirm conclusions presented here. *Tisbe pori*, from Israel (Mediterranean) has a similarly shaped curve for the minimum generation time with respect to temperature (Bethouhim-El and Kahan, 1972), but this species, native to warm temperate waters, is more sensitive to low temperatures than is *Tisbe sp.* from Los Angeles Harbor (Figure 1). Some caution, however, should be used in the interpretation of *Tisbe sp.* data, until positive identification of the species can be made. Battaglia and Lazzaretto (1967), found that changes in temperature alter the genetic composition of *Tisbe reticulata* from the Lagoon of Venice, Italy. One of the polymorphs was favored at warmer temperatures and another was favored under cooler conditions. In this study, *Tisbe sp.* which were able to reproduce at 13°C and 9°C had a slightly different appearance than those reproducing at 17°C and 21°C, and they may represent morphs or even species selected for by different temperatures.

The results of this study are also confirmed by findings that *Pachygrapsus crassipes* first zoeae from gravid females which had been kept for unspecified times at 18.5°C (Villalobos, 1971a) respired down to 10°C, but no respiration was recorded at 5°C. The prezoeal larvae, which were not used in the present study, had measurable respiration down to 13°C, but not at 8°C (Villalobos, 1971b).

In conclusion, diminished temperatures would affect organisms in Los Angeles Harbor. The damage to harbor fauna would be dependent on several factors tested for, as well as numerous others. Clearly, the actual amount of the temperature drop and the season in which the drop occurred would be important. Additional factors to be considered would include the volume of cold water to be released, the frequency of cold effluent releases (present ecological theory holds that unpredictable environmental stress brings about greater reduction of species diversity than does predictable stress (Slobodkin & Sanders, 1969)), and the actual minimum temperatures reached. In addition to the reduction in numbers for many species, there would probably be invasion by species not currently present in the harbor, and proliferation of some living there in small numbers at present. The ecological and economic effects of these changes are unknown, but it can be safely predicted that many will be viewed as detrimental by segments of the scientific and economic communities. There may be additional beneficial results, such as increasing the amount of dissolved oxygen and increasing nutrient turnover, but these will have to be weighed against large-scale mortalities, localized extinctions and partial replacement by organisms more capable of withstanding colder waters.

TABLE I

TWENTY-FOUR HOUR SURVIVAL EXPERIMENTS
USING FOUR LOS ANGELES HARBOR ORGANISMS

Acclimation Temperature (Degrees °C)	Experimental Temperature (Degrees °C)	Living	Dead	Total	Percent Survival
Tisbe sp.					
9	5	34	0	34	100.0
9	9	28	0	28	100.0
9	13	35	0	35	100.0
9	17	29	0	29	100.0
9	21	36	1	37	97.3
13	5	34	0	34	100.0
13	9	33	0	33	100.0
13	13	36	0	36	100.0
13	17	40	0	40	100.0
13	21	31	0	31	100.0
17	5	42	1	43	97.7
17	9	36	0	36	100.0
17	13	30	0	30	100.0
17	17	43	0	43	100.0
17	21	46	0	46	100.0

(continued next page)

TABLE I (Cont'd)

TWENTY-FOUR HOUR SURVIVAL EXPERIMENTS
USING FOUR LOS ANGELES HARBOR ORGANISMS

Acclimation Temperature (Degrees °C)	Experimental Temperature (Degrees °C)	Living	Dead	Total	Percent Survival
21	5	38	9	47	80.8
21	9	49	2	51	96.0
21	13	31	0	31	100.0
21	17	30	0	30	100.0
21	21	56	1	57	98.3
Cladonema sp. medusae					
17	5	0	18	18	0.0
17	9	34	1	35	97.2
17	13	46	2	48	95.9
17	17	16	0	16	100.0
17	21	16	0	16	100.0
Zoeae of *Pachygrapsus crassipes*					
21	5	229	1	230	99.7
21	9	97	0	97	100.0
21	13	97	0	97	100.0
21	17	101	1	102	99.0
21	21	86	0	86	100.0

(continued next page)

TABLE I (Cont'd)

TWENTY-FOUR HOUR SURVIVAL EXPERIMENTS
USING FOUR LOS ANGELES HARBOR ORGANISMS

Acclimation Temperature (Degrees °C)	Experimental Temperature (Degrees °C)	Living	Dead	Total	Percent Survival
Mytilus edulis					
21	5	23	0	23	100.0
21	9	22	0	22	100.0
21	13	20	0	20	100.0
21	17	22	0	22	100.0
21	21	22	0	22	100.0

TABLE II

DIFFERENCES IN SURVIVAL AFTER TWENTY-FOUR HOURS IN FOUR LOS ANGELES HARBOR ORGANISMS

Control Transfer (Degrees °C)	Experimental Transfer (Degrees °C)	Chi^2	Significance level
Tisbe sp.			
9 - 9	9 - 5	0	none
9 - 9	9 - 13	0	none
9 - 9	9 - 17	0	none
9 - 9	9 - 21	0.787	none
13 - 13	13 - 5	0	none
13 - 13	13 - 9	0	none
13 - 13	13 - 17	0	none
13 - 13	13 - 21	0	none
17 - 17	17 - 5	1.012	none
17 - 17	17 - 9	0	none
17 - 17	17 - 13	0	none
17 - 17	17 - 21	0	none
21 - 21	21 - 5	8.968	.99
21 - 21	21 - 9	0.468	none
21 - 21	21 - 13	0.550	none
21 - 21	21 - 17	0.532	none

(continued next page)

TABLE II (Cont'd)

DIFFERENCES IN SURVIVAL AFTER TWENTY-FOUR HOURS IN FOUR LOS ANGELES HARBOR ORGANISMS

Control Transfer (Degrees °C)	Experimental Transfer (Degrees °C)	Chi^2	Significance level
Cladonema sp.			
17 - 17	17 - 5	34.000	.999
17 - 17	17 - 9	0.466	none
17 - 17	17 - 13	0.688	none
17 - 17	17 - 21	0	none
Zoeae of *Pachygrapsus crassipes*			
21 - 21	21 - 5	0.375	none
21 - 21	21 - 9	0	none
21 - 21	21 - 13	0	none
21 - 21	21 - 17	0.848	none
Mytilus edulis			
21 - 21	21 - 5	0	none
21 - 21	21 - 9	0	none
21 - 21	21 - 13	0	none
21 - 21	21 - 17	0	none

TABLE III PREDICTED MINIMUM EFFECTS OF LOWERED TEMPERATURE ON HARBOR ORGANISMS

Temperature Change	*Tisbe sp.*	*Cladonema sp.*	*P. crassipes* zoeae	*Mytilus edulis*
21°C to 17°C	no direct effects; others not known	not known	lowered motility causing more zoeae to fall into sediment; mortality considerable	no direct effects; others not known
21°C to 13°C	motility lowered probably no major mortality due to rapid acclimation	not known	drastically lowered motility; mortality probably 100% from dropping out of water column	settling larvae and young with diminished ability to form byssus; importance to young considerable; importance to adults minimal
21°C to 9°C	drastically lowered motility, in part due to accumulated strings of detritus; *Tisbe* falling off hard substrates become buried; mortality very high	not known	same as above but likelihood much greater	settling of larvae poor or nonexistent; adults cannot move from anoxic layers if they occur; near 100% larval mortality; some adult mortality
21°C to 5°C	direct mortalities and deaths due to inability to clean detritus strings; mortality near 100%	not known	same as above but likelihood still much greater	same as above but likelihood much greater

TABLE III PREDICTED MINIMUM EFFECTS OF LOWERED TEMPERATURE ON HARBOR ORGANISMS (Cont'd)

Temperature Change	*Tisbe* sp.	*Cladonema* sp.	*P. crassipes* zoeae	*Mytilus edulis*
17° C to 13° C	lowered reproductive rate; probably no major effect on abundance	swimming impaired; considerable mortality due to settling into substrate	not known	not known
17° C to 9° C	much lower reproductive rate; motility lowered; effects considerable in combination	immotility and loss of ability to hold onto substrate leads to nearly 100% mortality	not known	not known
17° C to 5° C	lowered motility due in part to accumulation of detritus; no feeding; no reproduction; mortality 100%	100% direct mortality	not known	not known
13° C to 9° C	no direct effects; others not known	not known	not known	not known
13° C to 5° C	lowered motility; mortalities likely due to inability to acclimate to 5° C	not known	not known	not known
9° C to 5° C	no direct effects; others not known	not known	not known	not known

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THE EFFECT OF VARIOUS WATER TEMPERATURES ON THE SURVIVAL AND REPRODUCTION IN POLYCHAETOUS ANNELIDS: PRELIMINARY REPORT

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ABSTRACT. The effects of varying water temperatures, namely 5, 9, 13, 17, 23, 26, 29, 32, and 35 C, on survival, feeding, and reproduction were studied on three laboratory inbred species of polychaetous annelids. Laboratory procedures were outlined for both short term and long term experiments; the preliminary data were presented for *Capitella capitata* and *Neanthes arenaceodentata*. The maximum and minimum 28 day TL_m values were 7.8 and 26.6 C for *Capitella capitata* and 12.3 and 24.6 C for *Neanthes arenaceodentata*.

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THE EFFECT OF VARIOUS WATER TEMPERATURES ON THE SURVIVAL AND REPRODUCTION IN POLYCHAETOUS ANNELIDS: PRELIMINARY REPORT

INTRODUCTION. Investigations on the effects of lethal temperatures and temperature tolerance have been concerned primarily with rocky intertidal invertebrates by such workers as Gunter (1957), Southward (1958), Kinne (1963), and Newell (1964). Temperature studies with polychaetes have been in connection with oxygen consumption, as in the work by Dales (1961a, 1961b), Dales et al (1970), Mangum (1963, 1970), and Mangum et al (1969); in connection with salinity as in the work by Smith (1957); or in the relationship of geographical variation and temperature tolerances as shown by Kinne (1969a, 1969b). Some polychaete temperature work has been at the cellular level by Ivleva (1962) and Gorodilov (1961).

Huntsman and Sparks (1924) listed 31.6 C as the lethal maximum temperature for *Tomopteris catharina* in sea water. Kinne (1969a, 1969b) investigated two species of tube-dwelling polychaete worms, *Diopatra cuprea* and *Clymenella torquata*, for their resistance to high temperatures. He found that the 50% lethal maximum temperature for *D. cuprea* from Barnstable Harbor, Massachusetts, in August was 37.4 C and in July was 42.5 C. The same species from Beaufort Harbor, North Carolina, had a 50% lethal maximum temperature of 41.8 C in August while *D. cuprea* from Ocean Springs, Mississippi, had 40.1 C. The August 50% lethal maximums for *Clymenella torquata* were 36.2 C and 40.5 C for Barnstable and Beaufort, respectively. The lethal temperatures showed seasonal differences of 4.0 C between July and February for *Diopatra cuprea* while there was a 3.8 C difference between August and February for *Clymenella torquata*.

Mangum (1969a) noted the temperature adaptation response of an offshore onuphid polychaete species, *Hyalinoecia artifex*, in comparison with two closely related species, *Diopatra cuprea*, which inhabits a typical intertidal environment, and *Hyalinoecia tubicola*. *Diopatra cuprea* was experimentally exposed to a temperature range of 25 C with little or no mortality. Mangum stated that the ecologically meaningful upper temperature limit must lie between 32.5 C, at which the worms survive prolonged exposure of several months duration, and 35 C which kills over 75% within a week. The lower limit of thermal tolerance must extend below 0 C, since such temperatures are commonly recorded in the natural environment of *D. cuprea*. The mortality of *H. tubicola* was in excess of 50% below temperature of 4 C. Dales, et al, 1970, found that all specimens were killed at 2 C. *Hyalinoecia artifex* was able to acclimate to temperatures from 2.5 C to 12.5 C with little difficulty, but 15 C caused up to 50% mortality.

Mangum (1969b) noted, with respect to survival and feeding, that the temperature at which blockage of feeding response occurred in nereid polychaetes was correlated with the thermal environment from which the animals originated and probably with the geographic range of the species.

De Silva (1967) worked with four intertidal species of the polychaete genus *Spirorbis*. He listed 36 C as the lethal maximum temperature for *Spirorbis pagenstacheri*, which ranges in vertical distribution from high to low water. *Spirorbis borealis*, *Spirorbis tridentatus* and *Spirorbis corallinae*, which have vertical ranges extending from mid tide level down to the low tide mark, have lethal maximum temperatures of 32 C to 33 C. These figures were recorded from summer experiments with environmental water temperatures of approximately 16 C at Swansea, Wales.

The effects of heated or cooled effluents on polychaetous annelids has not been studied in the past. With the proposed construction of the liquid gas conversion facility in Los Angeles Harbor, it is of significance to know whether or not the decrease in water temperatures below ambient will have an adverse effect on marine organisms, in this case benthic polychaetous annelids. The purpose, therefore, of this study is to determine the effect of both increased and decreased temperatures on the survival and reproduction in polychaetous annelids.

MATERIALS AND METHODS--TEMPERATURE CONTROL. Maintenance of specific, constant temperatures was accomplished by use of refrigeration units and aquarium heaters. Experiments were conducted at 5, 9, 13, 17, 23, 26, 29, 32, and 35 C. A household refrigerator was used for the 5 C temperature experiments. Air, when required, was provided with plastic tubing connected to a compressed air system. A notch was cut in the insulation on the door of the refrigerator to allow air to pass through the tubing when the door was closed. Three constant-temperature cold baths maintained environmental conditions at 9, 13, and 17 C. The cold baths were constructed of 0.75 inch plywood, measured 1 x 4 x 8 feet, fiberglassed along the seams, and were coated with resin. Electric compressor-refrigeration units circulated refrigerant through copper tubes covered with plastic tubing. The tubing was fitted into the bottom of the cold bath box. The particular temperature was achieved with a rheostat. A wooden rack was fitted over the copper tubing, and the box was filled with five inches of fresh water. The experimental containers were placed on the wooden rack.

Room temperature was generally 23 C ± 1 C. Warmer temperatures were maintained with aquarium heaters fitted to 15 gallon aquaria. These aquaria were filled with about 10 gallons of fresh water and contained a wooden rack which supported the experimental glassware.

Capitella capitata. The polychaetous annelid, *Capitella capitata* (Fabricius), was chosen for this study because it is cosmopolitan in distribution, it is easily cultured in the laboratory, and its life history is well known (Eisig, 1898). *Capitella capitata* is usually found in brackish water, often in large numbers in the vicinity of outfalls discharging biological waste (Reish, 1957b). This worm has been used as an indicator of polluted conditions in southern California marine waters (Reish, 1959). It has been described as non-competitive since it abounds in regions usually lacking in other polychaete species, whereas large numbers are rarely found in areas with other polychaete species.

The life cycle of *Capitella capitata* is completed within 30 to 60 days under laboratory conditions which makes it a convenient test organism for long-term experimental studies. All experimental work was done with the Los Angeles Harbor strain of *C. capitata*. All specimens originated from a single female collected in Los Angeles Harbor in 1968. Reish found *C. capitata* in reproduction in the inner and outer harbor year around, although in lesser numbers in the winter. Actual sediment temperatures were unknown.

28 day TL_m Experiment. *Capitella capitata* from the laboratory colony were placed in shallow, white enamel pans. The worms were allowed to free themselves from their tube masses to minimize the amount of handling. By using a fine brush, the worms were then transferred to petri dishes containing filtered sea water. All specimens were examined under a dissecting microscope to check for injuries. All injured worms were discarded and any female with developing eggs moving freely in her coelom was returned to the stock colony. Median tolerance level (TL_m) is then determined.

One specimen was placed in a 500 ml erlenmeyer flask with 100 ml filtered sea water. Approximately 20 mg. of previously dried algae, *Enteromorpha*, was added to each flask as a food source. The flask was tightly closed with a rubber stopper.

Ten replications were placed in each of the following temperatures 5, 9, 13, 17, 23, 26, 29, 32, and 35 C. Each worm was checked every fourth day to see if it was living; if it had incorporated algae into tube construction; and if there were signs of feeding, as indicated by the presence of fecal pellets. The experiment was terminated at 28 days.

Long-term Reproduction Experiment. Clumps of *Capitella capitata* from the laboratory colony were examined under a dissecting microscope for females incubating eggs. When the developing eggs appeared grey-green in color, the female and her tube were placed in a separate stender dish. The mucous tube containing the eggs was then teased apart with forceps. The swimming trochophore larvae were then pipetted into a common container. This procedure was repeated until the number of larvae in the container equalled the number of larvae necessary for the experiment. At this point, 25 larvae were then placed in a separate stender dish along with sea water. This was repeated for a total of 1400 larvae. Eight, one-gallon jars containing 2500 ml filtered sea water, 0.1 g of dried *Enteromorpha*, and 25 larvae were set up in each of the following temperatures 5, 9, 13, 17, 23, 26, and 29 C. These jars were aerated with plastic tubing connected to aquarium air stones. Food was added once a week.

The worm masses at the bottoms of the gallon jars were examined at 15 days and every 2 to 3 days thereafter for presence of eggs. All females incubating eggs were removed to a separate dish and the eggs were counted. This experiment continued until all females had either laid eggs or had died.

F_1 and F_2 Generation Reproduction Experiment. The trochophore larvae were set up in the same manner as in the long-term reproduction experiment, except that as soon as an incubating female was observed in the late stages of incubation, her developing eggs were used for two sets of 25 larvae each, which were set up in gallon jars as before. This was repeated for two more females at each experimental condition.

The experiment was continued until the F_2 worms were observed to be incubating eggs. These worms were removed and the eggs counted.

Neanthes arenaceodentata. The polychaete *Neanthes arenaceodentata* was chosen for this study because of its short life cycle and the ease in culturing the worm under laboratory conditions. This species takes 3 to 4 months to complete its life cycle under laboratory conditions. Large quantities of this species of worm can be kept in small areas and one mating produces up to 200 to 500 worms. The life cycle and behavior have been studied by Reish (1957). These worms are cannibalistic to members of their own sex, and also, after egg-laying has been completed, the female may be eaten by the male. In any case, the female dies shortly after the eggs are laid and the male cares for the eggs. All experimental work was done with the Reish strain of *Neanthes arenaceodentata* which was derived from *N. arenaceodentata* specimens collected in Los Angeles Harbor in 1964. No information is known about reproductive seasonality of *Neanthes* in the Los Angeles Harbor.

28 day TL_m Experiment. *Neanthes arenaceodentata* from the laboratory colony were placed in shallow white enamel pans and allowed to free themselves from their tube masses to minimize the amount of handling. The worms were then transferred to petri dishes containing filtered sea water, where specimens were examined for injuries under a dissecting microscope. All injured worms were discarded and all females with developing eggs in their coelom were returned to the stock colony.

One specimen was placed in a 500 ml erlenmeyer flask with 100 ml filtered sea water. Approximately 0.3 g. of previously dried algae, *Enteromorpha*, was added to each flask as a food source. The flask was tightly closed with a rubber stopper. Ten replications were placed in each of the following temperatures 5, 9, 13, 17, 23, 26, 29, 32, and 35 C.

Each worm was examined every fourth day to see if it was living, if it had incorporated algae into tube construction, where the tube was located, if the worm was in the tube, and for signs of feeding as indicated by the presence of fecal pellets. The experiment was terminated at 28 days.

Long-term Egg Production Experiment. *Neanthes arenaceodentata* were removed from the laboratory colony and placed in shallow white enamel pans, where worms were allowed to free themselves from their tube masses to minimize the amount of handling. Four worms, each approximately 1.5 cm in length, were then transferred to each petri dish containing filtered sea water until approximately 300 worms were removed.

The *N. arenaceodentata* were examined for injuries under a dissecting microscope. All injured worms were discarded and replaced by appropriate worms from the stock colony.

Four worms were placed in a one gallon jar filled with 2500 ml of filtered sea water along with 0.3 g dried *Enteromorpha*. Four one gallon jars were set up in each of the following temperatures: 5, 9, 13, 17, and 23 C. These jars were then aerated with plastic tubing and aquarium air stones. Food was added once a week.

The worm masses at the bottoms of the gallon jars were examined at 15 days and every 2 to 3 days thereafter. After the egg masses were laid, the tube and egg mass were removed and the eggs counted. The experiment continued until the females had either laid eggs or had died.

F_1 and F_2 Generation Reproduction and Egg Production Experiment. The worms were set up in the same manner as in the long-term egg production experiment except, as soon as an egg mass was laid, all the other worms except the one incubating the eggs were removed. The eggs were allowed to develop to the 18-21 setigerous segment at which time feeding had commenced.

Ophyryotrocha sp. *Ophryotrocha* sp. is a new species from Los Angeles Harbor which is currently being described by Åkesson. This species has been maintained in the laboratory for approximately 1.5 years. These worms thrive on finely ground *Enteromorpha crinita* or commercially prepared fish food. The culture is maintained in one gallon glass jars with aeration.

This species is a protandric hermaphrodite. On the average, each worm develops four eggs which are laid and develop directly into young worms without a trochophore stage. The adults appear to develop only one batch of eggs during their lifetime. About one week is required to reach sexual maturity.

96 Hour Temperature TL_m Experiment. The 96 hour TL_m values for temperature and *Ophryotrocha* sp. were established. The temperatures used were 5, 9, 13, 17, 23, 26, 29, 32, and 35 C. There were five replications at each temperature.

Material from the bottom of a gallon jar used for culturing the laboratory population was pipetted into a petri dish. The petri dish was placed under a dissecting microscope and the worms were separated to a second petri dish containing sea water. These worms were checked for injuries, and all injured worms were discarded. Five worms each were placed in a 70 mm stender dish with approximately 20 ml of sea water. A small amount of finely ground *Enteromorpha crinita* was added to just cover the surface of the solution, and the dishes were then covered with a ground glass cover. Each stender dish was then placed in its respective temperature. At the end of 96 hours, the number of worms surviving was recorded.

2 Week Reproduction Experiment. The tests of the effects of various temperatures on the reproduction of *Ophryotrocha* were made in the same manner and at the same temperatures as the 96 hour test except that the reproduction tests were run for two weeks. The worms were fed once during the experiment.

RESULTS--*Capitella capitata*--28 Day TL_m Experiment. The data for the TL_m temperature experiment using *Capitella capitata* are summarized in Table I. The 28 day TL_m values were 7.8 for the lowered and 26.6 C for the elevated temperatures. Worms placed in 32 and 35 C essentially did not feed and were all dead within 8 days. Worms placed in 5 C also showed very little feeding activity, but 20 per cent survived the 28 day period.

Neanthes arenaceodentata--28 Day TL_m Experiment. The data for the TL_m experiment using *Neanthes arenaceodentata* are summarized in Table II. The minimum and maximum TL_m temperature values are 12.3 and 24.6 C, respectively. The worms did not feed in either a 5 or 35 C environment and died within four days. At 9 C, only one worm fed and all died within 20 days.

Long-term Reproduction Experiment. The data on the effects of temperatures on egg development and egg laying in adult *Neanthes arenaceodentata* are summarized in Table III. There were no survivors in 5 or 9 C. The only worms that have laid eggs to date were the 23 C group. The average number of eggs laid in the 23 C temperature was 452 and the average time for egg laying from the beginning of the experiment was 62 days. Worms at 13 and 17 C are living but have not yet laid eggs at 90 days.

SUMMARY. 1. The effects of various temperatures on survival and reproduction were tested under laboratory conditions using three species of polychaetous annelids cultured in the laboratory, *Capitella capitata*, *Neanthes arenaceodentata*, and *Ophryotrocha* sp.

2. The minimum and maximum 28 day TL_m temperature values for *Capitella capitata* were 7.8 and 26.6 C, respectively.

3. The minimum and maximum 28 day TL_m temperature values for *Neanthes* were 12.3 and 24.6 C, respectively.

4. *Neanthes* at 23 C produced an average of 452 eggs per female.

5. The laboratory procedures for the long term experiments through the F_2 generation were outlined for *Capitella capitata*, *Neanthes arenaceodentata*, and *Ophryotrocha* sp.

TABLE I

THE EFFECTS OF VARIOUS TEMPERATURES ON THE SURVIVAL AND FEEDING OF *CAPITELLA CAPITATA* OVER A 28 DAY PERIOD

	Temperature (°C)								
	5	9	13	17	23	26	29	32	35
Day									
0	10-0	10-0	10-0	10-0	10-0	10-0	10-0	10-0	10-0
4	10-1	10-4	10-2	9-7	9-7	9-9	8-8	2-1	0-0
8	10-1	10-4	10-7	9-8	8-6	9-9	5-5	0-1	0-0
12	10-1	10-4	10-7	9-8	8-6	9-9	5-5	0-1	0-0
16	5-1	9-4	10-7	9-8	8-8	9-9	4-5	0-1	0-0
20	3-1	9-4	10-8	9-9	8-8	9-9	4-5	0-1	0-0
24	3-1	9-8	9-9	9-9	8-8	9-9	4-5	0-1	0-0
28	2-1	8-8	9-9	9-9	8-8	8-9	1-5	0-1	0-0

The first number given indicates the number of specimens surviving.

The second number given indicates the number of specimens observed with fecal pellets.

TL_m	Temperature (°C)
Minimum	7.8
Maximum	26.6

TABLE II

THE EFFECT OF VARIOUS TEMPERATURES ON THE SURVIVAL AND FEEDING OF *NEANTHES ARENACEODENTATA* OVER A 28 DAY PERIOD

	Temperature (C)								
	5	9	13	17	23	26	29	32	35
Day									
0	10-0	10-0	10-0	10-0	10-0	10-0	10-0	10-0	10-0
4	0-0	10-0	10-0	10-4	10-10	10-10	10-10	9-5	0-0
8	0-0	10-0	10-3	10-10	10-10	9-10	10-10	2-8	0-0
12	0-0	8-1	10-7	10-10	10-10	9-10	8-10	0-8	0-0
16	0-0	1-1	9-7	10-10	10-10	7-10	2-10	0-8	0-0
20	0-0	0-1	8-8	10-10	10-10	4-10	0-10	0-8	0-0
24	0-0	0-1	7-9	10-10	9-10	3-10	0-10	0-8	0-0
28	0-0	0-1	6-9	10-10	8-10	2-10	0-10	0-8	0-0

The first number given indicates the number of specimens surviving.

The second number given indicates the number of specimens observed with fecal pellets.

TL_m	Temperature (C)
Minimum	12.3
Maximum	24.6

TABLE III

THE EFFECT OF VARIOUS TEMPERATURES ON THE REPRODUCTION AND NUMBER OF EGGS LAID IN *NEANTHES ARENACEODENTATA*

	Temperature (°C)				
	5	9	13	17	23
Initial number of worms in the series	16	16	16	16	16
Number of survivors of egg-laying*	0	0			8
Number of survivors at termination**	0	0			4
Number of females laying eggs	0	0			4
Total number of eggs laid	0	0			1808
Number of eggs (mean)	0	0			452
Range in number of eggs laid	0	0			93-632
Number of days required for egg laying	-	-			62

*Defined as the total number of worms that were alive at the time of egg-laying, in each worm's respective one gallon jar. The males may eat the females after the eggs are laid.

**Defined as that time when all females have laid eggs within a given series. Four was the maximum number of females which could lay eggs within a temperature series.

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MARINE STUDIES OF SAN PEDRO BAY, CALIFORNIA. PART III

TOLERANCE OF *LITTORINA PLANAXIS* AND *L. SCUTULATA* TO TEMPERATURE CHANGES

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ABSTRACT. This survey was conducted primarily to consider the effects of reduced water temperature on two intertidal snails, *Littorina planaxis* and *L. scutulata*. The parameters considered included mortality, attachment of animals to the substrate, size of animals, and season of the year. Additional experiments were conducted on elevated temperatures to consider whether the animals respond merely to temperature change itself, or whether the direction of temperature change is important. Within the range of 6°C to 29°C the response appears to be seasonal and size dependent in *L. planaxis*. *Littorina scutulata* is more tolerant of 6°C than 29°C. No mortality was recorded at 6°C while mortality was recorded in both species at 29°C. The rate of attachment was influenced by temperature in both species. This is important, because unattached animals are readily washed away from the population. *Littorina scutulata* had a higher attachment rate than *L. planaxis* at 6°C. When both species are subjected to a 12°C drop in temperature, there is often a short period when attachment rate is low so that intermittent exposure to such changes could result in a gradual loss of animals through wave action during such periods.

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TOLERANCE OF *LITTORINA PLANAXIS* AND *L. SCUTULATA* TO TEMPERATURE CHANGES

INTRODUCTION. Several possibilities are foreseen in predicting the effects of a cold water effluent on species. The effects may be of lethal or sublethal nature. The organisms may be continually exposed to the effluent, and thus merely experience a lower temperature range than that experienced prior to exposure to the effluent, or they may be exposed intermittently, which would mean they would be exposed to widely fluctuating water temperatures. As the Los Angeles Harbor is cleaned up, there may be changes in the tolerance of species to low temperature made possible by reduction of the stresses of pollution in the area. Organisms may initially suffer a high mortality on exposure to reduced temperature, but a population tolerant to lower temperatures may gradually become established in the area. There may be a seasonal variation in tolerance to low temperatures, or there may be variations associated with other components in the water.

Two species of the cosmopolitan genus *Littorina* occur in the Los Angeles Harbor, *L. planaxis* and *L. scutulata*. Both species of snails inhabit rocky substrates. Although *L. planaxis* occurs at higher intertidal levels, the zones of distribution of the two species overlap. *Littorina planaxis* is found up to 5 to 10 feet above spring high tide levels, but *L. scutulata* is found in a zone extending 2 or 3 feet on either side of the high tide mark (North, 1954).

Both species are wide-ranging in their distribution. *Littorina planaxis* ranges from Puget Sound to Baja California, and *L. scutulata* ranges from Alaska to Baja California (Ricketts, Calvin, 1968). Although both show a wide tolerance to different temperatures, *L. scutulata* has the more northerly range of the two species, suggesting a greater tolerance to low temperatures. Both species are also exposed to a wide range of both water and air temperature daily due to their high intertidal distribution.

Three criteria were used to define the effects of temperature on *Littorina planaxis* and *L. scutulata* populations: mortality, attachment of animals to the substrate, and size. The attachment of animals to the substrate is as important as mortality in the maintenance of field populations of these species, because if animals do not attach firmly to the substrate, they can be removed by wave action and effectively lost from the population.

MATERIALS AND METHODS. Animals used in the experiments were collected primarily in the Los Angeles Harbor on a rocky jetty dividing Inner and Outer Cabrillo Beach. In the Los Angeles Harbor *Littorina planaxis* was more abundant than *L. scutulata*, except in November when the reverse was true. The use of *L. scutulata* was often subject to availability. Animals of the two species collected from Little Fisherman's Harbor at Santa Catalina Island were also considered in cases where there was difficulty in obtaining sufficient numbers from the Los Angeles Harbor, and also to provide data on animals not exposed to Los Angeles Harbor pollution.

The procedure used for determining the activity rates was based on that established by Crapp (1971). Activity was assessed on a simple scale (0-3) of arbitrary values as follows:

3 = animals showing normal behavior, characterized by crawling out of water and attaching to the glass

2 = animals which were crawling on the floor or sides of the container but had not emerged from the water

1 = animals which were not crawling or attached, but which had an extended foot and were responsive to stimulation

0 = animals closed and showing no signs of life

Each of these stages was very obvious, but dead animals often could not be distinguished from stage 0 animals for several days until decomposition took place.

In the present study, Crapp's scale for degree of activity was modified. Levels 3 and 2 were combined; therefore, if the animals were attached to the glass, in or out of the water, they were considered "active". The 0 and 1 levels were combined, making the fact that they were not attached to the substrate and were readily moved a sign of an effective loss from the population. This distinction is appropriate, considering the importance of their ability to attach themselves to the substrate in order to withstand wave action.

Standard experimental temperatures were used throughout to determine seasonal changes in temperature tolerance. First, 4-6°C was chosen because it was 4-6°C below the lower levels of winter temperatures previously recorded in Los Angeles Harbor. Second, 15-17°C was selected because it was 4-6°C lower than summer temperature extremes from Palos Verdes (United States Department of Fisheries Data)and Los Angeles Harbor (M. Patterson, unpublished data). These temperatures were also used in on-going experiments on *Littorina* species from other areas. An upper temperature of 28-29°C was used in the current experiments. Throughout, these temperature experiments will be referred to as 6°C, 17°C, and 29°C.

Four series of experiments were conducted. Series 1 was aimed at determining seasonal differences in temperature tolerances over a period of one week; Series 2 was aimed at determining tolerances to intermittent (e.g. one high tide) exposure to low temperature seawater; Series 3 addressed itself to the applicability of this data as related to changing water quality in Los Angeles Harbor; Series 4 was designed to study the effects of longer term (2 months) exposure to reduced water temperatures.

In the first series of experiments, groups of 25 animals were placed in loosely covered glass pin bowls (5-6 cm in diameter, 4.5 cm deep) containing artificial seawater which was changed each day for one week. The animals were allowed to move in and out of the water freely. This meant that in the controlled temperature chambers they were exposed to air temperatures similar to water temperatures - a situation that seldom occurs in the field. As both species spend much of their time out of the water under laboratory conditions, it is difficult to relate to field conditions. Experimental temperatures were 6°C, 17°C, and 29°C. Activity and mortality were recorded daily. Also the size ranges of the animals were recorded. Data from Santa Catalina Island animals were also considered in this series.

In a second series of experiments using artificial seawater, groups of 35 animals were exposed to 6°C and 17°C. *Littorina scultulata*, the lower intertidal species, was kept under water by placing a mesh screen just below water level for 3 to 6 hours at both 6°C and 17°C, and then maintained at 17°C under conditions described for Series 1 experiments for seven days. *Littorina planaxis* was likewise kept under water for 3 hours at both 6°C and 17°C and then maintained at 17°C. Activity and mortality were recorded daily. The size range of each group of experimental animals was recorded.

In the third series of experiments, groups of 35 animals were kept under water for 3 hours at 6°C, 17°C, and 29°C, and then maintained at 17°C for a week. This experiment was conducted using artificial seawater, seawater collected from Los Angeles Harbor, Marineland filtered seawater, and Coal Oil Point seawater.

In the fourth series of experiments, groups of 35 animals were maintained for 2 months under two temperature conditions. Three groups of animals were exposed to 13°C, and two groups were exposed to water temperatures directly affected by the air temperatures. In this situation air and water temperatures were recorded. These experiments were conducted in water baths; therefore, the animals were allowed access to normal air temperatures. Size and weights were recorded before the experiment, after 1 month, and after 2 months. The water was changed every three days when activity and mortality were recorded. Los Angeles Harbor water was used in this experiment to provide a source of algal spores to allow an algal film to grow and thus provide food for the animals.

In all experiments seawater was maintained in all experimental chambers so that animals were not exposed to temperature shock when the water was changed. All animals were returned from the sides and lids to the bottom of the experimental containers each time the water was changed. The animals were not fed in the first three series of experiments.

Experiments were started as soon as possible after the animals were collected. Animals were transported dry in plastic bags and kept in these bags at room temperature until the experiment was started.

All experiments were conducted with the animals in glass pin bowls with loosely fitting petri dish lids that allowed air exchange but prevented the *Littorina* from escaping. Pin bowls were two-thirds (80 mls) full of water during these experiments.

RESULTS AND DISCUSSION. The average yearly temperature at Cabrillo Beach Inner Harbor (Harbor Project Station A9) is approximately 16°C. During the 17 months period (July 1972-November 1973) when these experiments were in progress, the maximum monthly surface temperature was 20°C and the minimum monthly surface temperature was 12°C (Table 1) at Station A9. Water temperatures showed similar trends, but fluctuated more at Santa Catalina Island.

While the purpose of these experiments was to determine the effects of cold temperatures on the two species of *Littorina*, the data for 29°C is considered important as a comparison to determine if a change in temperature alone is important and/or if the direction of that change is the important factor. In the first series of experiments there were no mortalities at either 6°C or 17°C; however, at 29°C, mortalities occurred in both species.

All mortality in *Littorina planaxis* was recorded among the larger animals (average shell length 7 to 8 mm and longer), while mortality was recorded in most experiments with *L. scutulata* at 29°C (Table 2. See Appendix 1 for details of size of animals used).

In experiments with *Littorina planaxis* there is no consistent seasonal trend in the attachment of animals to the substrate at high (29°C) or low (6°C) temperatures. In some instances (May 1, 1973) the number of *L. planaxis* attached to the substrate was lower at both 6°C and 29°C than at 17°C; in other instances (May 23, 1973), over 90% of the *L. planaxis* were attached to the substrate at all three temperatures; in other instances (July, 1972), all *L. planaxis* were attached at 17°C and 29°C and only 48% were attached at 6°C.

Experiments in May and November 1973 using *Littorina planaxis* of different sizes showed that at 29°C more smaller animals (average length less than 9 mm) were attached to the substrate than were larger animals (average length more than 9 mm) (Table 3, Appendix 1). It should be noted that in May all of the unattached animals were dead by 144 hours. All the survivors, therefore, were attached for the last two days of the experiment. There was no consistent relationship between size and attachment rate of *L. planaxis* at 6°C in these experiments.

Littorina scutulata consistently has a higher attachment rate at low temperatures (6°C) than at high temperatures (29°C) after 7 days exposure (Table 4). There appears to be a short acclimation period in some instances at 6°C (May and September 1973). The higher rate of attachment in *L. scutulata* is probably due to their more northerly distribution and selected habitat, suggesting a greater tolerance to low temperatures.

In the second series of experiments, *Littorina planaxis* and *L. scutulata* were kept under water for 3 hours at 6°C and 17°C. *Littorina scutulata* was also kept under water for 6 hours at both temperatures, and the animals were allowed to recover, moving freely in and out of the water at 17°C. Experiments were conducted in March with both species collected from winter water temperatures in Los Angeles Harbor. In September, experiments were conducted with *L. planaxis* only, collected from summer water temperatures in Los Angeles Harbor; *L. scutulata* was not available in sufficient numbers. Both sets of *L. planaxis* were approximately the same size range, with average lengths of 12.2 mm and widths of 9.0 mm.

The only significant reduction in attachment occurred after *Littorina scutulata* was kept under water for 6 hours at 6°C (Table 5). This species may normally be under water for up to 6 hours during high tides.

The attachment rate after animals were kept under water for 6 hours at 17°C was higher than that after animals were kept under water for 6 hours at 6°C (31 c.f. 20 animals). This suggests that prolonged exposure under water at low temperatures reduces the attachment rate. This could be a result of the shock of the 6°C exposure, because after recovery for 24 hours at 17°C all animals were attached. There was no reduction in attachment of *Littorina planaxis* kept under water for 3 hours after collection from either summer or winter water temperature requirements.

In preliminary oxygen consumption experiments it was shown that 35 *Littorina planaxis* in approximately the same volume of water as that used in the above experiment used up almost all of the available oxygen after 3 hours at 17°C. The conditions were not exactly the same as those in the actual experiment, where there was some interaction between air and water temperatures. Further experiments in this area should be conducted to determine if lack of oxygen is causing the reduced attachment after 6 hours exposure under water at the two temperatures.

In the third series of experiments, water from four sources was used to determine whether the chemical composition of the sea water influenced the survival or attachment rate of *Littorina*. Artificial seawater was used as a control. Water obtained from Coal Oil Point, Marineland, and Los Angeles Harbor have different sources of pollution. Coal Oil Point samples possibly contain petroleum hydrocarbons. Los Angeles Harbor water could be contaminated from many sources in the Los Angeles Harbor. Marineland water is filtered, but may be contaminated by sewage effluents. Animals were kept under water for 3 hours at the experimental temperatures (6°C, 17°C, 29°C). They were then allowed to recover for a week at 17°C and move freely in and out of each of the four different types of water. Both species were collected in winter water temperatures (12.2°C.). The average size of *L. planaxis* used from the Los Angeles Harbor was comparable with the average size of animals used in the other series of experiments (12.5 mm x 9.5 mm).

No mortalities occurred in the third series of experiments, and Table 6 shows that there were no effects on the rates of attachment. These animals, which were collected at winter temperatures, were not adversely affected by short term (3 hour) exposure to either elevated (29°C) or lowered (6°C) temperatures in seawater from four different sources.

In the fourth series of experiments, animals were maintained for two months in water baths at 13°C and were directly affected by air temperatures (Table 7). All groups of animals were able to migrate up out of the water onto the side of the bowl, where the air temperatures ranged from 24°C to 28°C during the experiments.

The Spearman Rank Correlation Coefficient (Siegel, 1956, p. 202) was calculated to determine if there was a significant change in attachment rates with time in these experiments. There was no significant change in *Littorina planaxis* maintained in the 13°C water bath. However, there was a significant reduction in attachment rate in *L. planaxis* maintained in the water bath at 21.5-24.5°C ($p = 0.5$, 0.1) and *L. scutulata* maintained in a water bath at 13°C ($p = 0.1$). One animal also died in each of the duplicate *L. planaxis* experiments maintained at the warmer temperature. The experiments were commenced in June 1973, when water temperatures were above 15.0°C. It is difficult at this stage to interpret the results of these experiments because there are at least two other possible explanations besides a direct temperature effect; our attempts to provide food also provided a very dense bacterial culture in the warmer water, and the *L. scutulata* had a smaller animal to water volume ratio than the *L. planaxis*.

SUMMARY. The data presented indicate the following:

1. A drop in temperature of 6°C in winter and 12°C in summer did not cause mortality in *Littorina planaxis* or *L. scutulata*.

2. The reduction in temperature resulted in a substantial reduction in attachment rate of *Littorina planaxis* during summer, and a lesser reduction in attachment rate of *L. scutulata* during summer.

3. Upon sudden exposure to low temperatures, both species undergo a period of inactivity. This is longer in *Littorina planaxis* than in *L. scutulata*.

4. Short-term exposure (e.g. tidal cycle) to temperatures of 6°C did not cause any mortality and did not affect the attachment rate during a subsequent recovery period.

5. Increased temperatures (29°C) caused some mortality. In *Littorina planaxis* this appeared to be related both to the size of the animals and the season.

6. Experiments with artificial seawater provided results that can be extrapolated to Los Angeles Harbor, except in areas and periods of high pollution.

7. Data from long term (2 months) experiments is inconclusive.

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TABLE 1

Surface Water Temperatures (°C)

		Cabrillo Beach Inner Harbor	Santa Catalina Island
1972	April	16.20	14.4
	May	14.50	16.1-16.7
	June	17.90	17.9
	July	18.00	18.9-19.4
	August	19.40	20.0
	September	20.00	19.4-20.0
	October	18.00	18.9-19.4
	November	15.80	17.9
	December	13.90	15.5-16.7
1973	January	13.40	17.0
	February	13.30	15.0
	March	15.10	13.9-15.0
	April	12.20	14.4
	May	15.20	13.3-15.5
	June	15.90	16.1-16.9
	July	19.00	16.9-18.0
	August	18.10	18.3-18.8
	September	16.70	18.0

Cabrillo Beach Inner Harbor data is Harbor Project Station A9 data. Santa Catalina Island data is United States Department of Fisheries Monthly Surface Sea Temperature data. Harbor Project data was recorded monthly and Department of Fisheries Data, twice a month.

TABLE 2

Mortality at 29°C

Average shell length (mm)	1972	1973				
	July	May	Aug.	Sept.	Nov.	
5 - 6	0				0	
6 - 7		0				
7 - 8				25	0	L. planaxis
8 - 9						
9 - 10					0	
10 - 11		13				
11 - 12			10		0	
5 - 6					1	
6 - 7				1		
7 - 8					0	
8 - 9		8				L. scutulata
9 - 10						
10 - 11			1		5	

TABLE 3

Number of Littorina planaxis Attached to Substrate in Series 1 Experiments

			Hours of Exposure							
		°C	1 hr.	24	48	72	96	120	144	168
Outer Cabrillo July, 1972		6	24	14	24	23	21	16	17	12
		17	24	25	25	25	25	25	25	25
		29	23	24	25	25	25	25	25	25
Catalina August, 1972 18 animals used at each temp.		6	15	2	5	10	10	8	10	0
		17	18	18	17	18	18	17	17	16
		29	14	18	18	17	18	18	18	17
Catalina January, 1973		6		25	25	25	25	25		
		11		25	25	25	25	25		
		17		25	25	25	25	25		
		29		25	25	25	25	25		
Catalina May 1, 1973 ( ) number dead		6	22	4	24	25	24	25	24	19
		17	25	23	25	23	24	24	24	25
		29	20	14	14	11	11 (7)	11 (2)	12 (4)	12
Catalina May 23, 1973		6	24	25	22	23	23	25	25	24
		6	24	24	23	25	24	22	22	24
		17	25	23	25	25	24	24	25	24
		17	25	25	25	24	24	25	25	24
		29	22	23	24	24	24	24	24	23
		29	22	25	25	25	22	25	25	25
		29	23	25	24	24	24	24	22	23
Inner Harbor August, 1973 + not counted		6		25	25	25	23	23	17	12
		17		25	25	24	24	24	24	25
		29		25	23	20	20	20	+	8 (10)
Catalina September, 1973		6	7	0	17	19	16	15	21	9
		17	25	25	24	25	24	25	25	24
		29	20	12	9	1	0 (25)	0	0	0
Inner Harbor November, 1973	Small	6		25	24	23	25	22	6	4
	Medium	6		22	23	22	21	18	2	1
	Large	6		20	16	20	21	22	11	9
	Small	17		25	25	24	25	23	25	25
	Medium	17		23	25	25	23	23	25	25
	Large	17		22	25	24	23	24	24	24
	Small	29		25	24	25	25	24	18	15
	Medium	29		25	25	24	24	25	24	24
	Medium	29		24	24	23	18	19	1	0 (25)
	Large	29		25	22	24	18	19	3	1

TABLE 4

Numbers of Littorina scutulata Attached to Substrate in Series 1 Experiments

		C°	1	24	48	72	96	120	144	168
Catalina		6		12	12	12	12	12		
January, 1973		11		12	12	12	12	12		
12 animals used		17		12	12	12	12	12		
at each temp		29		12	12	12	12	12		
Catalina		6	13	25	25	25	25	25	25	24
May 1, 1973		17	25	25	25	25	25	25	25	25
		29	21	24	23	23	17	7	9 (3)	8 (5)
Inner Harbor		6		25	25	25	24	24	23	24
August, 1973		17		25	25	25	25	25	24	25
+ not counted		29		24	25	24	23	23	+	18 (1)
Catalina		6	7	7	23	25	24	14	17	21
September, 1973		17	25	25	25	23	25	25	24	24
		29	20	16	12	5	2	1	0 (1)	0
Inner	Small	6		25	25	24	24	25	24	23
Harbor	Large	6		25	25	25	25	25	24	23
November,	Small	17		23	24	24	24	24	24	24
1973	Large	17		25	25	22	25	24	25	24
	Small	29		23	24	24	23	22 (1)	14	13
	Medium	29		23	25	24	25	25	23	21
	Large	29		24	18	15	16	13	2	2 (5)

( ) number dead

TABLE 5

Attachment of *Littorina* in Series 2 Experiments

Number of Hours Under Water	Hours of Exposure 3	6	24	48	72	96	120	144	168	Temperature Under Water
0	35		35	35	35	35	35	35	35	
	35		35	35	34	33	35	34	35	
3	30		35	35	35	33	29	35	35	17°C
6		31	35	35	34	35	35	34	35	
3	34		35	35	34	34	34	34	35	6°C
6		20	33	35	34	34	35	34	34	
	A. *Littorina scutulata* (March, 1973)									
0	34		35	35	35	35	33	35	35	
	34		35	35	35	35	35	35	35	17°C
3	35		35	35	35	33	35	35	35	
	34		35	35	34	34	34	35	35	
3	34		35	35	35	35	35	35	33	
	32		35	35	35	35	35	34	34	6°C
	B. *Littorina planaxis* (March, 1973)									
0	35		35	35	35	33	34	35	35	17°C
3	33		35	34	33	34	34	33	32	
	33		35	35	35	34	35	34	34	
3	35		34	35	34	35	34	35	35	
	33		34	34	32	34	33	35	34	6°C
	C. *Littorina planaxis* (September, 1973)									

35 animals used in each experiment

TABLE 6

Attachment rate of Littorina planaxis in seawater from different sources, after exposure under water to experimental temperatures for 3 hours and maintained at 17°C for 168 hours

	3	24	48	72	96	120	144	168	Exposure Temperature
H	35	33	33	35	32	34	35	35	
AW	35	33	35	34	34	35	35	35	6°C
COP	35	35	35	34	34	35	35	35	
Marl.	35	35	34	35	35	35	35	35	
H	35	35	35	35	34	35	35	35	
AW	35	34	34	35	33	35	35	35	17°C
COP	35	33	35	34	34	35	35	35	
Marl.	35	33	34	32	35	34	35	34	
H	35	35	35	35	34	35	35	34	
AW	33	34	34	34	35	34	35	35	29°C
COP	35	34	33	35	35	35	35	35	
Marl.	35	34	34	34	35	35	35	35	

35 animals used in each experiment

H - Harbor water
AW - Artificial seawater
COP - Coal Oil Point water
Marl. - Marineland water

TABLE 7

Attachment rates of Los Angeles Harbor *Littorina* over two month exposure

A. *Littorina planaxis* (35 animals)

°C	Number of Days																		
	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57
13	34	33	34	34	35	32	34	33	33	34	33	32	35	34	34	35	33	34	35
	35	34	35	35	33	34	34	34	33	33	35	34	34	33	33	33	32	33	35
21.5-24.5	35	34	32	33	34	34	33	32	35	34	33	32	30	34	31	34	31	30	28
	35	33	33	32	34	35	34	33	33	31	34	34	30	31	31	31	29	29	30

B. *Littorina scutulata* (32 animals)

°C	Number of Days																		
	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57
13	32	31	32	31	30	31	31	32	31	30	30	31	31	20	31	28	27	27	21

APPENDIX 1
Table A

Size of Littorina planaxis in Series 1 Experiments

Station date		Temp °C	Length (mm)			Width (mm)		
			Maximum	Minimum	Mean	Maximum	Minimum	Mean
Outer Cabrillo								
July, 1972		6	7	4	5.6	5	3	4.0
		17	8	5	5.9	6	4	4.4
		29	8	4	5.3	6	3	3.8
Total					5.6			4.1
Catalina		6	15	8	9.3	11	6	7.3
May 1, 1973		17	15	8	10.4	12	5	7.8
		29	14	8	10.7	10	5	8.2
		29	15	8	10.6	11	6	7.8
Total					10.1			7.8
Catalina		6	8	4	6.5	6	3	5.4
May 23, 1973		6	7	5	6.0	6	4	5.0
		17	7	5	5.9	6	4	5.1
		17	8	5	6.4	6	4	5.2
		29	8	4	6.3	6	3	5.1
		29	7	4	5.9	6	3	4.8
		29	8	4	5.7	6	3	4.6
Total					6.1			5.0
Inner Harbor		6	14	10	12.2	11	7	9.2
August, 1973		17	14	9	11.7	11	8	9.0
		29	14	9	11.4	10	6	8.9
		29	14	10	11.3	11	8	9.0
Total					11.6			9.0
Catalina		6	12	5	7.2	10	4	5.6
September,		17	11	5	7.2	8	4	5.6
1973		29	14	5	7.9	11	4	6.1
Total					7.4			5.8
Inner	Small	6	7	5	6.2	6	4	4.8
Harbor	Medium	6	11	8	9.8	9	6	7.6
November,	Large	6	13	10	11.5	11	7	9.2
1973	Small	17	7	5	6.1	6	4	4.8
	Medium	17	11	9	9.7	9	7	7.7
	Large	17	13	10	11.8	11	8	9.6
	Small	29	8	5	6.0	7	4	4.8
	Medium	29	9	7	8.0	7	5	6.6
	Medium	29	11	8	9.6	9	6	7.4
	Large	29	14	10	11.7	11	8	9.5
Total	Small				6.0			4.8
Total	Medium				9.3			7.3
Total	Large				11.7			9.4

APPENDIX 1
TABLE B

Size of *Littorina scutulata* in Series 1 Experiments

Station Date		Temp °C	Length (mm) Maximum	Length (mm) Minimum	Length (mm) Mean	Width (mm) Maximum	Width (mm) Minimum	Width (mm) Mean
Catalina		6	12	6	8.6	8	4	6.1
May 1, 1973		17	12	6	8.5	8	5	6.1
		29	11	5	7.9	8	3	5.6
		29	11	6	8.2	7	4	6.2
Total					8.3			5.7
Inner Harbor		6	13	8	10.2	9	6	7.3
August, 1973		17	14	7	9.7	10	5	6.8
		29	14	8	10.4	10	5	7.2
Total					10.3			7.1
Catalina		6	7	5	5.9	5	4	4.4
September,		17	8	5	6.4	6	4	4.8
1973		29	7	5	6.0	5	4	4.7
Total					6.1			4.6
Inner	Small	6	7	4	5.0	5	3	3.8
Harbor	Large	6	13	8	10.0	10	5	7.0
November,	Small	17	7	4	5.2	5	3	4.0
1973	Large	17	14	8	10.0	9	5	7.2
	Small	29	6	4	5.0	5	3	3.9
	Medium	29	8	6	7.1	6	5	5.3
	Large	29	14	8	10.3	10	6	7.4
Total	Small				5.1			3.9
Total	Medium				7.1			5.3
Total	Large				10.1			7.2

MARINE STUDIES OF SAN PEDRO BAY, CALIFORNIA. PART III.

PRELIMINARY INVESTIGATIONS OF THE EFFECTS OF RESUSPENDED SEDIMENT ON TWO SPECIES OF BENTHIC POLYCHAETES FROM LOS ANGELES HARBOR

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ABSTRACT. *Ophryotrocha* nr. *labronica* and *Capitella capitata* were used as bioassay organisms to determine the toxicity of resuspended sediment from Los Angeles Harbor. Sediment was collected from four stations representing a range of polluted conditions. A test solution or "standard elutriate" was prepared according to EPA guidelines. An additional series of dilutions and concentrations were prepared to produce a range of toxic elutriates. Mortality of *Capitella capitata* trochophores and metatrochs was less than 50% in all short term 96 hour exposures. Long term (28 day) exposures were unsuccessful. *Ophryotrocha* nr. *labronica* did not undergo any mortality during a short term 96 hour exposure. Long term (28 day) exposure decreased reproductivity at high concentrations of resuspended sediment. Lower concentrations of resuspended sediment produced a stimulatory effect on reproduction. *Echone limnicola* and *Cirriformia spirabranchia* could not be maintained in culture in sufficient numbers to be used as experimental animals during these studies. Preliminary evidence suggests that harbor dredge spoils may have a potential role as a "sea fertilizer" which could be distributed in specified areas to improve the productivity of the marine environment.

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A special thanks to the many people involved in the Los Angeles Harbor Environmental Projects who assisted in the sampling program. Dr. K. Chen and Chun-Ching Wang were consulted on the preparation of the "standard elutriate."

PRELIMINARY INVESTIGATIONS OF THE EFFECTS OF RESUSPENDED SEDIMENT ON TWO SPECIES OF BENTHIC POLYCHAETES FROM LOS ANGELES HARBOR

INTRODUCTION. Bioassays to determine the effect of dredging on benthic invertebrates have not been reported in the literature to date. The procedures for the short-term or routine toxicity bioassay outlined in the American Public Health Association Standard Methods (1971) were designed for evaluating acute toxicity of industrial wastes and other substances to fish. These tests require a series of dilutions of the substance in water and recording of the survival after 24, 48, and 96 hours of exposure.

The Environmental Protection Agency has issued a preliminary statement concerning criteria for the handling of sediments for bioassays, in which no provision is made for testing a range of sediment-to-water ratios, using standard bioassay techniques.

The requirements of the State Water Resources Control Board, under the "Ocean Plan," outlines the statistical treatment of the data obtained from short term and long term bioassays, although no mention is made about concentrating or diluting sediments to determine thresholds of sensitivity for the test organism.

Two approaches were used in this study to produce a range of toxicities for the test organism with regards to dredging operations. (1) Representative sediment was obtained from a range of stations that included highly polluted areas and relatively unpolluted areas. (2) A series of increasing and decreasing proportions of sediment to water ratios were made from a single station.

MATERIALS AND METHODS. Test animals were collected from Los Angeles Harbor sediment and returned to the laboratory. Culturing was attempted with several species found in the harbor sediment, which included *Echone limnicola, Cirriformia spirabrancha, Capitella capitata,* and *Ophryotrocha* nr. *labronica*. Cultures of the Los Angeles Harbor strains of *Capitella capitata* and *Ophryotrocha* nr. *labronica* were obtained from Dr. Donald J. Reish of California State University, Long Beach. Stock cultures were maintained in the laboratory in one gallon jars of sea water which was changed every three weeks. The cultures were aerated and maintained on *Enteromorpha* which was collected locally. The two species utilized in this study were selected on the basis of their previous culturing success and well documented life histories. *Capitella capitata* has been used extensively by Reish and *Ophryotrocha labronica* by Åkesson, 1970.

Ophryotrocha were maintained on *Enteromorpha* and "Tetra," an easily obtained tropical fish food. Individuals are hermaphroditic. Synchronous development can be initiated by isolating the worms at 15°C. After one week the worms can be returned to room temperature, which will initiate egg laying within a 24 hour period. Eggs are laid about every two weeks, and individuals require about two weeks to complete sexual maturity. The ease of culturing and the relatively short life cycle of this animal make it ideally suited for long-term toxicity tests.

Experimental setups consisted of placing one specimen within a small stendor dish filled with 16 ml of a test solution or "standard elutriate." Test animals were maintained without aeration or food for periods of up to 28 days.

Capitella capitata stock cultures were maintained in one gallon jars kept at room temperature. They utilize *Enteromorpha* as a primary food source and for facilitating their tube construction. The females incubate their eggs within a mucoid tube. The trochophore stage is reached about 4-6 days after egg laying, upon which they will begin to leave the tube as a free swimming metatrochophore. The metatrochophore stage will last 1-2 days before metamorphosis occurs, whereupon the juvenile will become attached to the bottom of the test container within a mucoid tube. Females have been observed to undergo several egg laying periods. The second egg laying occurs about 5-10 days after the first. Juvenile worms require about 30 days to reach sexual maturity. These animals cannot be maintained for more than one week without food; thus any long term experiments with *Capitella* require controlled feeding and oxygenation of the test solution.

Experimental procedures consisted of isolating the female worms that were incubating their eggs. The mucoid tube was teased open with forceps. The exposed trochophores or metatrochs were picked up with a pipette. Fifteen or twenty-five larva were placed in a test container which consisted of a 16 ounce jar containing freshly prepared "standard elutriate." Beginning the experiment with the larval stage exposes the more sensitive stages of the life cycle to the test solution during its highest toxicity potential. Experiments were conducted at room temperature and the test solutions were not aerated or changed during the course of an experiment.

In the present preliminary experimental series, sediment was collected from four stations within the harbor by means of a small Campbell grab and returned to the laboratory for storage at 4°C. Preparation of the "standard elutriate," or test solutions, followed guide lines suggested in a preliminary Environmental Protection Agency Report (1973) concerning dredge spoil criteria. The sediment was prepared in a series of 16 ounce jars containing one part bottom sediment to four parts of sea water (by weight). The jars were shaken for 30 minutes on a high speed Gyrotory Shaker. The solution was allowed to settle for one hour, upon which 300 ml of the supernatant or "standard elutriate" was decanted into appropriate test containers. Experiments were conducted with a series of sea water to sediment ratios of 100:1, 10:1, 4:1, and 2:1, to produce a greater range of elutriate toxicity levels.

RESULTS. In the short term (96 hour) test with *Ophryotrocha* nr. *labronica* mortality did not occur to any of the specimens placed in a "standard elutriate" from each of the four stations. An additional series of ratios of sea water to sediment was prepared from station 5C and station 6C, but no mortality occurred within a 96 hour period. Sublethal effects, which included reduced egg production and reduced growth rates, occurred at the higher concentrations of elutriate; however, this evidence was not quantified at this time.

The short term (96 hour) test with *Capitella capitata* produced mortality rates in both trochophores and metatrochs of less than 50% from all four stations (Table 1). Mortality was greatest for both trochophores and metatrochs with "standard elutriate" from station 6C. Station 4C produced the lowest mortality rates. Trochophores tested in "standard elutriate" had mortality rates slightly higher than metatrochs. Trochophore controls had considerably higher mortalities than metatroch controls, which may be due in part to their premature removal from the adult tube. (See map for sediment sample 3, 4, 5, and 6 locations and Table 2 for trace and heavy metal and pesticide analyses.)

The formula for determining toxicity concentrations for mortality of less than 50% has been suggested by the California State Water Resources Control Board as follows:

$$Tc\ (tu) = \frac{\log\ (100 - S)}{1.7}$$

S = percentage survival in 100% waste

In the long term (28 day) test with *Ophryotrocha* nr. *labronica* a range of sediment-to-water ratios including a "standard elutriate" were produced with sediment from station 4C. *Ophryotrocha* produced correspondingly fewer numbers of offspring in increasing concentrations of sediment with the exception of the 100:1 ratio of sea water to sediment (Figure 1). The 100:1 elutriate produced more offspring than the control group or any subsequent group tested on other ratios of sediment to sea water.

Long term (28 day) tests were conducted with *Ophryotrocha* to compare the effects of the "standard elutriate" from stations 4C and 6C (Figure 2). Adults placed in a "standard elutriate" from station 4C produced 66.6% more offspring than individuals exposed to a "standard elutriate" from station 6C. Control groups produced more offspring than any of the groups exposed to a "standard elutriate."

CONCLUSIONS. These preliminary experiments have provided an indication of a need for additional testing. The short term experiments should be repeated and, in addition, should include a third series of test chambers which would contain a portion of the "standard elutriate" that has been centrifuged or filtered to remove the particulate fraction. The particulate fraction may serve to improve water quality by the absorption of heavy metal and pesticide components from the water column.

Additional long-term tests need to be conducted to determine the significance of such sublethal effects as reduced fecundity and slower growth rates. Tests conducted for extended periods will necessitate providing food to the test animals and aeration of the test chambers. The effect of feeding and aeration should be monitored by daily measurement of dissolved oxygen (DO) and pH changes in the test chambers.

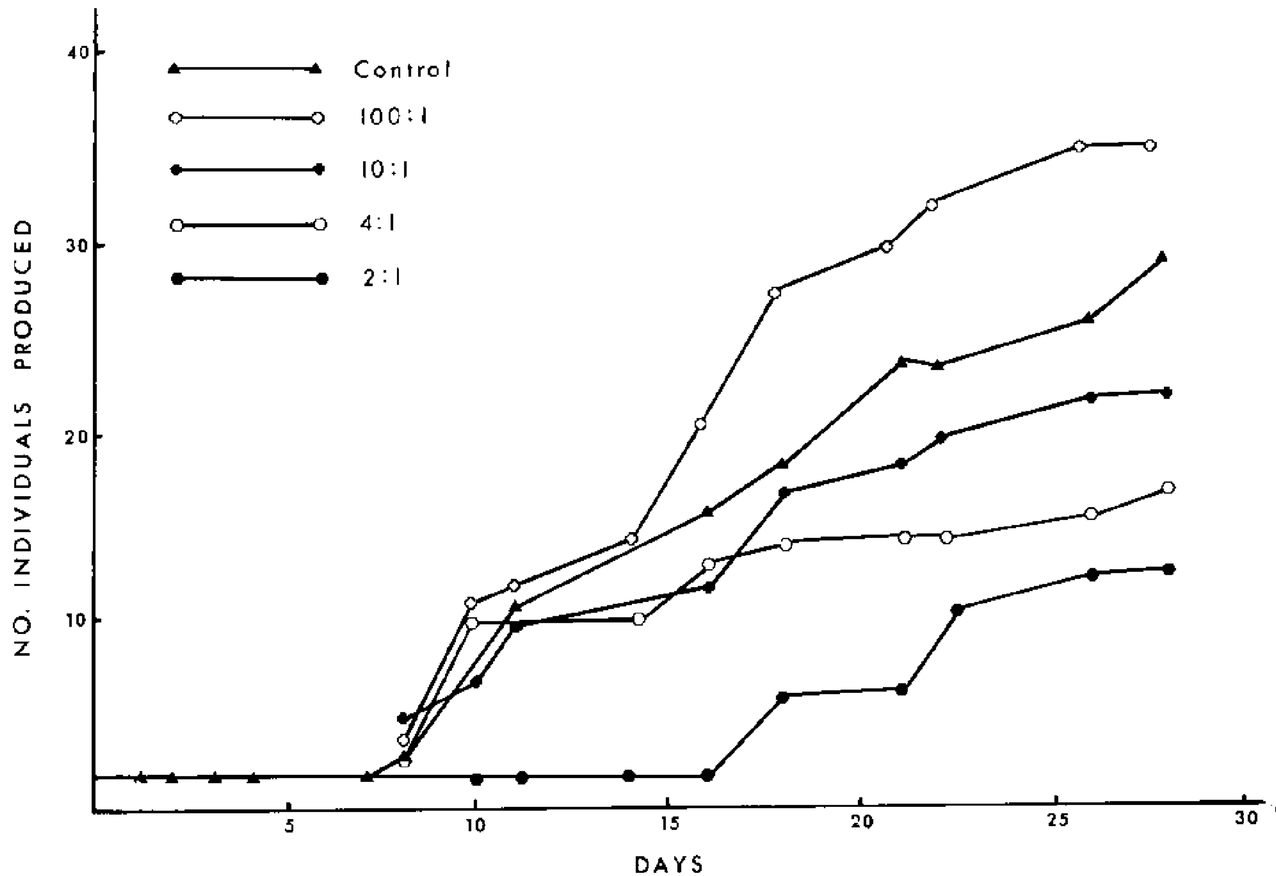

Figure 1. Number of *Ophyotrocha* nr. *labronica* produced within a 28 day period from Station 4C. Seawater to sediment ratios of 100:1, 10:1, 4:1 and 2:1 were tested.

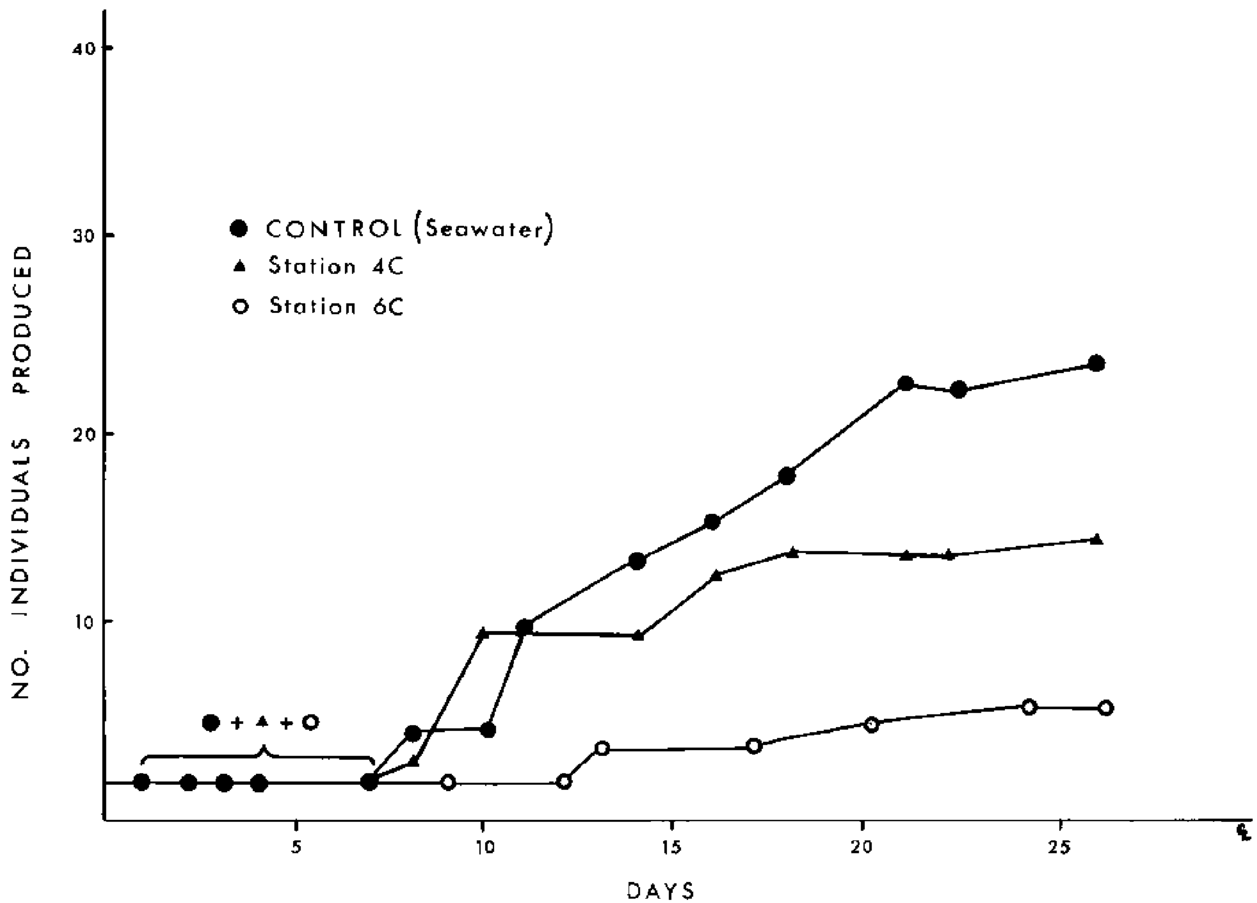

Figure 2. Standard Elutriate test. Number of *Ophyotrocha* nr. *labronica* produced within a 28 day period. Control seawater was collected at the surface near Station 4C.

Preliminary evidence indicates that a potentially stimulating effect on *Ophryotrocha* reproduction may result from some dredging activities. Additional tests will be necessary to better interpret the significance of this phenomenon and to determine whether there is stimulation in subsequent reproductive generations. Dredging spoils might serve an important roll as a "sea fertilizer" which could be distributed in specified areas to improve the productivity of the marine environment, should this prove true.

TABLE 1

Survival of *Capitella capitata* larva tested in "standard elutriate" from each station in short-term (96 hour) test.

Station	Larval Stage	Per Cent Survival: Control Group*	Per Cent Survival: Test Group	Toxicity Concentration
3C**	Trochophores	78%	68%	.888
3C	Metatrochs	100%	73%	.781
4C	Trochophores	88%	76%	.812
4C	Metatrochs	94%	87%	.650
5C	Trochophores	72%	68%	.888
5C	Metatrochs	100%	60%	.894
6C	Trochophores	75%	68%	.919
6C	Metatrochs	100%	60%	.944

*Low percentage of survival of trochophore controls may be due to premature removal from the adult tube.

**Stations sampled in triplicate; A samples were used for chemical analysis, B samples for benthic fauna identification, and C samples for toxicity studies.

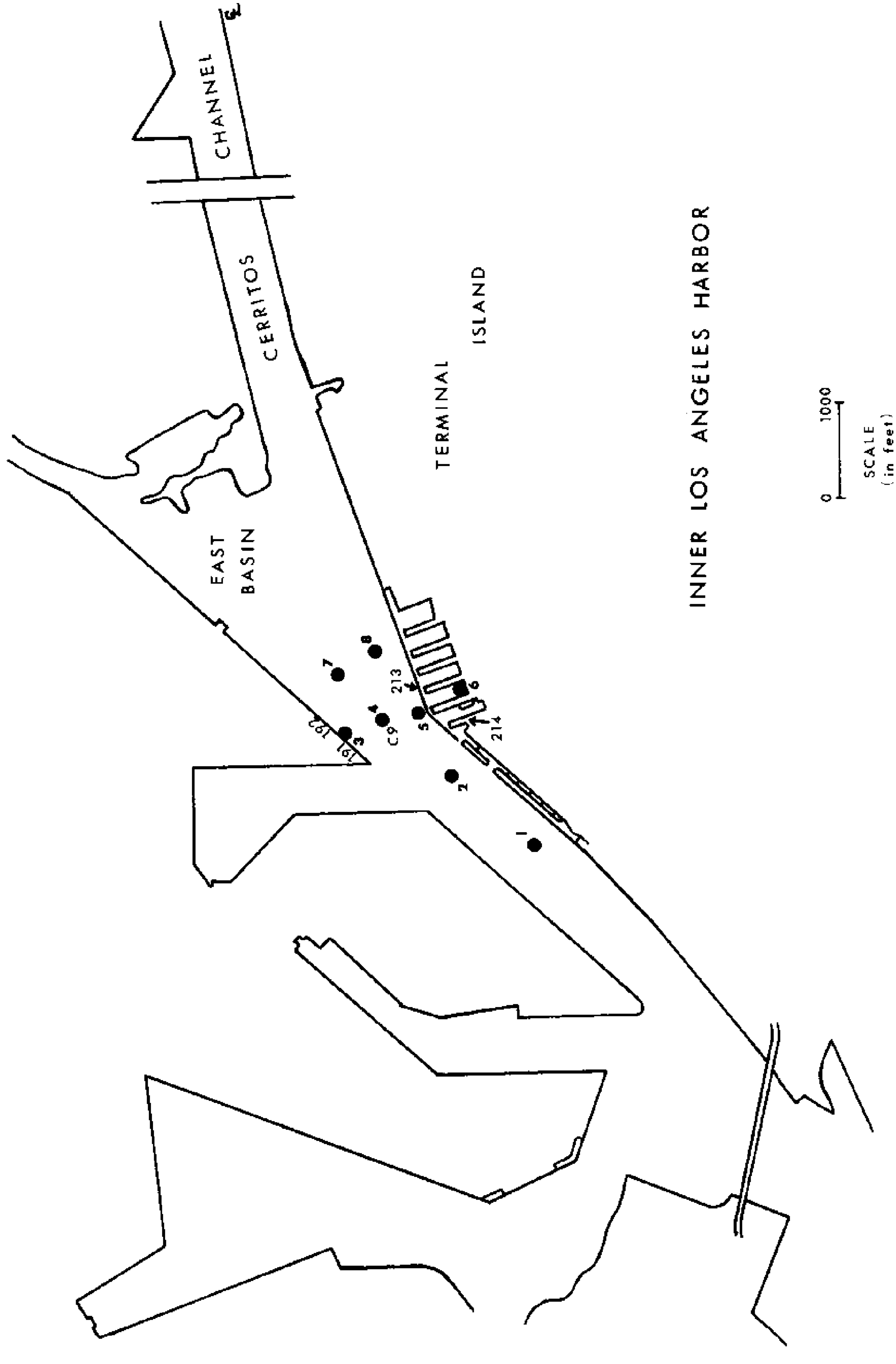
CERRITOS CHANNEL
TERMINAL ISLAND
EAST BASIN
1
2
3
4
5
6
7
8
C9
191
192
213
214
0
1000
SCALE
(in feet)
INNER LOS ANGELES HARBOR

Table 2. BOTTOM SEDIMENT ANALYSIS

GENERAL CHARACTERISTICS OF SEDIMENTS

So. Cal. Gas Pipeline Crossing	(%) Moisture	(%) Dry Matter	COD	(%) TOC	(%) TVS	IOD	Oil and Grease	Kjeldahl N	Org N	P	Sulfide
SCG 1	54.46	45.46	61916	2.310	6.41	1441	2751	327.4	295.7	1734	1427.0
SCG 2	51.44	48.56	54570	1.910	6.92	1019	3316	272.3	234.8	946	1171.0
SCG 3	54.31	45.69	39841	1.978	6.00	1112	2516	256.8	227.2	1346	1169.7
SCG 4	49.92	50.08	41093	1.391	6.19	757	2315	200.5	182.2	1056	767.0
SCG 5	54.19	45.81	53637	2.410	6.37	1207	3140	378.2	342.9	1326	1646.0
SCG 6	51.48	48.52	56643	4.410	9.33	4882	6651	252.5	252.5	806	5338.0
SCG 7	44.30	55.70	34479	1.230	6.59	934	2631	181.9	181.9	874	956.7
SCG 8	44.01	55.99	33967	1.940	6.01	564	2932	236.7	236.7	1006	765.6

Parts per million except as indicated.

HEAVY METALS

So. Cal. Gas Pipeline Crossing	Hg	Zn	As	Cd	Ni	Cu	Fe	Cr	Pb
SCG 1	1.590	517.29	24.10	5.84	69.23	229.05	41120	288.23	292.30
SCG 2	1.515	494.90	23.00	5.10	77.55	222.45	40310	306.12	316.30
SCG 3	1.173	482.26	21.48	4.55	58.23	234.76	30480	286.62	282.07
SCG 4	1.798	346.92	17.11	4.41	71.59	174.01	40200	203.70	209.25
SCG 5	2.788	513.91	20.14	6.05	76.18	250.30	47760	278.11	314.39
SCG 6	30.132	6891.27	32.46	8.73	84.42	1339.97	32160	378.25	1056.66
SCG 7	1.044	254.80	12.65	3.43	46.78	107.77	33830	129.49	167.08
SCG 8	0.929	224.58	13.75	3.64	38.14	127.12	24320	118.64	169.49

CHLORINATED HYDROCARBONS

So. Cal. Gas Pipeline Crossing	PP' DDE	OP' DDE	DDD	PP' DDT	OP' DDT	Total DDT	PCB 1242	PCB 1254	PCB 1260	Total PCB	Diel-drin
SCG 1	326.18	59.78	382.49	48.5	-	816.95	517.00	807.00	80.00	1404.00	4.27
SCG 2	317.10	84.84	507.82	82.37	8.24	1000.35	605.00	675.00	66.00	1346.00	5.67
SCG 3	276.96	79.33	341.87	101.00	12.43	811.59	552.00	550.00	54.00	1156.00	1.70
SCG 4	179.22	55.45	149.76	22.46	-	406.89	383.00	343.00	33.00	759.00	0.63
SCG 5	361.87	125.41	485.57	99.65	14.5	1087.00	170.00	1010.00	100.00	2280.00	9.34
SCG 6	558.88	447.36	810.22	112.53	25.72	1954.71	-	11150.00	-	11150.00	13.36
SCG 7	110.73	30.89	124.52	59.51	7.33	332.98	490.00	371.00	37.00	898.00	2.34
SCG 8	117.01	42.55	297.05	41.25	-	497.86	527.00	351.00	35.00	913.00	8.10

Pesticides reported in parts per billion. Arochlor, Lindane, BHC, Heptachlor, Kelthane, Methoxychlor, Chlordane, Toxaphene, Endrin: below detectable limits.

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